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DETECTION OF THE COLISTIN-RESISTANT MCR-1 GENE IN *ESCHERICHIA COLI* ISOLATES IN RETAIL PORK FROM FRESH MARKETS IN BANGKOK, THAILAND

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Abstract–This study aimed to investigate the presence of colistin resistance (the *mcr-1* gene) in *E. coli* isolates in retail pork. A total of 900 retail pork samples were collected from 36 fresh markets in Bangkok from January to October 2018. We collected *E. coli* that were isolated from MacConkey agar and Eosin Methylene Blue agar and screened the presence of the *mcr-1* gene by using PCR. Results showed the MICs to colistin of the 900 studied isolates, colistin resistance (MIC ≥ 2 mg/l) was identified in 9.44% (85/900) confirmed *E. coli* isolates. The size of the *mcr-1* amplicon was 309 bp. The *mcr-1* gene was detected in 37.65% (32/85) samples. The highest frequency was observed in the *mcr-1* genes from Thawi Watthana (75.00%). The data demonstrated that the *mcr-1* gene was highly prevalent in retail pork purchased from the fresh markets in Bangkok, suggesting that active surveillance of the *mcr-1* gene is imperative in curtailing its spread.

INTRODUCTION

Antimicrobial resistance (AMR) is a globally significant public health concern of this century. The condition poses substantial challenges to effective treatments against ever- increasing infectious diseases. The overuse of antibiotics in health and agriculture has put ubiquitous microbes under consistent selective pressure. As a result, these microbes have developed resistance to multiple antibiotics and are thriving in the environment (Aslam *et al.*, 2018).

Colistin, also known as polymyxin E, was recently reintroduced in human medical practices. It is one of the eventual last-resort drugs for the treatment of multidrug-resistant bacterial infections around the world. According to the Lancet Infectious Diseases, Yi-Yun Liuand co-workers were the first to report the detection of *E. coli* harboring the plas-mid bearing mobile colistin resistance gene 1 (*MCR-1*) (Lui *et al.*, 2016). In the case of plasmid-encoded resistance, resistance elements can be conveniently transmitted to humans via a horizontal gene transfer from livestock, where colistin is administered as a treatment for infected animals. The transmission of such resistance promotes a state known as multidrug-resistant for Enterobacteriaceae, and the condition would severely limit our current treatment options (Falgenhauer *et al.*, 2016). Furthermore, Current studies suggested that bacteria became resistant to colistin and that there has been a worldwide spread of gram-negative bacteria with the *mcr-1* gene, especially *E. coli*, among animals, food, environments, and humans (Skov and Monnet, 2016; Al-Tawfiq, Laxminarayan and Mendelson, 2017).

To the best of our knowledge, this study aimed to investigate the prevalence of the colistin-resistant *mcr-1* gene in *E. coli* that was isolated from retail pork in the fresh markets of Bangkok, Thailand.

MATERIALS AND METHODS

Sampling design and isolation of *E. coli*

A total of 900 retail pork samples were collected from the fresh markets in Bangkok from January to October 2018 as exhibited in Figure 1. This study was approved by the Institutional Review Board (IRB) and Ethics Committee of the Faculty of Physical Therapy, Srinakharinwirot University. The samples were transferred to the Department of Health Promotion, Faculty of Physical Therapy, Srinakharinwirot University, subcultured on MacConkey agar as well as Eosin Methylene Blue agar (EMB Agar) media (Merck, Germany) for a double-check, and a pure culture was subsequently taken. After incubation of plates at 37 °C for 18–24 hours, the isolated colonies were identified by Gram staining and subjected to biochemical confirmation with API 20 E (BioMerieux, France). Isolates were suspected of being resistant to colistin if they exhibited an inhibition zone diameter of 11 mg to a 10 µg colistin disk.

DNA extraction

Several monoclonal *E. coli* colonies were suspended in 100 µL of sterile distilled water and heated at 100 °C for 10 minutes and centrifuged at 10,000x g for 10 minutes. The resulting supernatant contained the *E. coli* genomic DNA.

DNA from *E. coli* isolates was extracted using a commercial DNA extraction kit (Qiagen DNA Mini Kit) and purified following the manufacturer's recommendation. All the extracted DNA templates were stored at -20 °C until further required.

Detection of *mcr-1* genes by polymerase chain reaction (PCR)

The detection of the *mcr-1* gene was conducted by a PCR amplification protocol, as described by Liu *et al.* (Lui *et al.*, 2016). Briefly, 2 mL of DNA was added to a total volume of 20 mL amplification reaction mixture with *mcr-1* gene-specific primers derived from the forward CLR5-F(5'-CGGTCAGTCCGTTTGTTC-3') and reversed CLR5-R(5'-CTTGGTCGGTCTGTAGGG-3'). Thirty-four cycles (94 °C for 20s, 50 °C for 20s, 72 °C for 30s, and 72 °C for 5 min) were performed. The PCR products were separated in 2% agarose gel. After staining with ethidium bromide, the gel was visualized under UV light. The PCR amplified fragments of the

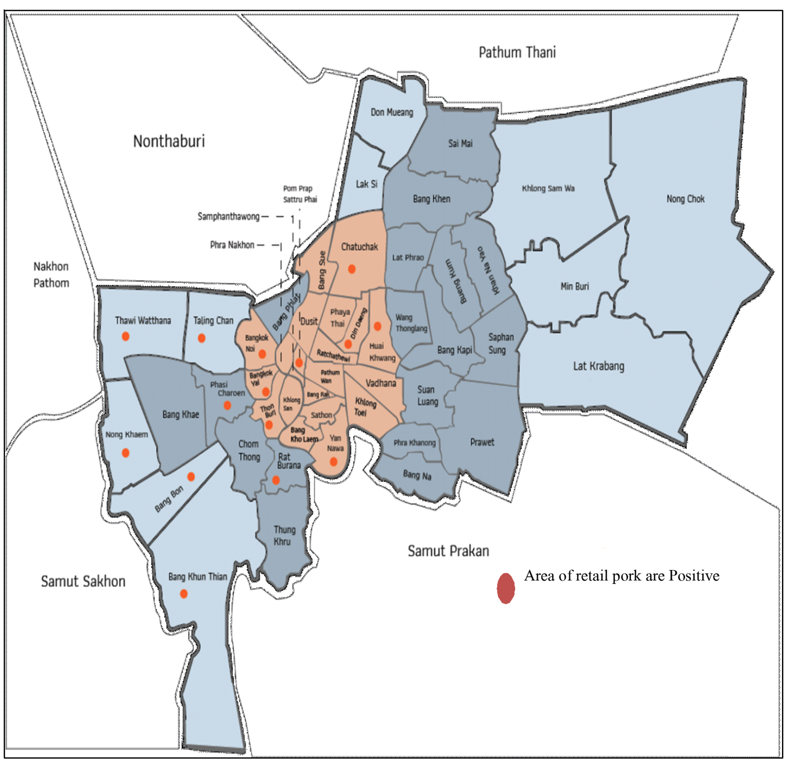

Fig. 1. The distribution of *mcr-1* in *Escherichia coli* isolates in retail pork from Bangkok's fresh markets. The *mcr*was detected in specimens from the 36 areas (*mcr-1*: 32/900). The colors (orange), shapes (circle), and positions of filled and empty graphics indicate the *mcr* positivity, the species sampled, and the cities involved, respectively.

mcr-1 gene of *E. coli* were 309 bp.

Statistical analysis

Descriptive statistics were employed to describe the percentage and the frequency of antimicrobial susceptibility, gene detection, the source of samples, and interview data.

RESULTS

Prevalence of colistin resistance among *Escherichia coli* isolates of different origin

The 900 samples were collected from the 36 fresh-market areas in Bangkok from January to October 2018, and *E. coli* isolates were investigated by culture methods on MacConkey agar and Eosin Methylene Blue agar. The isolated colonies were identified by Gram staining and subjected to biochemical confirmation with API 20E (BioMerieux, France). The MICs to colistin of the 900 studied isolates indicated that colistin resistance (MIC ≥ 2 mg/L) was found in 9.44% *E. coli* isolates (85/900). The size of the *mcr-1* amplicon was 309 bp. The *mcr-1* gene was detected in 37.65% (32/85) of the entire samples. The highest frequency was observed in *mcr-1* genes from Thawi Watthana (1.36%), as exhibited in Table 1.

Table 1. Prevalence of colistin resistance among *E. coli* isolates of different origin

No.	Area	No. of isolates	No. of *E. coli* positive isolates (%)	No. of *mcr*-1 positive isolates (%)
1	Khlong Toei	25	0(0%)	0(0%)
2	Chom Thong	25	4(16.00%)	2(50.00%)
3	Chatuchak	25	3(12.00%)	2(66.67%)
4	Don Mueang	25	5(20.00%)	2(40.00%)
5	Din Daeng	25	0(0%)	0(0%)
6	Taling Chan	25	5(20.00%)	1(20.00%)
7	Thawi Watthana	25	8(32.00%)	6(75.00%)
8	Thung Khru	25	5(20.00%)	1(20.00%)
9	Thon Buri	25	8(32.00%)	1(12.50%)
10	Bangkok Noi	25	3(12.00%)	2(66.67%)
11	Bangkok Yai	25	6(24.00%)	4(66.67%)
12	Bang Kapi	25	0(0%)	0(0%)
13	Bang Khun Thian	25	5(20.00%)	3(60.00%)
14	Bang Khen	25	6(24.00%)	2(33.33%)
15	Bang Khae	25	3(12.00%)	0(0%)
16	Bang Sue	25	0(0%)	0(0%)
17	Bang Na	25	0(0%)	0(0%)
18	Bang Bon	25	0(0%)	0(0%)
19	Bueng Kum	25	0(0%)	0(0%)
20	Prawet	25	0(0%)	0(0%)
21	Pom Prap Sattru Phai	25	6(24.00%)	2(33.33%)
22	Phra Nakhon	25	0(0%)	0(0%)
23	Phasi Charoen	25	4(16.00%)	1(25.00%)
24	Yan Nawa	25	5(20.00%)	1(20.00%)
25	Ratchathewi	25	0(0%)	0(0%)
26	Rat Burana	25	3(12.00%)	1(33.33%)
27	Lat Krabang	25	0(0%)	0(0%)
28	Lat Phrao	25	0(0%)	0(0%)
29	Wang Thonglang	25	0(0%)	0(0%)
30	SuanLuang	25	0(0%)	0(0%)
31	Saphan Sung	25	0(0%)	0(0%)
32	Samphanthawong	25	0(0%)	0(0%)
33	Sai Mai	25	0(0%)	0(0%)
34	Nong Khaem	25	2(8.00%)	0(0%)
35	Nong Chok	25	0(0%)	0(0%)
36	Huai Khwang	25	4(16.00%)	1(25.00%)
	Total	N=900	N=85(9.44%)	N=32(37.65%)

Table 2. The prevalence of colistin-resistant *mcr*-1 genebased on types of pork.

Type of pork	No. of *mcr*-1 positive isolates (n=32)	No. of *mcr*-1 positive isolates (%)
Pork meat	9	28.13%
Pork intestines	18	56.25%
Pork bones	0	0
Pork livers	1	3.13%
Pork lungs	4	12.50%

Prevalence of the colistin-resistant *mcr-1* gene based on types of pork.

Results from this study revealed the prevalence of colistin-resistant *mcr-1* gene based on types of pork. Among the 900 retail pork samples, 32 positive samples contained *mcr-1*. The highest prevalence of *mcr-1* was identified in pork intestines, with an overall prevalence of 28%. Furthermore, the prevalence of *mcr-1*, based on the pork types, ranked from 16.36%, 4.65%, and 2.13% in pork meat, lungs, and livers, respectively. Nonetheless, the samples from pork bones were found negative in terms of colistin resistance (Table 2).

DISCUSSION

While colistin is a last-line antibiotic for multidrug-resistant Gram-negative bacteria, its efficacy is being compromised by the recently detected mobile colistin-resistant *mcr-1* gene (Lui *et al.*, 2016). Hence, we surveyed colistin resistance rates in multidrug-resistant *E. coli* isolated from the 900 samples of retail pork from Bangkok's fresh markets. As presented, the PCR detection identified an unexpectedly high prevalence 37.65% (32/85) of *mcr-1* in the retail pork samples. This rate appears to be different from the results obtained in this study, and it could probably be due to the sampled meat was from different fresh markets in Bangkok. The analysis of the *mcr-1* genes showed that 37.65% of the 85 isolates presented higher Shiga toxin-producing *E. coli* (10.7%) than that of healthy pigs, higher than the 15% prevalence figure extracted from the Chinese retails, and remarkably higher than those from the pigs in our results (25.5%) (Bai *et al.*, 2016; Haenni *et al.*, 2016). Although colistin has not yet been approved for use with humans in China, it has been administered in animals as a therapeutic drug and a feed additive since the early 1980s.

However, limited comprehensive epidemiological data are available to describe the prevalence of *E. coli* and the *mcr-1* gene among retail pork in fresh markets in Bangkok, even though pork products are Thailand's primary source of animal protein.

To the best of our knowledge, the current study was the first report on the epidemiological prevalence and detection of the *E. coli mcr-1* gene in the context. Generally, the prevalence rate of *E. coli* and the *mcr-1* gene among pork samples was relatively low in this study, while similar results were observed from clinical, pigs, and retail pork in previous studies (Figueiredo *et al.*, 2016; Quesada *et al.*, 2016; Cui *et al.*, 2017).

However, since these retail pork samples were *E. coli* positive, and improper heating or cooking would increase risks of infections. In Thailand, only pre-packaged pork is governed by a pathogen limiting standard at the national level. In this study, all pork samples were collected from marketing sites.

This paper is the first report on the prevalence of the *mcr-1* gene in colistin-resistant *E. coli* isolates of retail pork in the fresh markets of Thailand. These data offer added insights into the mechanism of colistin resistance among Enterobacteriaceae pathogens.

Future work will be conducted to screen the other *mcr* variants (*mcr-2*, *mcr-3*, *mcr-4*, and *mcr-5*) in infectious enterobacterial isolates recovered from retail pork in fresh markets in Thailand.

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