# Prevalence of antibiotic resistant bacteria in healthy adults, foods, food animals, and the environment in selected areas in Thailand

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Objectives: The aim of this study was to determine the prevalence of antibiotic-resistant bacteria, especially extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli*, in samples from healthy adults, foods, food animals, and the environment in selected areas of Thailand.
Methods: Samples were collected from stool specimens from adult food factory and food animal farm workers, fresh and cooked foods sold at markets, rectal swabs of healthy pigs and chickens, fresh pork meat from slaughterhouses, water samples from canals as well as fish and shrimp farm ponds, and stagnant water sources on pig farms. Antibiotic susceptibility was determined using the disk diffusion or agar dilution methods. Extended-spectrum beta-lactamase production was assayed using a double disk diffusion method.
Results: Among 544 healthy adult food factory workers, 75.5% were positive for ESBL producing *E. coli*, while 77.3% of *E. coli* isolated from 30 healthy animal farm workers were positive. Amongst healthy food animals, ESBL producing status among *E. coli* isolates were more commonly detected in pigs (76.7%) than broilers (40%). Extended-spectrum beta-lactamase producing *E. coli* seemed to be more prevalent in fresh meat samples than in fresh vegetables, in fresh foods than in cooked foods, and in water samples collected from the animal farms than those from canals and fish and shrimp ponds.
Conclusions: Extended-spectrum beta-lactamase producing *E. coli* isolates are prevalent amongst healthy individuals, foods along the food production chain from farms to consumers, and in the environment in selected areas in Thailand.

**Keywords:** Antibiotic resistant bacteria, *E. coli*, Food chain

## Introduction

Antimicrobial resistance has been recognized as an important global health problem over the past few decades.[1] The main contributors to resistance are overuse of antibiotics in humans and animals, poor hygiene and sanitation, and inefficient prevention and control of infection in healthcare settings. Antimicrobial resistance has enormous health and economic impacts. Analysis of the estimated health burden of antimicrobial resistance in Thailand from 2009 to 2010 revealed that at least 90 000 patients per year were hospitalized with infections caused by antimicrobial-resistant bacteria and approximately 30 000 of these patients died.[2] Furthermore, antimicrobial-resistant bacterial infections resulted in at least 3 million excess days of hospitalization.[2] The cost of antibiotics to treat these infections was US$200 million, resulting in a total cost of US$1.3 billion.[2] Antimicrobial resistance is more prevalent in low- and middle-income countries, especially those in South and Southeast Asia.[3] It is well recognized that antimicrobial-resistant pathogens are a common cause of hospital-acquired infections; however, an increasing number of community-onset extended-spectrum beta-lactamase (ESBL)-producing bacterial infections, especially those caused by ESBL-Enterobacteriaceae, have been reported in many countries, including Thailand.[4–9] Risk factors for acquiring community-onset ESBL-producing *Escherichia coli* infections in Thailand include prior colonization with the bacterium and previous exposure to third-generation cephalosporins and fluoroquinolones.[9] Fecal carriage of ESBL-producing enterobacteria has increased significantly worldwide, with developing countries (especially in South and Southeast Asia) being

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the most affected. In these isolates, CTX-M enzymes are the dominant type of ESBL.[10] The prevalence of CTX-M-type ESBL-producing Enterobacteriaceae in feces of healthy individuals in three provinces of Thailand ranged from 29.3 to 76.2%.[11–13] Risk factors associated with fecal carriage of these bacteria included the use of antibiotics without prescription, history of hospitalization, and the use of antibiotics within the last 3 months.[11,12] The prevalence of colonization with ESBL-producing Enterobacteriaceae in the gastrointestinal tract of travelers from Sweden, the Netherlands, and Australia was 2.4–8.6% prior to travel, but increased to 30–49% following travel to Southeast Asia, India, and/or China.[13–16] Risk factors in these cases included traveling to the Indian subcontinent and Asia, and taking antibiotics while traveling.[13–16] Traveler's diarrhea associated with ESBL-producing pathogens was also common among patients returning from India, Egypt, and Thailand.[17] The aforementioned information indicated that the travelers probably acquired ESBL-producing Enterobacteriaceae from foods, water, and/or the environment in these Asian countries. Antibiotic-resistant bacteria, including ESBL-producing Enterobacteriaceae, have been detected in food animals, meats, water, and the environment in many Asian countries, including China, Hong Kong, India, Bangladesh, and Malaysia, and could be reservoirs for colonization and infection of human beings.[18–28] There are several previous microbiological surveys of food animals on farms, fresh meat samples from slaughterhouses and retail stores, exported fresh meat samples and vegetables, and stool samples from both healthy individuals and hospital patients from Thailand and several other countries.[29–41] Almost all of these reports emphasized the ability of foodborne bacteria in causing gastrointestinal infections, especially *Salmonella*, *Campylobacter*, and *Arcobacter* species, though only a few studies described antibiotic-resistant *E. coli*. Many *Salmonella* species were isolated from these samples, most of which were resistant to antibiotics. Associations of antibiotic-resistant foodborne bacteria with food animals, fresh meats sold at retail stores, healthy Thais, and Thai and international patients were observed. Contamination with *E. coli* in chicken meat obtained from supermarkets in Bangkok during 2010–2011 was 53%.[39] Some isolates of *E. coli* were resistant to ampicillin, cephalothin, gentamicin, co-trimoxazole, tetracycline, and/or ciprofloxacin, but all isolates were susceptible to ceftriaxone and carbapenems.[39] A study of 240 *E. coli* strains isolated from Thai patients and healthy adults revealed that some *E. coli* isolates produced ESBLs and shared the same antibiotic resistance genes.[41] The principal objective of our study focused on surveying foods along the production chain from farms to consumers and healthy individuals, including those who had direct contact with food animals, to determine the prevalence of important antibiotic-resistant bacteria that usually cause systemic infections in humans, especially ESBL-producing *E. coli*.

## Methods

### *Population and specimen collection*

The study was conducted from June 2012 to March 2013. Cary-Blair transport medium (non-nutritive medium) tubes were used to collect all swab specimens.

#### Healthy population

Stool samples were collected from 544 healthy adult workers in a food factory located in a central province and 30 healthy food animal farm workers in both an eastern and a northern province.

#### Healthy food animals

Rectal swabs were collected from 330 and 70 randomly chosen healthy pigs raised in a northern and an eastern province, respectively. One rectal swab was taken from each pig. Rectal swab samples were also collected from 141 randomly chosen healthy chickens, of which 80 samples were collected from broilers (70 samples from a northern province and 10 from an eastern province) and 61 were collected from laying hens (51 samples from a northern province and 10 from an eastern province). The rectal swabs taken from five chickens were pooled together in one swab. Therefore, the total number of broilers and laying hens that were swabbed was 400 and 305, respectively. Swabs were also taken from 18 fresh pork meat samples, 8 from slaughterhouses in a northern province and 10 from slaughterhouses in an eastern province.

#### Foods

Fresh and cooked foods were purchased from a market in Bangkok and from a market near the central province food factory described above. The fresh foods were chicken meat ($n = 14$), pork meat ($n = 15$), beef ($n = 11$), fish ($n = 14$), shrimp ($n = 13$), chicken eggs ($n = 13$), spring onions ($n = 15$), parsley ($n = 15$), and bean sprouts ($n = 13$). The cooked foods were chicken with rice ($n = 20$), grilled chicken ($n = 20$), grilled pork ($n = 19$), spicy green papaya salad ($n = 21$), spicy minced meat ($n = 21$), and spicy grilled meat ($n = 21$). All food samples were brought to the laboratory within a few hours of purchase and were thoroughly swabbed.

#### Environment

Water samples (50 ml for each sample) were collected from 15 canals (10 in Bangkok and 5 in a central province) and 87 fish and shrimp farm ponds in four coastal provinces. Twenty-five samples were also collected from stagnant water sources at animal food farms (10 in an eastern province and 15 in a northern province). The unspun water sample was used for culture.

### Bacterial culture and antibiotic susceptibility test

The stool samples from the 544 healthy adult workers in a food factory were inoculated onto MacConkey agar supplemented with ceftriaxone (1 mg/l) and MacConkey agar supplemented with ciprofloxacin (4 mg/l) to detect antibiotic resistant Gram-negative bacteria. Bacteria grown on the aforementioned antibiotic supplemented agars were further identified by matrix assisted laser desorption ionization time-of-flight (MALDI-TOF). Minimum inhibitory concentrations (MIC) of ceftriaxone and ciprofloxacin against Gram-negative bacteria were determined using an agar dilution method. Extended-spectrum beta-lactamase-producing bacteria were detected using a double disk diffusion method.[42] An extra zone was determined between a disk of amoxicillin-clavulanate (20 μg/10 μg) and a 30-μg disk of each cephalosporin (ceftriaxone, ceftazidime, cefepime) placed at a distance of 20 mm from center to center of the disks on Mueller-Hinton agar plates. Clear extension of the edge of the cephalosporin inhibition zone toward the amoxicillin-clavulanate disk was interpreted as positive for ESBL production. Colistin susceptibility was also performed using a disk diffusion method.[43]

Stool samples from healthy food animal farm workers, water samples, and swabs from fresh and cooked foods, healthy food animals, and fresh pork meat samples were inoculated onto MacConkey agar, MacConkey agar supplemented with ceftriaxone, mannitol salt agar (selective for *Staphylococci*), and enterococcal agar supplemented with vancomycin [selective for vancomycin-resistant *Enterococcus* species (VRE)]. The inoculated plates were incubated at 35°C for 24 hours. Isolated colonies were further identified by biochemical tests. Extended-spectrum beta-lactamase production was detected by the double disk diffusion method. Antibiotic susceptibility was determined using a disk diffusion method. The tested antibiotics were ceftriaxone, cefoxitin, ceftazidime (for *Pseudomonas aeruginosa*), imipenem, ertapenem, meropenem, gentamicin, amikacin, nalidixic acid, ciprofloxacin, tetracycline, erythromycin, piperacillin/tazobactam, cefoperazone/sulbactam, and colistin where appropriate and available.

### Data analyses

The data were described by descriptive statistics and the comparison of categorical data between groups was performed using Chi-square or Fisher's exact test as appropriate. Data were analyzed using PASW statistics for windows, version 18.0 (SPSS Inc., Chicago, USA).

## Results

### Healthy adult food factory workers

The mean (SD) age of 544 healthy adult workers in a central province was 34.6 years (9.8 years), and 245 (45%) were female. *E. coli* grown on ceftriaxone- and ciprofloxacin-supplemented agar was found in stool samples of 497 (91.4%) workers. The total number of *E. coli* isolates was 906, as some workers had more than one isolate in their stool sample. Resistance to ceftriaxone, ciprofloxacin, and colistin, and the proportion of ESBL-producing isolates among *E. coli* are shown in Table 1. Although the prevalence of resistance to either ceftriaxone or ciprofloxacin in *E. coli* isolates was very high (60.7% of isolates), the prevalence of resistance to both ceftriaxone and ciprofloxacin in the same *E. coli* isolate was much less common (15.1% of isolates). The prevalence of ceftriaxone and ciprofloxacin resistance in one, two, and three same strains of *E. coli* was 6.6, 9.0 and 0.2% of the workers, respectively. The $MIC_{50}$, $MIC_{90}$, and MIC range for ceftriaxone against isolated *E. coli* were 8, 64, and 0.06–512 mg/l, respectively. The $MIC_{50}$, $MIC_{90}$, and MIC range for ciprofloxacin against isolated *E. coli* were 8, 32, and 0.06–128 mg/l, respectively. Extended-spectrum beta-lactamase-producing *E. coli* were detected in 75.5% of food factory workers, and accounted for 47.4% of isolates. The prevalence of colistin-resistant *E. coli* was 8.8%.

### Healthy animal farm workers

Enterobacteriaceae were recovered from the stool samples of 27 of the 30 healthy animal farm workers (90%), and 66.7% of these produced ESBLs (Table 2).

**Table 1 Antibiotic resistance to ceftriaxone, ciprofloxacin, and colistin and the proportion of ESBL-producing isolates amongst resistant *Escherichia coli*, isolated from stool samples of 544 healthy adult workers containing 906 *E. coli* isolates**

| Antibiotic resistance (by population) | No. of subjects ($n$ = 544) | Antibiotic resistance (by isolate) | No. of isolates ($n$ = 906) |
|---|---|---|---|
| Ceftriaxone resistance (MIC > 4) in any isolates, $n$ (%) | 424 (77.9) | Ceftriaxone resistance (MIC > 4) (by isolate) | 493 (54.4) |
| Ciprofloxacin resistance (MIC > 4) in any isolates, $n$ (%) | 450 (82.7) | Ciprofloxacin resistance (MIC > 4) | 550 (60.7) |
| Ceftriaxone and ciprofloxacin resistance in the same isolate, $n$ (%) | 86 (15.8) | Ceftriaxone and ciprofloxacin resistance in the same isolate | 137 (15.1) |
| ESBL production in any isolates, $n$ (%) | 412 (75.5) | ESBL production in any isolates | 429 (47.4) |
| | | Colistin resistance (inhibition zone diameter < 11 mm) | ($n$ = 352) 31 (8.8) |

ESBL: extended-spectrum beta-lactamase; MIC: minimum inhibitory concentration.

**Table 2 Bacteria isolated from stool samples of healthy animal farm workers and their extended-spectrum beta-lactamase (ESBL) production status**

| | Per population | | | Per isolate | | |
|---|---|---|---|---|---|---|
| Bacteria | Total workers (*n* = 30) | Eastern province (*n* = 10) | Northern province (*n* = 20) | Total isolates (*n* = 59) | Eastern province (*n* = 19) | Northern province (*n* = 40) |
| Enterobacteriaceae | 27/30 (90%) | 9/10 (90%) | 18/20 (90%) | 49/59 (83.1%) | 17/19 (89.5%) | 32/40 (80.0%) |
| ESBL-producing isolates | 18/27 (66.7%) | 4/9 (44.4%) | 14/18 (77.8%) | 27/49 (55.1%) | 9/17 (53%) | 18/32 (56.3%) |
| *Escherichia coli* | 22/27 (81.4%) | 7/9 (77.7%) | 15/18 (83.3%) | 25/59 (42.4%) | 7/19 (36.8%) | 18/40 (45.0%) |
| ESBL-producing isolates | 17/22 (77.3%) | 3/7 (42.9%) | 14/15 (93.3%) | 18/25 (72.0%) | 3/7 (42.9%) | 15/18 (83.3%) |
| *Enterobacter* spp. | 2/27 (7.4%) | 1/9 (11.1%) | 1/20 (5%) | 2/59 (3.4%) | 1/19 (5.3%) | 1/40 (2.5%) |
| ESBL-producing isolates | 1/2 (50%) | 1/1 (100%) | 0/1 (0%) | 1/2 (50%) | 1/1 (100%) | 0/1 (0%) |
| *Klebsiella* spp. | 19/27 (70.4%) | 8/9 (88.9%) | 11/18 (61.1%) | 19/59 (32.2%) | 8/19 (42.1%) | 11/40 (27.5%) |
| ESBL-producing isolates | 8/19 (42.1%) | 5/8 (62.5%) | 3/11 (27.3%) | 8/19 (42.1%) | 5/8 (62.5%) | 3/11 (27.3%) |

The overall prevalence of ESBL-producing status among *E. coli* was 77.3%. The prevalence of ESBL-producing *E. coli* was higher in a northern province than in an eastern province (93.3% vs. 42.9%, $P = 0.02$). *P. aeruginosa* and *Acinetobacter baumannii* were observed in 6.7 and 10% of farm workers, respectively. Neither *Staphylococcus aureus* nor VRE was isolated. Among 59 Enterobacteriaceae isolates obtained from the 30 animal farm workers, 55.1% were ESBL producers (Table 2), and the overall prevalence of ESBL-producing *E. coli* was 72.0%. The prevalence of ESBL-producing *E. coli* was higher in a northern province than in an eastern province (83.3 vs. 42.9%, $P = 0.48$). The overall prevalence of ceftriaxone-resistant *E. coli* was 72.0%. The prevalence of ceftriaxone-resistant *E. coli* in farm workers in a northern province was higher than from workers in an eastern province (83.3 vs. 42.9%, $P = 0.07$). Gentamicin-resistance and nalidixic acid-resistance in *E. coli* were 48.0 and 20.0%, respectively. The overall prevalence of *E. coli* with resistance to cefoxitin, amikacin, or ciprofloxacin was less than 10.0%. No carbapenem-resistant Enterobacteriaceae (CRE) or colistin-resistant Enterobacteriaceae were observed, while tetracycline resistance was very common (84.0%). All isolates were resistant to erythromycin, with the largest inhibition zone diameter for erythromycin against these isolates was only 9 mm.

### Healthy food animals

Overall proportions and ESBL-producing status of Enterobacteriaceae isolates from 400 pigs, 141 chickens (80 from broilers and 61 from laying hens), and 18 fresh pork meat samples collected from slaughterhouses are shown in Table 3. Extended-spectrum beta-lactamase-producing Enterobacteriaceae and *E. coli* isolates were most common in pigs (75.1 and 76.7%, respectively), followed by broilers (38.8 and 40%, respectively), fresh pork meat samples (20 and 33.3%, respectively), and laying hens (3.6 and 4%, respectively). Other ESBL-producing status among Enterobacteriaceae isolates were less frequently isolated compared with *E. coli*, except for *Klebsiella* species isolated from pigs in Table 3. *P. aeruginosa* was uncommonly found in pigs and broilers. *A. baumannii* and *P. aeruginosa* were found in 40% of pork meat samples but were not isolated from laying hen samples. Methicillin-resistant *S. aureus* (MRSA) was found in a pig from a northern province, but no VRE was detected across all samples. The prevalence of ESBL-producing status among *E. coli* and other Enterobacteriaceae strains is shown in Table 3. Extended-spectrum beta-lactamase-producing *E. coli* isolates were common in pigs (76.7%), less common in broilers (40%), and pork meat (33.3%) and uncommon in laying hens (4%). Extended-spectrum beta-lactamase-producing status among *Klebsiella* species isolates were common in pigs but were much less common in broilers (71.4 vs. 11.1%, $P = 0.01$) and were not found in laying hens and pork meat samples. The prevalence of ESBL-producing Enterobacteriaceae and *E. coli* in pigs in a northern province (77.4 and 80.2%) tended to be higher than that in an eastern province (62.3 and 64.7%). The prevalence of ESBL-producing Enterobacteriaceae and *E. coli* in broilers in a northern province (40.0 and 41.1%, respectively) tended to be higher than that in an eastern province (35.0 and 36.8%, respectively). Ceftriaxone-resistant *E. coli* strains were commonly isolated from rectal swabs of pigs (59.3%), and no significant differences in resistance rates were observed between a northern province (59.7%) and an eastern province (59.7 vs. 57.1%, $P = 0.69$). For antibiotic resistance of *E. coli* isolated from rectal swabs, cefoxitin resistance was more common in an eastern province than in a northern province (35.7 vs. 2.4%, $P < 0.01$). Carbapenem resistance was found in two isolates of *E. coli* from rectal swabs of pigs collected from each province. Gentamicin resistance rates in both provinces were similar (55.4 vs. 58.6%, $P = 0.63$), whereas the amikacin resistance rate in an eastern province was higher than that in a northern province (12.9 vs. 0.6%, $P < 0.01$). Nalidixic acid and ciprofloxacin resistance rates in an eastern province (51.4 and 71.4%, respectively) were also higher than those in a northern province (12.1 and 32.7%, respectively, $P < 0.01$).

**Table 3 Bacteria isolated from rectal swabs of healthy pigs, healthy chickens, and fresh pork meat samples collected from slaughterhouses in a northern province and an eastern province**

| Bacteria | Per sample | | | | Per isolate | | | |
|---|---|---|---|---|---|---|---|---|
| | | Chicken ($n$ = 141) | | | | Chicken ($n$ = 202) | | |
| | Pig ($n$ = 400) | Broiler ($n$ = 80) | Laying hen ($n$ = 61) | Fresh pork meat ($n$ = 18) | Pig ($n$ = 918) | Broiler ($n$ = 126) | Laying hen ($n$ = 76) | Fresh pork meat ($n$ = 25) |
| Enterobacteriaceaes | 369/400 (92.2%) | 80/80 (100%) | 55/61 (90.3%) | 15/18 (83.3%) | 721/918 (78.5%) | 118/126 (93.7%) | 72/76 (92.5%) | 15/25 (60%) |
| ESBL-producing isolates | 277/369 (75.1%) | 31/80 (38.8%) | 2/55 (3.6%) | 3/15 (20%) | 435/721 (60.3%) | 32/118 (27.1%) | 2/72 (2.8%) | 3/15 (20%) |
| *Escherichia coli* | 314/369 (85.1%) | 75/80 (93.3%) | 50/55 (90.1%) | 6/15 (40.0%) | 502/721 (69.6%) | 78/118 (66.1%) | 54/72 (75%) | 5/15 (33.3%) |
| ESBL-producing isolates | 241/314 (76.7%) | 30/75 (40%) | 2/50 (4.0%) | 2/6 (33.3%) | 349/502 (69.5%) | 30/78 (38.4%) | 2/54 (3.7%) | 2/5 (60%) |
| *Enterobacter* spp. | 111/369 (30.1%) | 3/80 (3.7%) | 9/55 (16.4%) | 5/15 (33.3%) | 132/721 (18.3%) | 3/118 (2.5%) | 10/72 (13.9%) | 5/15 (33.3%) |
| ESBL-producing isolates | 53/111 (47.7%) | 0/3 (0%) | 0/9 (0%) | 1/5 (20%) | 66/132 (50%) | 0/3 (0%) | 0/10 (0%) | 1/5 (20%) |
| *Klebsiella* spp. | 28/369 (7.6%) | 18/80 (22.5%) | 5/55 (9.1%) | 2/15 (13.3%) | 28/721 (3.9%) | 18/118 (15.2%) | 5/72 (6.9%) | 2/15 (13.3%) |
| ESBL-producing isolates | 20/28 (71.4%) | 2/18 (11.1%) | 0/5 (0%) | 0/2 (0%) | 20/28 (71.4%) | 2/18 (11.1%) | 0/5 (0%) | 0/2 (0%) |

ESBL: Extended-spectrum beta-lactamase.

Tetracycline resistance was common, and the resistance rate was higher in an eastern province than in a northern province (84.3 vs. 62.4%, $P < 0.01$). Colistin resistance was observed in 0.3–2.9% from rectal swabs of pigs.

Ceftriaxone-resistant *E. coli* isolates were common (40%) in broilers, and the resistance rate seemed to be higher in an eastern province than in a northern province (50.0 vs. 38.3%, $P = 0.51$). Cefoxitin resistance was more common in an eastern province than in a northern province (10.0 vs. 0%, $P = 0.14$). Gentamicin and amikacin resistance were most frequently identified in isolates from samples collected in an eastern province (60 and 90%, respectively), while nalidixic acid and ciprofloxacin resistance were also prevalent in an eastern province (40 and 70%, respectively) and a northern province (53.3 and 61.7%, respectively). Tetracycline resistance was very common across all samples (72.9%). No carbapenem or colistin-resistant isolates were detected.

Ceftriaxone resistance was more common in *E. coli* isolated from rectal swabs of laying hens in an eastern province than in a northern province (50.0 vs. 39.3%, $P = 0.73$). Cefoxitin, gentamicin, and amikacin resistance were uncommon (<10%), and no carbapenem-resistant isolates were detected. Nalidixic acid resistance was common in both the eastern (40%) and northern (52.4%) provinces. Ciprofloxacin resistance was more common in an eastern province than in a northern province (60 vs. 13.1%, $P < 0.01$). Tetracycline resistance was very common (73.2%) and resistance rate seemed to be greater in an eastern province than in a northern province (90 vs. 70.5%, $P = 0.27$). Colistin resistance was rare (1.4%).

### Fresh foods

No bacteria were isolated from chicken eggs, but Enterobacteriaceae and *A. baumannii* were found in the rest of the fresh food samples (Table 4). Extended-spectrum beta-lactamase-producing status among Enterobacteriaceae and *E. coli* isolates were most common in pork (53.3 and 61.5%, respectively) and chicken (45.4 and 50.0%, respectively) samples. Extended-spectrum beta-lactamase-producing status among *Klebsiella* species were prevalent in fish (66.7%), followed by pork (44.4%), chicken (40.0%), beef (36.4%), bean sprouts (30.8%), and shrimp (20.0%). Extended-spectrum beta-lactamase-producing status among *Enterobacter* species were less common in spring onion (20.0%) and fish (16.7%). No MRSA or VRE was detected. Contamination of fresh food samples purchased from markets in Bangkok with many bacterial species seemed to be more prevalent than those from a central province (Table 4). Contamination with *E. coli* seemed to be more prevalent in fresh meat samples than fresh vegetables, whereas contamination with *P. aeruginosa* seemed to be more prevalent in fresh vegetables

**Table 4 Bacterial isolates from fresh food samples purchased from markets in Bangkok and a central province**

| Fresh food ($n$ = 110) | Location | Enterobacteriaceae | ESBL-producing isolates | *Escherichia coli* | ESBL-producing isolates | *Klebsiella* spp. | ESBL-producing isolates | *Enterobacter* spp. | ESBL-producing isolates |
|---|---|---|---|---|---|---|---|---|---|
| Chicken ($n$ = 14) | Bangkok ($n$ = 10) | 9/10 (90%) | 5/9 (55.6%) | 7/9 (77.8%) | 4/7 (57.1%) | 5/9 (55.6%) | 2/5 (40%) | 5/9 (55.6%) | 0/5 (0%) |
| | Central province ($n$ = 4) | 2/4 (50%) | 0/2 (0%) | 1/2 (50%) | 0/1 (0%) | – | – | 1/2 (50%) | 0/1 (0%) |
| Pork ($n$ = 15) | Bangkok ($n$ = 10) | 10/10 (100%) | 4/10 (40%) | 9/10 (90%) | 4/9 (44.4%) | 6/10 (60%) | 2/6 (33.3%) | 3/10 (30%) | 0/3 (0%) |
| | Central province ($n$ = 5) | 5/5 (100%) | 4/5 (80%) | 4/5 (80%) | 4/4 (100%) | 3/5 (60%) | 2/3 (66.7%) | 1/5 (20%) | 0/1 (0%) |
| Beef ($n$ = 11) | Bangkok ($n$ = 10) | 10/10 (100%) | 4/10 (40%) | 2/10 (20%) | 0/2 (0%) | 10/10 (100%) | 4/10 (40%) | 7/10 (70%) | 0/7 (0%) |
| | Central province ($n$ = 1) | 1/1 (100%) | 0/1 (0%) | 1/1 (100%) | 0/1 (0%) | 1/1 (100%) | 0/1 (0%) | – | – |
| Fish ($n$ = 14) | Bangkok ($n$ = 10) | 9/10 (90%) | 3/9 (33.3%) | 2/9 (22.2%) | 0/2 (0%) | 4/9 (44.4%) | 3/4 (75%) | 6/9 (66.7%) | 0/6 (0%) |
| | Central province ($n$ = 4) | 3/4 (75%) | 2/3 (66.7%) | – | – | 2/3 (66.7%) | 0/2 (0%) | 1/3 (33.3%) | 1/1 (100%) |
| Shrimp ($n$ = 13) | Bangkok ($n$ = 10) | 8/10 (80%) | 1/8 (12.5%) | – | – | 5/8 (62.5%) | 1/5 (20%) | 2/8 (25.0%) | 0/2 (0%) |
| | Central province ($n$ = 3) | – | – | – | – | – | – | – | – |
| Spring onion ($n$ = 15) | Bangkok ($n$ = 10) | 9/10 (90%) | 0/9 (0%) | – | – | 9/9 (100%) | 0/9 (0%) | 1/9 (11.1%) | 0/1 (0%) |
| | Central province ($n$ = 5) | 4/5 (80%) | 0/4 (0%) | – | – | 4/4 (100%) | 0/4 (0%) | 1/4 (25.0%) | 1/1 (100%) |
| Parsley ($n$ = 15) | Bangkok ($n$ = 10) | 9/10 (90%) | 0/9 (0%) | – | – | 8/9 (88.9%) | 0/8 (0%) | 1/9 (11.1%) | 0/1 (0%) |
| | Central province ($n$ = 5) | 2/5 (40%) | 0/2 (0%) | – | – | 2/2 (100%) | 0/2 (0%) | – | – |
| Bean sprout ($n$ = 13) | Bangkok ($n$ = 10) | 10/10 (100%) | 3/10 (30%) | – | – | 9/10 (90%) | 3/9 (33.3%) | 1/10 (10%) | 0/1 (0%) |
| | Central province ($n$ = 3) | 2/3 (66.7%) | 2/2 (100%) | – | – | 2/2 (100%) | 1/2 (50%) | – | – |

ESBL: extended-spectrum beta-lactamase.

than fresh meat samples. At least one strain of each of *E. coli*, *Klebsiella* species and *Enterobacter* species was resistant to ceftriaxone, cefoxitin, gentamicin, nalidixic acid, and/or ciprofloxacin. No resistance to amikacin, imipenem, ertapenem, or colistin was detected. The overall prevalence of antibiotic resistance in fresh vegetables seemed to be less than that in fresh meat samples. Ceftriaxone resistance was more common in bean sprouts. At least one strain of *Klebsiella* species or *Enterobacter* species in fresh foods was resistant to ceftriaxone, cefoxitin, gentamicin and/or nalidixic acid. No resistance to imipenem, ertapenem, ciprofloxacin, or colistin was detected. The numbers of bacteria isolated from the samples purchased from markets in Bangkok and a central province were too small to compare their antibiotic resistance rates.

### Cooked foods

Bacterial contamination of all cooked food samples purchased from markets in Bangkok and a central province is summarized in Table 5. Only one sample of each cooked food was purchased from a market in a central province. Contamination with Enterobacteriaceae, *A. baumannii*, or *P. aeruginosa* was common in chicken with rice, spicy minced meat, and spicy grilled meat. No Enterobacteriaceae, *A. baumannii*, or *P. aeruginosa* were detected in grilled chicken or grilled pork. No MRSA or VRE was detected in any sample. At least one strain of *E. coli*, *Klebsiella* species, or *Enterobacter* species was resistant to ceftriaxone, cefoxitin, gentamicin, nalidixic acid, and/or ciprofloxacin. No resistance to imipenem, ertapenem, amikacin, or colistin was detected.

### Fish and shrimp pond water

Enterobacteriaceae were recovered from one-third of fish and shrimp ponds, and *E. coli*, *Klebsiella* species, and *Enterobacter* species were the major groups (Table 6). Extended-spectrum beta-lactamase-producing strains of Enterobacteriaceae were not detected, and resistance to the tested antibiotics was not prevalent. At least one strain of *E. coli*, *Klebsiella* species, or *Enterobacter* species was resistant to ceftriaxone, cefoxitin, imipenem, ertapenem, gentamicin, amikacin, nalidixic acid, ciprofloxacin, or colistin.

### Canal water

Bacteria isolated from water samples collected from 15 canals in Bangkok and a central province are shown in Table 7. Enterobacteriaceae were found in 50.0 and 60.0% of canals in these locations, respectively, and ESBL-producing Enterobacteriaceae were isolated from canal water in Bangkok. Resistance to ceftriaxone, cefoxitin, imipenem, ertapenem, gentamicin, amikacin, nalidixic acid, and/or ciprofloxacin was found in some Enterobacteriaceae isolated from the canals in Bangkok. Resistance to colistin was not

**Table 5 Bacteria isolated from cooked food samples purchased from markets in Bangkok and a central province**

| Cooked food ($n$ = 122) | Location | Enterobacteriaceae | ESBL-producing isolates | *Escherichia coli* | ESBL-producing isolates | *Klebsiella* spp. | ESBL-producing isolates | *Enterobacter* spp. | ESBL-producing isolates |
|---|---|---|---|---|---|---|---|---|---|
| Chicken with rice ($n$ = 20) | Bangkok ($n$ = 19) | 9/19 (47.4%) | 0/9 (0%) | 1/9 (11.1%) | 0/1 (0%) | 8/9 (88.9%) | 0/8 (0%) | 2/9 (22.2%) | 0/2 (0%) |
| | Central province ($n$ = 1) | 1/2 (50%) | 0/1 (0%) | – | – | – | – | 1/1 (100%) | 0/1 (0%) |
| Grilled chicken ($n$ = 20) | Bangkok ($n$ = 19) | 1/19 (5.3%) | 0/1 (0%) | – | – | – | – | 1/1 (100%) | 0/1 (0%) |
| | Central province ($n$ = 1) | – | – | – | – | – | – | – | – |
| Grilled pork ($n$ = 19) | Bangkok ($n$ = 19) | 2/19 (10.6%) | 0/2 (0%) | – | – | 1/2 (50%) | 0/1 (0%) | 1/2 (50%) | 0/1 (0%) |
| | Central province ($n$ = 0) | – | – | – | – | – | – | – | – |
| Spicy minced meat ($n$ = 21) | Bangkok ($n$ = 20) | 5/20 (25%) | 1/5 (20%) | 2/5 (40%) | 1/2 (50%) | 1/5 (20%) | 0/1 (0%) | 2/5 (40%) | 0/2 (0%) |
| | Central province ($n$ = 1) | 1/1 (100%) | 0/1 (0%) | – | – | 1/1 (100%) | 0/1 (0%) | – | – |
| Spicy grilled meat ($n$ = 21) | Bangkok ($n$ = 20) | 5/20 (25%) | 1/5 (20%) | 2/5 (40%) | 1/2 (50%) | 2/5 (40%) | 0/2 (0%) | 1/5 (20%) | 0/1 (0%) |
| | Central province ($n$ = 1) | 1/1 (100%) | 0/1 (0%) | – | – | 1/1 (100%) | 0/1 (0%) | – | – |
| Spicy green papaya salad ($n$ = 21) | Bangkok ($n$ = 20) | 11/20 (55%) | 0/11 (0%) | 1/11 (9.1%) | 0/11 (0%) | 5/11 (45.5%) | 0/5 (0%) | 8/11 (72.7%) | 0/8 (0%) |
| | Central province ($n$ = 1) | 1/1 (100%) | 0/1 (0%) | – | – | 1/1 (100%) | 0/1 (0%) | – | – |

ESBL: Extended-spectrum beta-lactamase.

observed. No MRSA or VRE were detected in any of the samples.

### *Stagnant water from food animal farms*

Bacteria isolated from water samples collected from 25 stagnant water sources on food animal farms in an eastern province and a northern province are shown in Table 7. Enterobacteriaceae were found in most of the stagnant water samples, and up to 67.0% of the *E. coli* isolates produced ESBLs. Some strains of *E. coli*, *Klebsiella* species, or *Enterobacter* species were resistant to ceftriaxone, cefoxitin, gentamicin, nalidixic acid, ciprofloxacin, or colistin. No resistance to imipenem, ertapenem, or amikacin was observed.

## Discussion

This study aimed to determine variability in distribution of important antibiotic-resistant bacteria capable of causing systemic infections in humans, especially ESBL-producing *E. coli*, in foods, the environment, and healthy people. Therefore, a variety of foods along the production chain from farm to consumer, along with healthy individuals, including those who had direct contact with food animals, were chosen for investigation. Samples were collected from eight provinces located in the central, northern, eastern, and coastal areas of Thailand. The prevalence of resistant bacteria in samples collected from some areas may not be accurate because the number of samples was small. This limitation arose because some of the selected areas, such as the area near the food factory in a central province, were small and had only a limited number of retail vendors. Moreover, resistant bacteria present at low levels might not be detected in some samples because the method of bacterial isolation was qualitative culture.

The prevalence of ESBL-producing *E. coli* in the stool samples of healthy food factory workers from a central province (75%) was quite high when compared with a study conducted in 2004 that included 120 healthy adults from Bangkok, which found no ESBL production by the *E. coli* isolates.[41] However, the observed high prevalence of ESBL-producing *E. coli* in this study was comparable to another report using samples collected in 2010, which found ESBL-producing *E. coli* in 63.5–73.2% of healthy adults.[12] The overall prevalence of ESBL-producing status among *E. coli* in the stool samples of healthy animal farm workers observed in this study was also very high (77.3%). However, the prevalence in healthy animal farm workers from an eastern province was 42.9%, whereas it was 93.3% in the samples from a northern province. These findings suggest that the fecal carriage of ESBL-producing bacteria in healthy individuals varies between different geographical areas, occupations, foods, and the environment, as observed in two previous studies in different areas.[11,13] Colonization of

**Table 6 Bacteria isolated from 87 water samples collected from fish and shrimp ponds and their extended-spectrum beta-lactamase (ESBL) production status in four coastal provinces**

| | Enterobacteriaceae | ESBL-producing isolates | *Escherichia coli* | *Klebsiella* spp. | *Enterobacter* spp. | *Acinetobacter baumannii* | *Pseudomonas aeruginosa* |
|---|---|---|---|---|---|---|---|
| Total fish and shrimp ponds ($n = 87$) | 29/87 (33.3%) | 0/29 (0%) | 9/29 (31%) | 7/29 (24.1%) | 20/29 (69%) | 5/9 (55.6%) | 1/9 (11.1%) |

healthy adult workers with ESBL-producing *E. coli* may be associated with consumption of food and water contaminated with ESBL-producing bacteria, or self-medication with antibiotics. Extended-spectrum beta-lactamase-producing *E. coli* was also present in the foods sold at the market near the factory where stool samples were collected from workers. Typing of ESBL-producing *E. coli* isolated from these workers and food samples will be performed in future studies. Information on self-administration of antibiotics by individual workers was not available. However, the factory infirmary informed us that amoxicillin and norfloxacin were commonly given to workers who had common colds and diarrhea. Food animal farm workers may have acquired antibiotic resistant bacteria from pigs and chickens, and typing of ESBL-producing *E. coli* strains isolated from food animal farm workers and pigs and chickens will also be performed in future studies.

This survey revealed that antibiotic-resistant bacteria, including ESBL-producing *E. coli*, are present in pigs and chickens, and can be isolated throughout the production chain from farms, slaughterhouses, and markets. This observation implied that contamination of pork products with ESBL-producing *E. coli* is probably the result of fecal contamination. However, this hypothesis should be confirmed by molecular typing of resistance genes of the resistant bacteria isolated along the food production chain. Differences in prevalence of antibiotic resistance among pigs, broilers, and laying hens, and amongst the samples collected from an eastern province and a northern province were likely a result of differences in food animal raising environments and antibiotics that were used in these food animals. The pig farmers were more concerned of infections than the chicken farmers. Therefore, they usually used antibiotics to feed the pigs for prevention of infections. The prevalence of ESBL-producing *E. coli* in rectal swabs of pigs was also higher than that of broilers and laying hens.

Extended-spectrum beta-lactamase-producing *E. coli* was found more frequently in broilers than laying hens. The reason for such an observation is unclear, as both types of chickens received periodic antibiotics. Broilers were raised for 5–6 weeks, whereas laying hens were raised for several years. Some of the antibiotics that were mass-administered to promote growth or prevent or treat infections in these animals were tetracycline, tulathromycin, amoxicillin, enrofloxacin, ceftifur, lincomycin, and spectinomycin. Differences in patterns of antibiotic resistance of bacteria isolated from food animals in an eastern province and a northern province could be caused by differences in the types of antibiotics used. The high prevalence of antibiotic resistance in food animals in the surveyed areas might be associated with inappropriate use of antibiotics, including fluoroquinolones and third generation cephalosporins, in animal husbandry. The World Organization for Animal Health (OIE) has developed a list of antimicrobial agents of veterinary importance.[44] This list includes recommendations on restricting the use of antimicrobials that are critically important for both animal and human health in food-producing animals. These include fluoroquinolones and third

**Table 7 Bacteria isolated from water samples collected from 15 canals in Bangkok and a central province and from 25 stagnant water sources in food animal farms in an eastern province and a northern province**

| Bacteria | Total canal water ($n = 15$) | Bangkok ($n = 10$) | Central province ($n = 5$) | Total stagnant water ($n = 25$) | Eastern province ($n = 10$) | Northern province ($n = 15$) |
|---|---|---|---|---|---|---|
| Enterobacteriaceae | 8/15 (53.3%) | 5/10 (50%) | 3/5 (60%) | 21/25 (84%) | 9/10 (90%) | 12/15 (80%) |
| ESBL-producing isolates | 3/8 (37.5%) | 3/5 (60%) | – | 3/21 (14.3%) | 3/9 (33.3%) | 0/12 (0%) |
| *Escherichia coli* | 4/8 (50%) | 4/5 (80%) | – | 9/21 (42.9%) | 6/9 (66.7%) | 3/12 (25%) |
| ESBL-producing isolates | 1/4 (25%) | 1/4 (25%) | – | 3/9 (33.3%) | 3/6 (50%) | 0/3 (0%) |
| *Enterobacter* spp. | 2/8 (25%) | 2/5 (100%) | – | 5/21 (23.8%) | 2/9 (22.2%) | 3/12 (25%) |
| ESBL-producing isolates | 2/2 (100%) | 2/2 (100%) | – | 0/5 (0%) | 0/2 (0%) | 0/3 (0%) |
| *Klebsiella* spp. | 7/8 (87.5%) | 5/5 (100%) | 2/3 (66.7%) | 11/21 (52.4%) | 4/9 (44.4%) | 7/12 (58.3%) |
| ESBL-producing isolates | 3/7 (42.9%) | 3/5 (60%) | – | 0/11 (0%) | 0/4 (0%) | 0/7 (0%) |
| *Acinetobacter baumannii* | 8/13 (61.5%) | 7/8 (87.5%) | 1/5 (20%) | 6/9 (66.7%) | – | 6/7 (85.7%) |
| *Pseudomonas aeruginosa* | 4/13 (30.8%) | 1/8 (12.5%) | 3/5 (60%) | 2/9 (22.2%) | 1/2 (50%) | 1/7 (14.3%) |

ESBL: Extended-spectrum beta-lactamase.

and fourth generation celphaosporins. Furthermore, the OIE recommends that the potential animal use of antimicrobials that are currently used only in human beings be carefully considered, to preserve their effectiveness. Intervenltions to promote rational use of antibiotics in animal husbandry, including regulatory and educational measures, are being implemented in the eastern and northern provinces of Thailand.

The presence of antibiotic resistant bacteria in feces of pigs and chickens in this study has several implications. Resistant bacteria in pig or chicken manure can spread to humans following consumption of foods contaminated with viable resistant bacteria, and such consumption has impacts for human health. Resistant bacteria can cause foodborne diseases, especially acute gastroenteritis. Resistant bacteria can initially cause a silent carrier state and may later cause infections that are not recognized as being of foodborne origin, such as urinary tract or abdominal infections caused by ESBL-producing *E. coli*. Moreover, antibiotic resistant bacteria could be transmitted to other individuals, the food production chain, and the environment. Consumption of non-viable resistant bacteria can also induce antibiotic resistance in gastrointestinal bacteria in humans and other animals by gene transfer.[45] Furthermore, it is a common practice in many communities in Thailand to use pig and chicken manure to feed fish in ponds or canals, and to fertilize vegetables and fruits. These practices could transfer antibiotic resistant bacteria from the feces of some food animals to other foods and the environment. Food-producing animals are reservoirs for pathogens, with the potential to transfer resistance to humans. The magnitude of such transmission remains unknown, and probably varies between different bacterial species and modes of food animal consumption.

We collected spring onions, parsley, and bean sprouts in this survey because these vegetables are usually consumed without appropriate heating. Extended-spectrum beta-lactamase-producing Gram-negative bacteria were recovered from some of these samples. Similarly, Enterobacteriaceae with resistance to third-generation cephalosporins and fluoroquinolones were isolated from fresh culinary herbs exported from Thailand and were transferable to consumers.[40] Therefore, consumption of fresh vegetables also poses a risk for acquiring resistant bacteria.

Our study found that antibiotic-resistant bacteria were much less prevalent in many cooked food samples than in fresh meat and vegetables. Therefore, consumption of cooked food should be less likely to transfer live antibiotic-resistant bacteria. Basic sanitation and hygiene, including consumption of cooked foods and clean water, as well as hand washing, are very important measures for containment and prevention of antibiotic resistance. However, avoidance of contamination with antibiotic resistant bacteria across the food production chain is also important, because consumption of killed resistant bacteria may induce viable non-resistant bacterial strains to become resistant to antibiotics.[45] Moreover, consumption of foods that contain residual antibiotics could still induce non-resistant bacteria colonizing the gut to become antibiotic-resistant. Therefore, restriction of inappropriate use of antibiotics in animal husbandry, aquaculture, and agriculture is important.

Antibiotic-resistant bacteria were commonly found in water samples collected from canals in Bangkok, and in water sources on food animal farms. Antibiotic-resistant bacteria were less commonly detected in water samples collected from fish and shrimp farms in four coastal provinces, even though 12 antibiotics are registered with the Thailand Food and Drug Administration for treatment of infections in aquatic animals: enrofloxacin, sarafloxacin, oxolinic acid, oxytetracycline, sulfadimethoxin-ormethoprim, sulfadimethoxin-trimethoprim, sulfadimethoxin, sulfamonomethoxin, salfadiazine, trimethoprim, ormethoprim, and toltrazuril. However, the Ministry of Public Health has only allowed tetracycline to be used in aquaculture. The low prevalence of antibiotic resistant bacteria collected from fish and shrimp ponds could be due to an infrequent use of antibiotics in aquaculture or selection bias, because the survey was performed using samples collected from farms recommended by the Department of Fisheries. Sun exposure of pond water could also be a contributing factor, as all fish and shrimp ponds are open water sources.

It should be noted that VRE was not found in any of the collected samples, and MRSA was detected in only one rectal swab sample taken from a pig from a farm in a northern province. A molecular study to determine if this MRSA isolate is healthcare-associated or community-associated will be performed. Although infection or colonization with CRE has been reported in hospitalized patients in many hospitals in Thailand,[46,47] the prevalence of CRE in healthy individuals, foods, food animals, and the environment in this study was still extremely low. Molecular typing of these CRE isolates will also be performed in order to determine the mechanism of resistance to carbapenem.

This study collected samples from eight provinces, and many of the samples were not collected within the same areas. Therefore, we intend to collect additional samples along the food production chain in the same eastern and northern provinces, so that relationships of resistance mechanisms, especially for ESBL-producing genes, between foods, the environment, and humans can be established with more confidence.

The present survey results indicated that the prevalence of resistant bacteria in different types of samples collected from different areas varied widely.

For example, resistant bacteria detected in the rectal swabs of pigs in a northern province were more prevalent than those collected in an eastern province, and the patterns of antibiotic resistance in bacteria collected from pigs and chickens in an eastern province were different from those in a northern province. Therefore, antimicrobial surveillance programs to integrate data from bacterial isolates originating from humans, food-producing animals, and retail meats in different locations are necessary. Moreover, the prevalence of antibiotic resistant bacteria also depends on antibiotic consumption. The World Health Organization has recommended that countries should develop antimicrobial resistance surveillance programs to collect and integrate antimicrobial use and antimicrobial resistance data along the producer-to-consumer continuum.[48] We are collaborating with the Department of Livestock Development and Department of Fisheries, Ministry of Agriculture and Cooperatives of Thailand to establish comprehensive surveillance systems for antibiotic resistance, antibiotic residues, and antibiotic use in animal husbandry, aquaculture, and agriculture.

## Disclaimer Statements

**Contributors** Adhiratha Boonyasiri: Literature search, figures, study design, data collection, data analysis, data interpretation, writing and editing.
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