Prevalence of ESBL-Producing *Escherichia coli* Isolated from elderly living at home setting in Mae Chai district, Phayao, Thailand

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Abstract

The prevalence of ESBL-producing bacteria has become a problem worldwide. This study aimed to investigate the existence of ESBL-producing *E. coli* isolates and their drug resistant genes that have become normal flora of the elderly population living in Phayao province. The fecal specimens from 252 elderly people were collected and drug resistant phenotypic screening was done by plating the samples on MacConkey agar supplemented with (1 μg/mL) cefotaxime or ceftazidime. The suspected *E. coli* isolates were identified with biochemical tests. Then, the phenotype of ESBL was confirmed by combination disk method and analyzed for bla_{TEM}, bla_{SHV}, and $bla_{CTX-M-1}$ by PCR. In this study, 75 isolates (29.8%) were confirmed as suspected ESBL-producing *E. coli*. All confirmed isolates were multidrug resistant (MDR), and resistant to AMP, CTX, CRO, and CPD. However, all of them were susceptible to IMP, ETP, and MEM. There were 5 phenotypic β-lactam resistant patterns detected. The most predominant pattern was AMP CTX CAZ CRO FEP CPD ATM (55.4%). The genotypic characterization showed predominance of $bla_{CTX-M-1}$ (35.7%) while there was no bla_{SHV} detected. Moreover, the combination of bla_{TEM} and $bla_{CTX-M-1}$ together was detected at about 43%. This study showed there was high prevalence of ESBL-producing *E. coli* in elderly people residing in one regional community. These results suggest that ESBL may be transmitted via feces in communities of close-living quarters without good cleaning and hygiene practices.

Keywords: ESBL-producing *E. coli*, elderly people, multidrug resistance, bla_{CTX-M}, Thailand

Introduction

The increase of extended-spectrum β-lactamases (ESBL)-production bacteria is a common problem worldwide. The region of Southeast Asian shows high prevalence of ESBLs and multidrug-resistant (MDR) bacteria, especially members of Enterobacteriaceae family (Chang et al., 2020, Nguyen et al., 2019, Wyres et al., 2020). This bacteria family developed a complex mechanism of resistance and evaded the therapeutics available in hospitals (Hafza et al., 2018). In Thailand, the prevalence of community-acquired infection of ESBL-producing Enterobacteriaceae trends continue to increase (Kanoksil, Jatapai, Peacock, & Limmathurotsakul, 2013). Moreover, reports of fecal carriage associated with ESBL-producing Enterobacteriaceae in Thai people is high and decreased after the AMR campaign (Boonyasiri et al., 2014; Khamsarn et al., 2016; Seenama, Thamlikitkul, & Ratthawongjirakul, 2019). A study in the northeast part of Thailand indicates that the incidence rate of community-acquired bacteremia was highest in infants and the elderly, and *E. coli* was the most common pathogen identified (Kanoksil et al., 2013). When compared to young patients, elderly patients have high mortality rates (24.6%) within 28 days after infection with ESBL-producing *E. coli* and *K. pneumoniae* (Ku et al., 2014). In Thailand, a study shows high incidence of ESBL-producing *E. coli* infection during 2008-2014 in Sa Kaeo and Nakhon Phanom provinces, based on community-onset among elderly persons aged over 70 years (Sawatwong et al., 2019).

E. coli usually harbors ESBL genes that encoding for CTX-M, TEM- and SHV-type ESBLs, which increase their prevalence in clinical isolates (Pishtiwan & Khadija, 2019). Several studies have reported the presence of ESBL-producing *E. coli* in food-producing animals and their foods. In addition, the type of ESBL and associated plasmids could potentially transfer from animals to human through the food chain (Ben Sallem et al., 2012). However, the prevalence of ESBL in healthy elderly in Phayao province, Thailand, is limited. Thus, the purpose of this study was to determine the ESBL-producing *E. coli* isolates in the healthy elderly in Phayao and investigate the existence of the ESBL genes. This study could be provided an information of the effects on ESBL-producing bacteria spreading and introduced an awareness of antimicrobial drugs used.

Methods and Materials

Specimens collection

This study included the fecal specimens from 252 elderly people with an age >60 years old and residing in Mae Chai district, Phayao Province during September to October, 2018. The participants' specimens were collected using rectal swab by themselves after a standardized instruction with one sample per person. All specimens were collected in Cary-Blair medium and kept in icebox, then transferred to the Clinical Microbiology Laboratory, School of Allied Health Sciences, University of Phayao. The study was conducted with the approval of the University of Phayao human ethics committee, Phayao University (2/037/58).

Bacteria isolation and ESBL-producing *E. coli* detection

All specimens suspected ESBL-producing isolates were inoculated in two selective media plates, MacConkey agar supplemented with (1 μg/mL) cefotaxime or ceftazidime. After 18-24 hours of incubation at 37°C, the isolates were characterized for suspected ESBL-producing *E. coli* using biochemical tests (Oxoid), including triple sugar iron medium, motile-indole-lysine medium, urea medium, Simmons' citrate medium, malonate medium, methyl-red medium and Voges-Proskauer medium. Then, the ESBL production ability was screened by using a combination disk method according to the Clinical and Laboratory Standards Institute (CLSI), 2016 criteria (CLSI, 2016). If an isolate was resistant to at least 3 or more antimicrobial classes, it was defined as multidrug-resistant bacteria (MDR). According to the CLSI guideline, all suspected isolates were subjected to ESBL confirmation test.

Confirmation of ESBL-producing *E. coli*

All suspected isolates were confirmed by combination disk diffusion methods with both 30 μg of cefotaxime and ceftazidime, with and without clavulanic acid (10 μg). The difference of inhibition zone was interpreted, as described by the CLSI, 2016 recommendation (CLSI, 2016). *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 were used as positive and negative controls. The isolates were identified as an ESBL producer when increase in inhibition zone >5 mm with clavulanic acid compares to without clavulanic acid.

Antimicrobial susceptibility testing

All the ESBL-producers were tested for antimicrobial susceptibility by disk diffusion methods according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI), 2016 (CLSI, 2016). In these guidelines, 21 antimicrobial agents (Oxoids) were used including ampicillin (10 μg), amoxicillin-clavulanate (20/10 μg), cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefepime (30 μg), cefoxitin (30 μg), cefpodoxime (10 μg), aztreonam (30 μg), ertapenem (10 μg), imipenem (10 μg), meropenem

(10 μg), gentamicin (10 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), fosfomycin (200 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), norfloxacin (10 μg), tetracycline (30 μg) and tigecycline (15 μg). Moreover, *E. coli* ATCC 25922 was used as a quality control.

DNA extraction and ESBL genotypic characterization

All ESBL-positive isolates were extracted for DNA. The purified colonies were suspended in 500 μL sterile deionized water and heated at 95^{O}C for 10 min. Then, the cell suspensions were pelleted by centrifugation at 6,000 rpm for 10 min (Dallenne, Da Costa, Decré, Favier, & Arlet, 2010). The supernatant was transferred into a new tube and used as template for PCR analysis.

The ESBL positive isolates were examined for ESBL genes including bla_{TEM}, bla_{SHV}, and $bla_{CTX-M-1}$ following the protocol described previously (Xu, An, Wang and Zhang, 2015). PCR was performed at 94 ^{O}C for 5 min, followed by 30 cycles at 94^{O}C for 20 secs, 55-58^{O}C for 10 secs, 72^{O}C for 45 secs, and a final extension at 72^{O}C for 5 min. The PCR component contained 2 μL of template DNA, 1 μL of each primer (10 pmol/μL), 10 μL 2 × PCR Master mix solution (i-TaqTM, iNtRON biotechnology). Final total volume of 20 μL was reached with the addition of ddH_2O. *K. pneumoniae* ATCC 700603 was used as a positive control.

Results

Detection of ESBL-producing *E. coli*

A total of 252 samples of feces were screened for ESBL-producing *E. coli*. By using the MacConkey agar supplemented with cefotaxime (or ceftazidime) and conventional biochemical test, the total of 75 isolates (29.8%) were identified as suspected ESBL-producing *E. coli*. All suspected isolates were confirmed with combination disk method and identified as ESBL producer.

The identified ESBL isolates were confirmed for antimicrobial susceptibility using 21 agents according to the CLSI guidelines using disk diffusion method. The isolates revealed 100% of antimicrobial resistance to AMP, CTX, CRO, and CPD followed by to ATM (97.3%), TE (88%), CAZ (62.7%), FEP (54.7%), SXT (54.7%), C (54.7%), CN (44%), CIP (18.7%), LEV (18.7%), and NOR (16%). Moreover, the resistance to FOX, AMC, TGC and FOS were found at the prevalence of 1.3, 2.7, 2.7 and 5.3%, respectively. In this study, the resistance to IMP, ETP, and MEM were not found (Table 1).

All ESBL-producing *E. coli* were multidrug resistant (MDR). Resistance to AMP, CTX, CRO, CPD, ATM, TE, FEP, and CAZ were identified, with a prevalence of 9.3%. Furthermore, resistance in the pattern of AMP CTX CRO CPD ATM TE FEP CAZ SXT and AMP CTX CRO CPD ATM TE FEP CAZ C CN SXT were identified with the prevalence of 5.3%. Additionally, 5 phenotypic β-lactam resistant patterns were correlated with the ESBL gene detection. The most common phenotypic pattern was AMP CTX CAZ CRO FEP CPD ATM (55.4%) followed by AMP CTX CRO CPD ATM (19.6%), AMP CTX CAZ CRO CPD ATM (16.1%), AMP CTX CRO FEP CPD ATM (7.1%), and AMP AMC CTX CRO FEP CPD ATM (1.8%) (Table 2).

Genotypic characterization of ESBL genes

All confirmed ESBL-producing *E. coli* were characterized for genotypic-resistant genes. Of 75 isolates, 73 were successfully extracted, and the ESBL genes were detected from 56 isolates (76.7%). Twenty isolates (35.7%) revealed only the $bla_{CTX-M-1}$ gene and 12 isolates (21.7%) were found carrying only the bla_{TEM} gene.

The bla_{SHV} gene was not detected in this study. Twenty-four isolates (42.9%) were positive for both bla_{TEM} and $bla_{CTX-M-1}$ genes.

Table 1 Antimicrobial resistance pattern of *E. coli* from Mae Chai district, Phayao Province, Thailand

Antimicrobial class	Antimicrobial agents	No. of resistance isolates (%)
β-lactam		
Penicillins	Ampicillin (AMP)	75 (100.0)
β-lactam/β-lactamase inhibitor	Amoxicillin-clavulanate acid (AMC)	2 (2.7)
Cephems	Cefotaxime (CTX)	75 (100.0)
	Ceftazidime (CAZ)	47 (62.7)
	Ceftriaxone (CRO)	75 (100.0)
	Cefepime (FEP)	41 (54.7)
	Cefpodoxime (CPD)	75 (100.0)
	Cefoxitin (FOX)	1 (1.3)
Monobactams	Aztreonam (ATM)	73 (97.3)
Penems	Imipenem (IMP)	0
	Ertapenem (ETP)	0
	Meropenem (MEM)	0
Non- β-lactam		
Aminoglycosides	Gentamicin (CN)	33 (44.0)
Folatepathway	Trimethoprim-sulfamethoxazole (SXT)	41 (54.7)
Fosfomycins	Fosfomycin (FOS)	4 (5.3)
Phenicols	Chloramphenicol (C)	41 (54.7)
Fluoroquinolones	Ciprofloxacin (CIP)	14 (18.7)
	Levofloxacin (LEV)	14 (18.7)
	Norfloxacin (NOR)	12 (16.0)
Tetracyclines	Tetracycline (TE)	66 (88.0)
	Tigecycline (TGC)	2 (2.7)

Table 2 Phenotypic and genotypic characteristics of ESBL-producing *E. coli*

Phenotypic β-lactam resistant pattern	No. of isolate (%)	No. of each genotypic resistant pattern (%)			
		bla			
		TEM	SHV	CTX-M-1	TEM+CTX-M-1
AMP CTX CRO CPD ATM	11 (19.6)	7 (12.5)	0	0	4 (7.1)
AMP CTX CAZ CRO CPD ATM	9 (16.1)	0	0	6 (10.7)	3 (5.4)
AMP CTX CRO FEP CPD ATM	4 (7.1)	4 (7.1)	0	0	0
AMP CTX CAZ CRO FEP CPD ATM	31 (55.4)	0	0	14 (25.0)	17 (30.4)
AMP AMC CTX CRO FEP CPD ATM	1 (1.8)	1 (1.8)	0	0	0
Total	56 (100)	12 (21.4)	0	20 (35.7)	24 (42.9)

Discussion

The main objective of the present study was to determine the prevalence of ESBL-producing *E. coli* isolates in healthy elderly persons in Phayao, Thailand, and to investigate the existence of the ESBL genes. From a total of 252 of samples, 29.8% were found to be ESBL- producing *E. coli*. Our results were consistent with a study conducted in Nan, Nakhon Si Thammarat, and Kanchanaburi provinces, Thailand; this study demonstrated a 32-53.9% prevalence of ESBL-producing Enterobacteriaceae (Luvsansharav et al., 2011). However, participants in this report were with median age of 54 years (range 25-86 years), which was different to our study. In addition, a recent study from Nakhon Phanom and Sa Kaeo provinces, Thailand, during 2008-2014 reported the prevalence of the community onset cases identified as ESBL-producing *E. coli* was 25.2% (Sawatwong et al., 2019). Among those data, the mortality rate was 3 time higher among case patients aged 70 years and older with ESBL-producing *E. coli* than among patients with non ESBL-producing *E. coli*. When compared to reports from other parts of the world, our study was inspired from a report from Sweden that documented the prevalence of ESBL-producing Enterobacteriaceae among elderly living in their own house, and nursing home residents were 8.7, and 11% respectively (Blom, Ahl, Mansson, Resman, & Tham, 2016). Thus, the discrepancies may due to the difference in selected population. However, the ESBL-producing Enterobacteriaceae detected in this study were higher than a report in Amsterdam, the Netherlands (8.6%) (Reuland et al., 2016), Saudi Arabia (13.1%) (Kader, Angamuthu, & Kamath, 2007), and Libya (13.4%) (Ahmed, Ali, Mohamed, Moussa, & Klena, 2014). Interestingly, these other reports documented that use of antacids can increase the risk of carriage of ESBL-producing Enterobacteriaceae (Reuland et al., 2016). These findings suggest that the ESBL-producing bacteria isolated from healthy individuals may vary by different areas, food, and the environment (Boonyasiri et al., 2014; Mitchell et al., 2021).

Our study showed that all ESBL-positive isolates (100%) were resistant to ampicillin (AMP), cefotaxime (CTX), ceftriaxone (CRO), and cefpodoxime (CPD) and showed more than 90% resistance to aztreonam (ATM). The most phenotypic characterization found in the study was AMP, CTX, CAZ, CRO, FEP, CPD, ATM (55.4%). Whereas, all the ESBL-positive isolates were susceptible to imipenem (IMP), ertapenem (ETP), and meropenem (MEM). These antimicrobials are classified as penicillins (AMP), third generation of cephalosporin (CTX, CRO, and CPD), and monobactam (ATM). In addition, these antibiotics are widely used in Thailand, both in humans and in the poultry industry, and tend to be one of the causes of multidrug resistance in hospitals (Apisarnthanarak et al., 2006; Sawatwong et al.,2019; Seenama, Thamlikitkul, and Ratthawongjirakul 2019; Thamlikitkul & Apisitwittaya, 2004).

Among the ESBL-producing isolates, 76.7% were found to contain the related ESBL genes including bla_{TEM} and $bla_{\text{CTX-M-1}}$. The most prevalent gene in our study was found to be $bla_{\text{CTX-M-1}}$ gene. Globally, the most common type of ESBL appeared to be $bla_{\text{CTX-M}}$, when compared to bla_{TEM} and bla_{SHV} (Jorgensen, McElmeel, Fulcher, & Zimmer, 2010; Kawamura et al., 2017; Pishtiwan & Khadija, 2019). The $bla_{\text{CTX-M-1}}$ as the most detected ESBL gene was similar to several studies conducted in many countries in Southeast Asia such as Thailand (Bubpamala et al., 2018; Saekhow & Sriphannam, 2021), Laos, and Vietnam (Nakaya et al., 2015), showed that $bla_{\text{CTX-M-1}}$ was the most common sub-genotypes of CTX-M. In, Thailand, the first detection of the $bla_{\text{CTX-M}}$ gene was reported in 1998 and 1999 at 52 % (Chanawong et al., 2007). A study from Bangkok and Pratumthani province, Thailand, reported the prevalence of $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} was 99.2%, 77.0%

and 3.8%, respectively (Kiratisin, Apisarnthanarak, Laesripa, & Saifon, 2008); these showed very high prevalence when compared to the present study. Interestingly, another report stated a 20% presence of CTX-M group I genes among asymptomatic individuals, and this pattern is similar to the patient isolates from the study of our region. These suggests that the ESBL-producing bacteria obtained in hospital setting could be widely and rapidly spread to communities and increase the prevalence of ESBL-producing bacteria among patients in the future.

A recent study (Pishtiwan & Khadija, 2019) reported the similar prevalence of $bla_{\text{CTX-M}}$ (32.4%), while the prevalence of bla_{TEM} (81.0%) was higher than our study. However, our study was conducted in healthy people while these reports identified the isolates from specimens of urine at a Thalassemia center in Iraq. We observed $bla_{\text{CTX-M}}$ and bla_{TEM} together in detected ESBL-positive isolates in this study with about 42.9% which is similar to a study conducted in Thailand (Seenama et al., 2019). They found ESBL- producing *E. coli* carried $bla_{\text{CTX-M}}$ co-existing with bla_{TEM} as the predominant ESBL genes (56.6%) observed among the *E. coli* isolated from both healthy humans and swine. These indicated that ESBL-*E. coli* found in both types of hosts may serve as a reservoir for antimicrobial resistance in community acquired setting. Furthermore, several studies reported contaminated with ESBL producing *E. coli* containing $bla_{\text{CTX-M}}$ and bla_{TEM} gene together in raw chicken meat (Kola et al., 2012; Pehlivanlar Önen, Aslantaş, Şebnem Yılmaz, and Kürekci, 2015). These finding may suggest the rapid increase in the prevalence of ESBL producers in humans related to plasmid transfer between different bacteria adapted to chicken and humans (Pishtiwan & Khadija, 2019). Spreading of ESBL-producing organisms to the community could be related to the nosocomial acquisition and these the ESBL-producing organisms could be carried asymptomatically for prolonged period and propagate outside the hospital (Ahmed et al., 2014). In the present study ESBL-encoding genes were not detected in 17 ESBL-producing *E. coli* isolates. As the reason that only three ESBL genes including bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M-1}}$were identified in this study. Because of several genes associated to the antimicrobial resistance, it could be possible that those 17 ESBL isolates had other ESBL genes or mechanisms of drug resistance. However, unfortunately, there were two unsuccessfully extracted DNA samples which may because of poor stocking samples process. Moreover, we could not obtain any documents about recent individual's exposure to antibiotics or some of the antibiotics frequently obtained without prescriptions.

Conclusion and Suggestions

In conclusion, 252 fecal samples, 75 isolates (29.8%) were suspected ESBL-producing *E. coli.* From a total of 75 ESBL-positive isolates, 100% of them were resistant to AMP, CTX, CRO and CPD as well as susceptible to IMP, ETP, and MEM. All the ESBL-positive isolates detected in this study were MDR. The most common detected β-lactam resistant pattern was AMP CTX CAZ CRO FEP CPD ATM (55.4%). The genotypic characterization of the ESBL gene predominant showed only that of the $bla_{\text{CTX-M-1}}$ gene (35.7%), whereas, bla_{SHV} was not detected in this study. Moreover, bla_{TEM} and $bla_{\text{CTX-M-1}}$ gene were detected 43% of the time. Our findings showed a high prevalence of ESBL-producing *E. coli* among asymptomatic elderly people in Mae Chai district, Phayao Province. This suggests that the high prevalence may be related to antibiotic abuse. Thus, prescription and use of antimicrobial drugs should be guided by the awareness of the effects on the ESBL-producing bacterial rate of incidence and both ease and means of community spread.

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References

Ahmed, S. F., Ali, M. M., Mohamed, Z. K., Moussa, T. A., & Klena, J. D. (2014). Fecal carriage of extended-spectrum β-lactamases and AmpC-producing *Escherichia coli* in a Libyan community. *Annals of clinical microbiology and antimicrobials, 13*, 22. http://dx.doi.org/10.1186/1476-0711-13-22

Apisarnthanarak, A., Danchaivijitr, S., Khawcharoenporn, T., Limsrivilai, J., Warachan, B., Bailey, T. C., . . . Thammasart University Antibiotic Management, T. (2006). Effectiveness of education and an antibiotic-control program in a tertiary care hospital in Thailand. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America, 42*(6), 768-775. http://dx.doi.org/10.1086/500325

Ben Sallem, R., Ben Slama, K., Estepa, V., Jouini, A., Gharsa, H., Klibi, N., . . . Torres, C. (2012). Prevalence and characterisation of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates in healthy volunteers in Tunisia. *European journal of clinical microbiology & infectious diseases: official publication of the European Society of Clinical Microbiology, 31*(7), 1511-1516. http://dx.doi.org/10.1007/s10096-011-1471-z

Blom, A., Ahl, J., Mansson, F., Resman, F., & Tham, J. (2016). The prevalence of ESBL-producing Enterobacteriaceae in a nursing home setting compared with elderly living at home: a cross-sectional comparison. *BMC infectious diseases, 16*, 111. http://dx.doi.org/10.1186/s12879-016-1430-5

Boonyasiri, A., Tangkoskul, T., Seenama, C., Saiyarin, J., Tiengrim, S., & Thamlikitkul, V. (2014). Prevalence of antibiotic resistant bacteria in healthy adults, foods, food animals, and the environment in selected areas in Thailand. *Pathogens and global health, 108*(5), 235-245. http://dx.doi.org/10.1179/2047773214Y.0000000148

Bubpamala, J., Khuntayaporn, P., Thirapanmethee, K., Montakantikul, P., Santanirand, P., & Chomnawang, M. T. (2018). Phenotypic and genotypic characterizations of extended-spectrum beta-lactamase-producing *Escherichia coli* in Thailand. *Infection and drug resistance, 11*, 2151-2157. https://doi.org/10.2147/IDR.S174506

Chanawong, A., Lulitanond, A., Kaewkes, W., Lulitanond, V., Srigulbutr, S., & Homchampa, P. (2007). CTX-M extended-spectrum β-lactamases among clinical isolates of Enterobacteriaceae in a Thai university hospital. *The Southeast Asian journal of tropical medicine and public health, 38*(3), 493-500.

Chang, K., Rattanavong, S., Mayxay, M., Keoluangkhot, V., Davong, V., Vongsouvath, M., . . . Dance, D. (2020). Bacteremia caused by extended-spectrum β-lactamase-producing Enterobacteriaceae in Vientiane, Lao PDR: A 5-Year Study. *The American journal of tropical medicine and hygiene, 102*(5), 1137-1143. http://dx.doi.org/10.4269/ajtmh.19-0304

CLSI, (2016). *Performance standards for antimicrobial disk susceptibility tests*, *CLSI supplement M100* (26th ed.). Wayne: Clinical and Laboratory Standards Institute.

Dallenne, C., Da Costa, A., Decré, D., Favier, C., & Arlet, G. (2010). Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. *The Journal of antimicrobial chemotherapy*, *65*(3), 490-495. https://doi.org/10.1093/jac/dkp498

Hafza, N., Challita, C., Dandachi, I., Bousaab, M., Dahdouh, E., & Daoud, Z. (2018). Competition assays between ESBL-producing *E. coli* and *K. pneumoniae* isolates collected from Lebanese elderly: An additional cost on fitness. *Journal of infection and public health.*, *11*(3), 393-397. http://dx.doi.org/10.1016/j.jiph.2017.09.010

Jorgensen, J. H., McElmeel, M. L., Fulcher, L. C., & Zimmer, B. L. (2010). Detection of CTX-M-type extended-spectrum β-lactamase (ESBLs) by Testing with microscan overnight and ESBL confirmation panels. *Journal of Clinical Microbiology*, *48*(1), 120-123. http://dx.doi.org/10.1128/jcm.01507-09

Kader, A. A., Kumar, A., & Kamath, K. A. (2007). Fecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in patients and asymptomatic healthy individuals. *Infection control and hospital epidemiology*, *28*(9), 1114-1116. https://doi.org/10.1086/519865

Kanoksil, M., Jatapai, A., Peacock, S. J., & Limmathurotsakul, D. (2013). Epidemiology, microbiology and mortality associated with community-acquired bacteremia in northeast Thailand: a multicenter surveillance study. *PLoS One*, *8*(1), e54714. http://dx.doi.org/10.1371/journal.pone.0054714

Kawamura, K., Nagano, N., Suzuki, M., Wachino, J. I., Kimura, K., & Arakawa, Y. (2017). ESBL-producing *Escherichia coli* and its rapid rise among healthy people. *Food safety*, *5*(4), 122-150. http://dx.doi.org/10.14252/foodsafetyfscj.2017011

Khamsarn, S., Nampoonsak, Y., Busamaro, S., Tangkoskul, T., Seenama, C., Rattanaumpawan, P., . . . Thamlikitkul, V. (2016). Epidemiology of antibiotic use and antimicrobial resistance in selected communities in Thailand. *Journal of the Medical Association of Thailand*, *99*(3), 270-275.

Kiratisin, P., Apisarnthanarak, A., Laesripa, C., & Saifon, P. (2008). Molecular characterization and epidemiology of extended-spectrum-β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates causing health care-associated infection in Thailand, where the CTX-M family is endemic. *Antimicrobial agents and chemotherapy*, *52*(8), 2818-2824. http://dx.doi.org/10.1128/aac.00171-08

Kola, A., Kohler, C., Pfeifer, Y., Schwab, F., Kühn, K., Schulz, K., . . . Steinmetz, I. (2012). High prevalence of extended-spectrum-β-lactamase-producing Enterobacteriaceae in organic and conventional retail chicken meat, Germany. *Journal of Antimicrobial Chemotherapy*, *67*(11), 2631-2634. http://dx.doi.org/10.1093/jac/dks295

Ku, N. S., Kim, Y. C., Kim, M. H., Song, J. E., Oh, D. H., Ahn, J. Y., . . . Choi, J. Y. (2014). Risk factors for 28-day mortality in elderly patients with extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* bacteremia. *Archives of gerontology and geriatrics*, *58*(1), 105-109. http://dx.doi.org/10.1016/j.archger.2013.07.002

Luvsansharav, U. O., Hirai, I., Niki, M., Sasaki, T., Makimoto, K., Komalamisra, C., . . . Yamamoto, Y. (2011). Analysis of risk factors for a high prevalence of extended-spectrum {β}-lactamase-producing Enterobacteriaceae in asymptomatic individuals in rural Thailand. *Journal of medical microbiology, 60*(Pt 5), 619-624. http://dx.doi.org/10.1099/jmm.0.026955-0

Mitchell, J., Purohit, M., Jewell, C. P., Read, J. M., Marrone, G., Diwan, V., & Stålsby Lundborg, C. (2021). Trends, relationships and case attribution of antibiotic resistance between children and environmental sources in rural India. *Scientific reports, 11*(1), 22599. https://doi.org/10.1038/s41598-021-01174-w

Nakayama, T., Ueda, S., Huong, B. T., Tuyen, l., Komalamisra, C., Kusolsuk, T., Hirai, I., & Yamamoto, Y. (2015). Wide dissemination of extended-spectrum β-lactamase-producing *Escherichia coli* in community residents in the Indochinese peninsula. *Infection and drug resistance, 8*, 1-5. https://doi.org/10.2147/IDR.S74934

Nguyen, V. T., Jamrozy, D., Matamoros, S., Carrique-Mas, J. J., Ho, H. M., Thai, Q. H., . . . Ngo, T. H. (2019). Limited contribution of non-intensive chicken farming to ESBL-producing *Escherichia coli* colonization in humans in Vietnam: an epidemiological and genomic analysis. *The Journal of antimicrobial chemotherapy, 74*(3), 561-570. http://dx.doi.org/10.1093/jac/dky506

Pehlivanlar Önen, S., AslantaŞ, Ö., Şebnem Yılmaz, E., & Kürekci, C. (2015). Prevalence of β-lactamase producing *Escherichia coli* from retail meat in Turkey. *Journal of Food Science, 80*(9), M2023-M2029. http://dx.doi.org/10.1111/1750-3841.12984

Pishtiwan, A. H., & Khadija, K. M. (2019). Prevalence of bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes among ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* isolated from thalassemia patients in Erbil, Iraq. *Mediterranean journal of hematology and infectious diseases, 11*(1), e2019041. http://dx.doi.org/10.4084/mjhid.2019.041

Reuland, E. A., Al Naiemi, N., Kaiser, A. M., Heck, M., Kluytmans, J. A. J. W., Savelkoul, P. H. M., . . . Vandenbroucke-Grauls, C. M. J. E. (2016). Prevalence and risk factors for carriage of ESBL-producing Enterobacteriaceae in Amsterdam. *The Journal of antimicrobial chemotherapy, 71*(4), 1076-1082. http://dx.doi.org/10.1093/jac/dkv441

Saekhow, P., & Sriphannam, C. (2021). Prevalence of extended-spectrum beta-lactamase-producing *Escherichia coli* strains in dairy farm wastewater in Chiang Mai: *Veterinary Integrative Sciences*, *19*(3), 349-362. https://doi.org/10.12982/VIS.2021.030

Sawatwong, P., Sapchookul, P., Whistler, T., Gregory, C. J., Sangwichian, O., Makprasert, S., . . . Rhodes, J. (2019). High burden of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* bacteremia in older adults: a seven-year study in two rural Thai provinces. *The American journal of tropical medicine and hygiene, 100*(4), 943-951. http://dx.doi.org/10.4269/ajtmh.18-0394

Seenama, C., Thamlikitkul, V., & Ratthawongjirakul, P. (2019). Multilocus sequence typing and bla_{ESBL} characterization of extended-spectrum β-lactamase-producing *Escherichia coli* isolated from healthy humans and swine in northern Thailand. *Infection and drug resistance, 12*, 2201-2214. http://dx.doi.org/10.2147/IDR.S209545

Thamlikitkul, V., & Apisitwittaya, W. (2004). Implementation of clinical practice guidelines for upper respiratory infection in Thailand. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases*, *8*(1), 47-51. http://dx.doi.org/10.1016/j.ijid.2003.09.001

Thamlikitkul, V., Tangkoskul, T., & Seenama, C. (2019). Fecal carriage rate of extended-spectrum β-lactamase-producing Enterobacteriaceae as a proxy composite indicator of antimicrobial resistance in a community in Thailand. *Open forum infectious diseases*, *6*(10), ofz425. http://dx.doi.org/10.1093/ofid/ofz425

Wyres, K. L., Nguyen, T. N. T., Lam, M. M. C., Judd, L. M., van Vinh Chau, N., Dance, D. A. B., . . . Holt, K. E. (2020). Genomic surveillance for hypervirulence and multi-drug resistance in invasive *Klebsiella pneumoniae* from South and Southeast Asia. *Genome medicine*, *12*(1), 11-11. http://dx.doi.org/10.1186/s13073-019-0706-y

Xu, G., An, W., Wang, H., & Zhang, X. (2015). Prevalence and characteristics of extended-spectrum β-lactamase genes in *Escherichia coli* isolated from piglets with post-weaning diarrhea in Heilongjiang province, China. *Frontiers in microbiology*, *6*, 1103. https://doi.org/10.3389/fmicb.2015.01103