ຂໍ້ມູນລະບາດວິທະຍາທາງດ້ານຖິ່ນກຳເນີດຂອງເຊື້ອ ESBL producing Escherichia coli and Klebsiella pneumoniae

ເຊີນຮ່ວມຮັບຟັງ **ທ່ານ ຄຳໃບ ນອຍລັດ,** ວິຊາການ ຂະແໜງວິເຄາະ, ສູນວິເຄາະ ແລະ ລະບາດວິທະຍາ, ກະຊວງສາທາລະນະສຸກ. ເຊິ່ງທ່ານຈະໄດ້ຮັບຟັງ ການບັນລະຍາຍ ກ່ຽວກັບຂໍ້ມູນລະບາດວິທະຍາທາງດ້ານຖິ່ນກຳເນີດຂອງເຊື້ອ ESBL producing Escherichia coli ແລະ Klebsiella pneumoniae ຢູ່ ສປປ ລາວ ການບັນລະຍາຍຈະຈັດຂຶ້ນໃນວັນທີ:

21 ມີນາ 2025

ເວລາ: 14:00PM – 15:00PM ໂມງ (GMT +7)

zoom

https://us06web.zoom.us/j/4593736274?omn=83132928941

ທ່ານ ຄຳໃບ ນອຍລັດ

ວິຊາການ ຂະແໜງວິເຄາະ,
ສູນວິເຄາະ ແລະ ລະບາດວິທະຍາ, ກະຊວງສາທາລະນະສຸກ.

Epidemiology of Clinical Isolates of Extended-Spectrum Beta-Lactamase (ESBL)-producing Escherichia coli and Klebsiella pneumoniae

Listen to Mr. Khambai Nuaylath, Technical Officer of Laboratory unit, National Center for Laboratory and Epidemiology, Ministry of Health gave a lecture on the point prevalence Epidemiology of Clinical Isolates of Extended-Spectrum Beta-Lactamase (ESBL)-producing Escherichia coli and Klebsiella pneumoniae in Lao PDR. The lecture will be held on:

21 March 2025

TIME: 14:00PM – 15:00PM (GMT +7)

zoom

https://us06web.zoom.us/j/4593736274?omn=83132928941

Mr. Khambai Nuaylath
Technical Officer Laboratory unit, National center for Laboratory and Epidemiology, Ministry of Health, Lao PDR

Epidemiology of Clinical Isolates of Extended-Spectrum Beta-Lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* in Lao PDR

Fellow: Dr. Khambai Nouaylath
AMR Laboratory Surveillance in Human
National Center for Laboratory and Epidemiology, Ministry of Health, Lao PDR

Mentor: Associate Professor Rujipas Sirijatuphat
Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand

Consultant: Professor Visanu Thamlikitkul
Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand

Mahidol University
Faculty of Medicine
Siriraj Hospital

The Fleming Fund

MOTT MACDONALD

Background

- AMR burden is enormous in Southeast Asia in terms of morbidity, mortality and economic loss
- Regarding to Strategy for Antimicrobial Resistance (AMR) surveillance system in Lao PDR, AMR burden should be estimated
- Most of these resistant bacteria were extended-spectrum beta-lactam-beta-lactamase (ESBL) producing Enterobacteriaceae, especially *Escherichia coli* and *Klebsiella pneumoniae*
- ESBL-producing Enterobacteriaceae are the most common antibiotic-resistant bacteria causing gastrointestinal colonization in healthy people and both community-acquired and hospital-acquired infections in patients in many countries, especially in Asia including Lao PDR

Objective

- The objective of this study was to determine epidemiology in terms of the phenotypes and genotypes of clinical isolates of ESBL- producing *E. coli* and ESBL- producing *K. pneumoniae* in Lao PDR in 2020

- ຈຸດປະສົງຂອງການສຶກສາຄັ້ງນີ້ ແມ່ນເພື່ອກຳນົດການລະບາດຂອງ ພະຍາດກ່ຽວກັບປະກົດການ ແລະ genotypes ຂອງການໂດດດ່ຽວທາງ ດ້ານຄລີນິກຂອງ ESBL- ຜະລິດ E. coli ແລະ ESBL- ຜະລິດ K. pneumoniae ໃນ ສປປ ລາວ ໃນປີ 2020.

Methodology

- The study has 2 parts on determination of
  - Phenotype of ceftriaxone-resistant *E. coli* and *K. pneumoniae* clinical isolates by CLSI/ EUCAST standard disk diffusion method and Double Disks Synergy
  - Genotype of ceftriaxone-resistant *E. coli* and *K. pneumoniae* clinical isolates with ESBLs by detecting ESBL genes (bla_{CTX-M}, bla_{TEM}, bla_{SHV}) by polymerase chain reaction (PCR)
- Study design: Prospective cohort study
- Period of Study: February - December 2020
- Study Sites for collection of bacteria:
  - Mahosot Hospital
  - Sethathirath Hospital
  - Khammuane Provincial Hospital
  - National Center for Laboratory and Epidemiology (NCLE)

Methodology

Sample size

- 200 isolates of ceftriaxone-resistant *E. coli* and ceftriaxone-resistant *K. pneumoniae* recovered from clinical specimens of different patients sent to microbiology service laboratories of Mahosot Hospital (N=50), Setthathirath Hospital (N=50), Khammoun Hospital (N=50) during February and December 2020, and the isolates kept at the National Center for Laboratory and Epidemiology or NCLE (N=50)
- Ceftriaxone-resistant *E. coli* and ceftriaxone-resistant *K. pneumoniae* isolates from participating hospitals were transported in Amies transport medium to NCLE
- The isolates were stored in nutrient broth with 20% glycerol at -80°C at NCLE for subsequent ESBL detection, antibiotic susceptibility test, and ESBL resistance genes detection

Oligonucleotide sequences of genes encoding β-lactamase used in this study by PCR

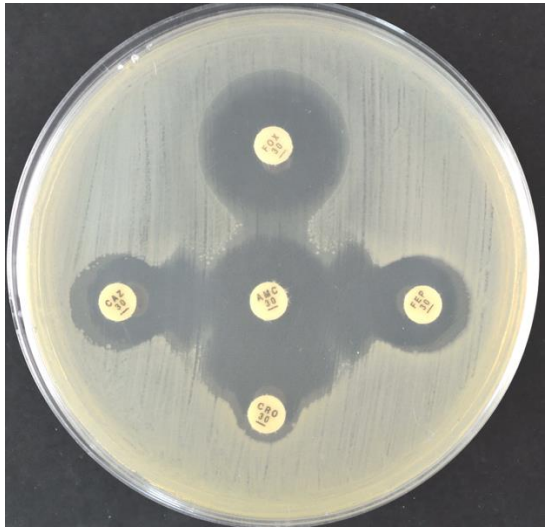
Gene	Primers sequence (5'→3')	Product size (bp)	Annealing Temp. (°C)
CTX-M_F CTX-M_R	5′-SCSA TGTGCAGYACCAGTAA-3′ 5′-CCGCRATATGRTTGGTGGTG-3′	544 bp	55°C
TEM_F TEM_R	5′-GGTTATGCGTTATATTCGCC-3′ 5′-TTAGCGTTGCCAGTGCTC-3′	867 bp	55°C
SHV_F SHV_R	5′-ATGAGTATTCAACATTTCCG-3′ 5′-CTGACAGTTACCAATGCTTA-3′	863 bp	55°C


RESULTS

1. Phenotype Study Results

Double Disks Synergy

- All 200 isolates of ceftriaxone-resistant E. coli and ceftriaxone-resistant K. pneumoniae isolated from clinical specimens were found to be ESBL-producers according to the results from the double disks synergy test.



Rate of resistance (%) to various antibiotics in ESBL-producing *E. coli*

Site of Surveillance	No. isolates	AMP	AMC	CRO	CAZ	FOX	FEP	CHL	IMP	MEM	CN	CIP	NA	SXT	TE
KM	**19**	100	63.1	100	63.1	21.0	68.4	31.5	0	0	47.3	36.8	42.1	68.4	68.4
MHS	**41**	100	63.4	100	95.1	31.7	80.5	12.2	0	0	58.5	63.4	53.7	85.4	80.5
NCLE	**20**	100	55.0	100	50.0	15.0	70.0	30.0	0	0	45.0	40.0	20.0	70.0	55.0
SET	**43**	100	39.5	100	58.1	2.3	74.4	20.9	0	0	39.5	72.1	74.4	88.4	88.4

Abbreviations: KM, Khammoun Hospital; MHS, Mahosot Hospital; NCLE, National Center for Laboratory and Epidemiology; SET, Setthathirath Hospital; AM, Ampicillin; AMC, Amoxicillin/Clavulanic Acid; CRO, Ceftriaxone; CAZ, Ceftazidime; FOX, Cefoxitin; CHL, Chloramphenicol; CIP, Ciprofloxacin; FEP Cefepime; CN, Gentamicin; NA, Nalidixic Acid; IMP, Imipenem; MEM, Meropenem; SXT, Co-trimoxazole (SXT); TE, Tetracycline

Rate of resistance (%) to various antibiotics in ESBL-producing *K. pneumoniae*

Site of Surveillance	No. isolates	AMP	AMC	CRO	CAZ	FOX	FEP	CHL	IMP	MEM	CN	CIP	NA	SXT	TE
KM	**31**	100	74.2	100	12.9	6.5	96.8	9.7	0	0	93.5	48.4	9.7	19.4	12.9
MHS	**9**	100	88.9	100	100	0	88.9	22.2	0	0	77.8	77.8	44.4	88.9	88.9
NCLE	**30**	100	38.0	100	20.0	10.0	96.7	13.3	0	0	76.7	46.7	13.3	20.0	16.7
SET	**7**	100	57.1	100	71.4	14.3	100	28.6	0	0	28.6	100	57.1	57.1	71.4

Abbreviations: KM, Khammoun Hospital; MHS, Mahosot Hospital; NCLE, National Center for Laboratory and Epidemiology; SET, Setthathirath Hospital; AM, Ampicillin; AMC, Amoxicillin/Clavulanic Acid; CRO, Ceftriaxone; CAZ, Ceftazidime; FOX, Cefoxitin; CHL, Chloramphenicol; CIP, Ciprofloxacin; FEP Cefepime; CN, Gentamicin; NA, Nalidixic Acid; IMP, Imipenem; MEM, Meropenem; SXT, Co-trimoxazole (SXT); TE, Tetracycline

Rate of resistance (%) to various antibiotics in ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae*

Organism	No. isolates	AMP	AMC	CRO	CAZ	FOX	FEP	CHL	IMP	MEM	CN	CIP	NA	SXT	TE
E. coli	**123**	100	53.9	100	66	19.3	71.4	24.9	0	0	47.6	54.8	46.2	71.1	74.3
K. pneumoniae	**77**	100	74.2	100	50	11.2	95.6	18.4	0	0	66.3	68.2	28.6	43.5	44.7

Abbreviations: AM, Ampicillin; AMC, Amoxicillin/Clavulanic Acid; CRO, Ceftriaxone; CAZ, Ceftazidime; FOX, Cefoxitin; CHL, Chloramphenicol; CIP, Ciprofloxacin; FEP Cefepime; CN, Gentamicin; NA, Nalidixic Acid; IMP, Imipenem; MEM, Meropenem; SXT, Co-trimoxazole (SXT); TE, Tetracycline

2. Genotype Study Results

Among 200 study isolates, *bla*$_{\text{CTX-M}}$ or *bla*$_{\text{TEM}}$ genes were detected in only 96 isolates (48.0%) in which 1 isolate had both genes and 95 isolates had only one gene, whereas the aforementioned genes were not detected in 104 isolates (52.0%)

Prevalence of *bla*$_{CTX-M}$ genes in ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* isolates

Site of Surveillance	*E. coli*	*bla*$_{CTX-M}$ genes N (%)	*K. pneumoniae*	*bla*$_{CTX-M}$ genes N (%)
KM	**19**	3 (15.8)	**31**	11 (35.5)
MHS	**41**	22 (53.7)	**9**	5 (55.6)
NCLE	**20**	11 (55.0)	**30**	10 (33.3)
SET	**43**	27 (62.8)	**7**	4 (57.1)
Total	**123**	**63 (51.2)**	**77**	**30 (39.0)**

Abbreviations: KM, Khammoun Hospital; MHS, Mahosot Hospital; NCLE, National Center for Laboratory and Epidemiology; SET, Setthathirath Hospital

Prevalence of *bla*$_{TEM}$ genes in ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* isolates

- *bla*$_{TEM}$ genes were detected in only 9.3% of ESBL-producing *E. coli* isolates collected
- Setthathirat Hospital giving the overall prevalence of *bla*$_{TEM}$ genes of 3.3% in all ESBL-producing *E. coli*
- None in all ESBL-producing *K. pneumoniae* isolates contained *bla*$_{TEM}$ genes
- Therefore, the overall prevalence of *bla*$_{TEM}$ genes in all *E. coli* and *K. pneumoniae* isolates was 2%.

Prevalence of *bla*$_{SHV}$ genes in ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* isolates

- ***bla***$_{SHV}$ ***genes in ESBL-producing Escherichia coli and ESBL-producing Klebsiella pneumoniae isolates***
- ***bla***$_{SHV}$ **genes were not detected in all isolates of ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae*.**

Discussion

- Since the first ESBL-producing E. coli identified in 2004, there has been a steady increase in the proportion of bacteremia caused by ESBLE in Vientiane. We previously reported that 9% of E. coli causing
- In 2020 was more than 500, this study included only 150 isolates of ceftriaxone-resistant *E. coli* and ceftriaxone-resistant *K. pneumoniae* recovered from the patients in these three surveillance hospitals
- Although there are many types of ESBL genes associated with resistance to the third-generation cephalosporins, this study focused on only 3 genes such as $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} because they were the most common genes observed in ceftriaxone-resistant *E. coli* and ceftriaxone-resistant *K. pneumoniae* with ESBLs in many previous reports [5, 6, 15, 17-19].
- The aforementioned genes were detected in only 48% of the isolates in this study. The most common detected gene was $bla_{\text{CTX-M}}$ which was found in only 51.2% of ceftriaxone-resistant *E. coli* isolates and only 39.0% of ceftriaxone-resistant *K. pneumoniae* isolates. This frequency of $bla_{\text{CTX-M}}$ gene was lower than several previous reports.
- The prevalence of bla_{TEM} and bla_{SHV} genes that was detected in ceftriaxone-resistant *E. coli* and ceftriaxone-resistant *K. pneumoniae* isolates in this study was also lower than the previous reports.

Conclusions

- All isolates of ceftriaxone-resistant *E. coli* and ceftriaxone-resistant *K. pneumoniae* that were included in this study produced ESBLs

➢ Some isolates were still susceptible to some beta-lactam and non-beta-lactam antibiotics but none of them was resistant to carbapenems

- The most common gene detected in ceftriaxone-resistant *E. coli* and ceftriaxone-resistant *K. pneumoniae* isolates was $bla_{\text{CTX-M}}$ which was found in 51.2% of ceftriaxone-resistant *E. coli* isolates and 39.0% of ceftriaxone-resistant *K. pneumoniae* isolates whereas bla_{TEM} was much less common and none of them had bla_{SHV}

Limitation

- Due to logistics and a limited resource and personnel according to the COVID-19 pandemic, we can not collect sample size more than 150 samples
- We can not perform sequence genotype e.g CTX-M1-157….etct

Limitation

- ໃນການເກັບຮັກສາເຊື້ອແບັກເຕີເຣຍໄວ້, ຢາກສືບຕໍ່ຊອກຫາ Whole-genome genotyping, ໃຫ້ກັບບັນດາໄຊ້ທີ່ສົ່ງຕົວຢ່າງມາຢັ້ງຢືນຢູ່ ສວລ
- ມີແຜນໃນການເຮັດໂປສເຕີ ແລະ ຕີພິມບົດ ເພື່ອເຜີຍແຜ່ກ່ຽວກັບຫມາກຜົນຂອງການສຶກສາ
- ສືບຕໍ່ເຮັດການສຶກສາການເພີ່ມຂຶ້ນຂອງເຊື້ອໃນຊ່ວງ 2020-2024 ວ່າຈະມີຄວາມແຕກຕ່າງທາງດ້ານເປີເຊັນ ຫຼາຍຫນ້ອຍປານໃດ