PREVALENCE AND ANTIMICROBIAL RESISTANCE OF BACTERIAL ISOLATES IN LAO PDR FROM 2012 TO 2015

BY

MISS PHANTHANEEYA TEEPRUKSA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (BIOCLINICAL SCIENCES)

GRADUATE STUDIES

CHULABHORN INTERNATIONAL COLLEGE OF MEDICINE

THAMMASAT UNIVERSITY

ACADEMIC YEAR 2016

PREVALENCE AND ANTIMICROBIAL RESISTANCE OF BACTERIAL ISOLATES IN LAO PDR FROM 2012 TO 2015

BY

MISS PHANTHANEEYA TEEPRUKSA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (BIOCLINICAL SCIENCES)

GRADUATE STUDIES

CHULABHORN INTERNATIONAL COLLEGE OF MEDICINE

THAMMASAT UNIVERSITY

ACADEMIC YEAR 2016

THAMMASAT UNIVERSITY

CHULABHORN INTERNATIONAL COLLEGE OF MEDICINE

THESIS

BY

MISS PHANTHANEEYA TEEPRUKSA

ENTITLED

PREVALENCE AND ANTIMICROBIAL RESISTANCE OF BACTERIAL ISOLATES IN LAO PDR FROM 2012 TO 2015

was approved as partial fulfillment of the requirements for the degree of Master of Science (Bioclinical Sciences)

on May 15, 2017

Chairman

(Professor Vithoon Viyanant, Ph. D)

Member and Advisor

(Assistant Professor Wanna Chaijaroenkul, Ph.D.)

Member

(Professor Kesara Na-Bangchang, Ph.D.)

Director, Graduate Studies

(Professor Kesara Na-Bangchang, Ph.D.)

Dean

(Associate Professor Kammal Kumar Pawa, M.D.)

Thesis Title	PREVALENCE AND ANTIMICROBIAL RESISTANCE OF BACTERIAL ISOLATES IN LAO PDR FROM 2012 TO 2015
Author	Miss Phanthaneeya Teepruksa
Degree	Master of Science (Bioclinical Sciences)
Major Field/Faculty/University	Bioclinical Sciences Chulabhorn International College of Medicine Thammasat University
Thesis Advisor	Assistant Professor Wanna Chaijaroenkul, Ph.D.
Thesis Co-Advisor (If any)	Mayuri Tarasuk, Ph.D.
Academic Years	2016

ABSTRACT

Antimicrobial resistance (AMR) is a concerned in area all over the world. Increasing of AMR effect to public health, community and economy. This study is retropective on prevalence of bacterial infection and AMR of bacterial isolates from 2012 to 2015. Data reviewed of laboratory information and results to find the association between risk factor (gender, age, region, year, source and types of samples) and occurrences of bacteria infection were analysed. Total of 6789 specimens were collected and selected for analysis, 1341, 1873, 1588 and 1987 specimens from year, 2012, 2013, 2014 and 2015 respectively. The prevalence of at least one bacterial infection from 2012 to 2015 were 45.1%, 30.8%, 30.6% and 33.8%, respectively. The decreasing infection trend was statistical significant. This might be the result from successful health policy in Lao PDR. The factors that influenced the infection rate were sex, age, region, source of collection and year. There were 11 types of bacterium continued the susceptibility test. The overall results of susceptibility test were susceptible to commonly used drugs. However, there were four organisms; *E. coli*, *Klebsiella* spp, *Neisseria gonorrhoeae* and *Shigella* spp

showed high resistance rate and also showed the multidrug resistance phenomenon. Monitoring of AMR trend in the country is one of key supporting information for planning and control measure of AMR. Expanding site of specimen collection is required for represent of the country data.

Keywords: Antimicrobial resistance, Bacteria, Lao PDR

ACKNOWLEDGEMENTS

I would like to express my grateful to Ass. Prof Wanna Chaijaroenkul for her meaningful guidance, support and assistance during my enrollment until completed of this thesis.

I am also appreciated to Prof Kesara Na Bangchang, Dr. Mayuri Tarasuk and Miss Supanida Tanunta for valuable support and cooperation.

Finally, I would like to acknowledge my deepest thanks to Dr. Phengta Vonphrachanh, Director of National Center for Laboratory and Epidemiology (NCLE), Dr. Noikaseumsy Sithivong, Deputy chief of Laboratory and Bacteriology unit staff of NCLE for their valuable information. It is an honor to work with you all.

Moreover, all the reagents and equipments used in this study was supported by the United State Agency for International Development (USAID) - Emerging Pandemic Threats Program (EPT) and the European Union (EU) through the World Health Organization (WHO), Lao PDR.

Miss Phanthaneeya Teepruksa

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
ADH	Adenine dihydrolase
AFB	Acid-fast bacilli
AM	Ampicillin
AMC	Amoxicillin-clavulanate
AMR	Antimicrobial resistance
AN	Amikacin
APW	Alkaline peptone water
AST	Antimicrobial susceptibility testing
AWD	Acute watery diarrhea
BA	Blood agar
BA-P	Potassium-tellurite blood agar
BE	Bile esculin
C	Chloramphenicol
CAZ	Ceftazidime
CCDA	Charcoal ceforazone deoxycholate agar
CFU	Colony-forming unit
CH	Chocolate agar
CIP	Ciprofloxacin
Citrate	Simmon citrate agar
CLSI	Clinical Laboratory Standard Institute
CRE	Carbapenem-resistance Enterobacteriaceae
CRO	Ceftriaxone
DHPS	Dihydropteroate synthase
DHFR	Dihyrofolate reductase
DNA	Deoxyribonucleic acid
E	Erythromycin
EAEC	Enteroaggregative *E. coli*

EHEC	Enteropathogenic *E. coli*
EIEC	Enteroinvasive *E. coli*
ELISA	Enzyme linked immunosorbent assay
EPEC	Enteropathogenic *E. coli*
ESBL	Extended Specrtum Beta-lactamase
ETEC	Enterotoxigenic *E. coli*
EU	The European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
E-test	Epsilometer test
FOX	Cefoxitin
GM	Gentamicin
H2O2	Hydrogenperoxide
HIB	Heart infusion broth
I	Intermediate
ICU	Intensive Care Unit
Lao PDR	Lao People's Democratic Republic
LDC	Lysine decarboxylation
LPS	Lipopolysaccharides
MC, MAC	MacConkey agar
MDR	Multidrug resistance
MHT	Muller hinton agar
MIC	Minimal inhibitory concentration
mRNA	messenger RNA
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MSRV	Rappaport-Vassiliadis Medium, Semisolid Modification
NA	Nutrient agar
NaCl	Sodium chloride
NAL	Nalidixic acid
NCLE	National Center for Laboratory and Epidemiology

NFB	Non-Fermenter Gram Negative Bacilli
NS	Nonsusceptible
ODC	Ornithine decarboxylation
OFX	Ofloxacin
PABA	Para-aminobenzoic acid
PBPs	Penicillin- binding proteins
PCR	Polymerase chain reaction
PE	Penicillin
PPR	Pigment production
PYR	Pyrolidonylarylamidase
R	Resistant
RNA	Ribonucleic acid
S	Susceptible
SDD	Susceptible-Dose Dependent
SIM	Sulfide Indol Motile agar
SN	Selenite broth
SOP	Standard Operating Procedure
spp	Species
SS	Salmonella-Shigella agar
SXT	Trimethoprim-sulfamethoxazole
TCBS	Thiosulfate citrate bile salts agar
TD	Tindale agar
TE	Tetracycline
TM	Thayer martin agar
tRNA	transfer RNA
TSI	Triple Sugar Iron agar
Urea	Urease
USAID-EPT	United State Agency for International Development-Emerging Pandemic Threats Program
UTI	Urinary tract infection
VP	Voges-Proskauer

VTEC	Verocytotoxin-producing E. coli
WHO	World Health Organization
XLD	Xylose lysine desoxycholate agar
μg	Micrograms
μM	Micormeters

CHAPTER 1
INTRODUCTION

Antimicrobial resistance (AMR) is resistance of a microorganism to an antimicrobial drug that originates effectively control or kills bacterial growth. The evolution of resistant strains is a natural phenomenon that occurs when microorganisms replicate themselves erroneously or when resistant traits are exchanged between them. The major factor cause of AMR is the use and misuse of antimicrobial drugs, which accelerates the emergence of drug-resistant strains. Spreading of AMR in all parts of the world has become a serious public health problem that requires action across all government sectors and society. Various key organizations such as; the Centers for Disease Control and Prevention, the World Health Organization (WHO), and the World Economic Forum, have focused on the report, conferences and actions of antibiotic resistance within past decade. The recently report of WHO on global surveillance of antimicrobial resistance reveals that "threatens the achievements of modern medicine. A post-antibiotic era — in which common infections and minor injuries can kill — is a very real possibility for the 21st century". The global plan will aim to propose implementation of antibiotic stewardship in health care facilities and the community; development of rapid, point-of-care diagnostics; recruitment of academic and industry partners to increase the pipeline of antibiotics, vaccines, and alternative approaches; and international collaboration for prevention, surveillance, and control of antibiotic resistance. Therefore, study of AMR pattern of importance bacteria is essential to diagnostic and effective treatment.

Lao PDR has a limited sources, fund and information on AMR study from the government sectors and as well bacteriology traditional culture was not popular in country. In 2007, the diarrheal illness caused by *Vibrio cholera* was outbreak in Lao PDR. Later, the surveillance program of the causative of diarrhea in Lao PDR by National Center for Laboratory and Epidemiology (NCLE) has been started. From 2009-2011, the surveillance was focused on *Vibrio*, *Salmonella*, *Shigella* and *Campylobacter*. Subsequently, the monitoring program has been expanded to six

genera: *Vibrio*, *Salmonella*, *Shigella*, *Plesiomonas*, *Aeromonas* and *Campylobacter* which started on 2012. The AMR surveillance program will provide important information to control bacterial resistance especially for the nation policy.

The objective of this research project was to investigate the prevalence and antimicrobial resistance of bacterial isolated from clinical samples in Lao PDR during 2012 to 2015, to compare the trend of AMR over 4 year.

CHAPTER 2
REVIEW OF LITERATURE

2.1 Lao People's Democratic Republic

Lao People's Democratic Republic, Lao PDR or Lao, the land-locked country surrounding with five neighbors: Burma, China, Vietnam, Cambodia and Thailand. Lao PDR is dividing to 3 regions including Northern region (Phongsaly, Borkeo, Louangnamtha, Oudomxay, Xayabury, Louangprabang, Huaphanh and Xiengkhuang province), Central region (Vientiane province, Xaysomboon, Vientiane capital, Bolikhamxay, Khammouane and Savannakhet province) and Southern region (Sekong, Saravanh, Attapue and Champasack province) (**Figure 2.1**). Lao PDR has limited accessibility to in-country healthcare service, as 67.9 percent of the country is mountain and forest area. Over 75 percent of the population has access to primary health care with average 2.17 health worker per 1000 populations. Also more than 40 percent of the populations are the small ethnics group who may have difficulty to access the medical service. In rural areas, people are travel the long distances to seek medical help and health care services. The country's geography plays a significant role in defining access to health facilities and obtains treatment in a neighboring country. There are an estimated 5,000 pharmacies nationwide selling drugs and offered advice on prescriptions. However, these pharmacies are unregulated and their owners unlicensed. As a consequence, misprescription is common, both of inappropriate drugs and incorrect dosages (1-3).

2.2 Bacteria

Bacteria, the oldest and the most abundant living organisms are simple structure that known as prokaryotic cells. These prokaryotic cells contain genetic material both DNA and RNA with no nuclear membrane; which is different from eukaryotic cells such as fungi, protists, plants and animals (**Figure 2.2**).

Figure 2.1 LAO PDR map (http://www.mapscd.com/wp-content/uploads/laos.jpg)

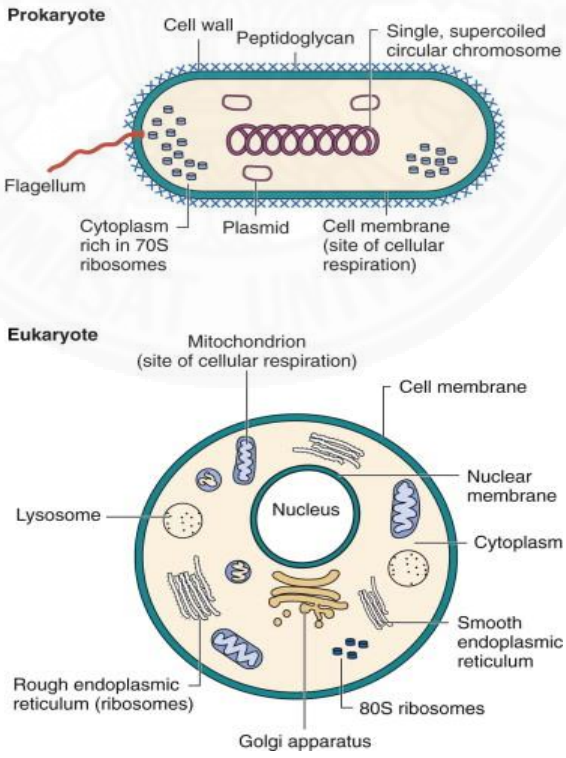

Figure 2.2 Major features of prokaryotes and eukaryotes (4).

2.2.1 Bacterial structure and classification

The bacteria classification is based on the macroscopic and microscopic appearance. The growth characteristics of bacteria on different nutrient and selective media can provide distinction phenomenon such as color, size, shape and smell. The microscopic appearance, including the size, shape, and configuration of the organisms and arrangement of cells which are essential for bacterial identification. Morphologies of bacteria include cocci (round), coccobacilli (oval), bacilli (rod), curved and spiral shapes (**Figure 2.3**). Bacterial cell arrangement includes singly, in pairs, tetrads, and clusters or in chains. Generally, bacteria cells are divided to two parts: cell envelope and cell interior.

2.2.1.1 Cell envelope

Cell envelope is the outer structure composed of cell wall, periplasmic space and cytoplasmic membrane. Cell wall is the common component of all bacteria except Mycoplasma, composed of outer membrane and peptidoglycan.

2.2.1.2 Cell wall

Cell wall components are also unique to bacteria. Most of bacteria can differentiate into two general groups by cellular structures, cell wall. Differences of cell wall separate by the gram stain testing: gram positive bacteria stain a deep blue or violet color and gram negative bacteria stain a pink to red color (**Figure 4**). Gram positive bacteria contain multilayer peptidoglycans with teichoic acid or lipoteichoic acid in the other hand gram negative bacteria cell wall contain thinner layer of peptidoglycan with periplasmic space between outer membrane and cytoplasmic membrane, outer membrane consist of lipopolysaccharide and porins (5, 6). The important differences in membrane characteristics between gram-positive and gram-negative bacteria are shown in **Table 2.1**.

Outer membranes are only found in gram negative bacteria function as the barrier to environment. The membrane is bi-layered structure of lipopolysaccharide with porins the protein structure. Lipopolysaccharides is endotoxin and somatic (O-antigen) (**Figure 2.4**).

Table 2.1 Bacterial cell wall structures

Structure	Chemical Constituents	Functions
Gram-Positive Bacteria		
Peptidoglycan	Glycan chains of GlcNAc and MurNAc cross-linked by peptide bridge	Cell shape and structure; protection from environment and complement killing
Teichoic acid	Polyribitol phosphate or glycerol phosphate cross-linked to peptidoglycan	Strengthens cell wall; calcium ion sequestration; activator of innate host protections, induce septic shock
Lipoteichoic acid	Lipid-linked teichoic acid	
Gram-Negative Bacteria		
Peptidoglycan	Thinner version of that found in gram-positive bacteria	Cell shape and structure
Periplasmic space		Enzymes involved in transport, degradation, and synthesis
Outer membrane		Cell structure; protection from host environment
Proteins	Porin channel	Permeation of small, hydrophilic molecules; restricts some antibiotics
	Secretory devices (types I, II, III, IV)	Penetrates and delivers proteins across membranes, including virulence factors
	Lipoprotein	Outer membrane link to peptidoglycan
Lipopolysaccharides (LPS)	Lipid A, core polysaccharide, O antigen	Outer membrane structure; Endotoxin, potent activator of innate host responses
Phospholipids	With saturated fatty acids	

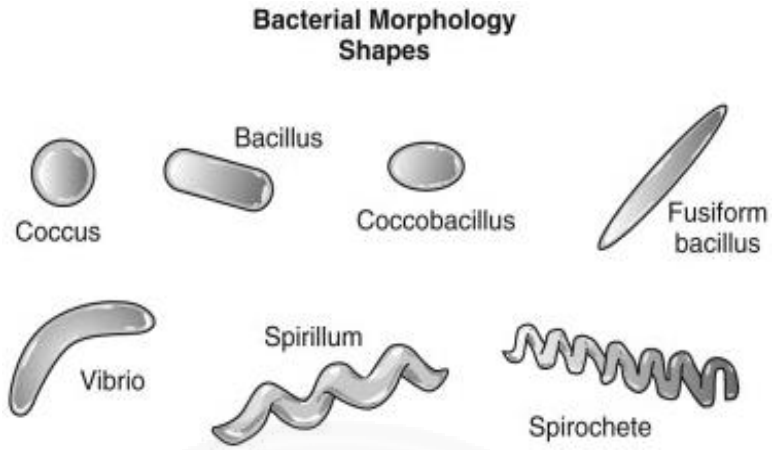

Figure 2.3 Bacterial morphologies (4).

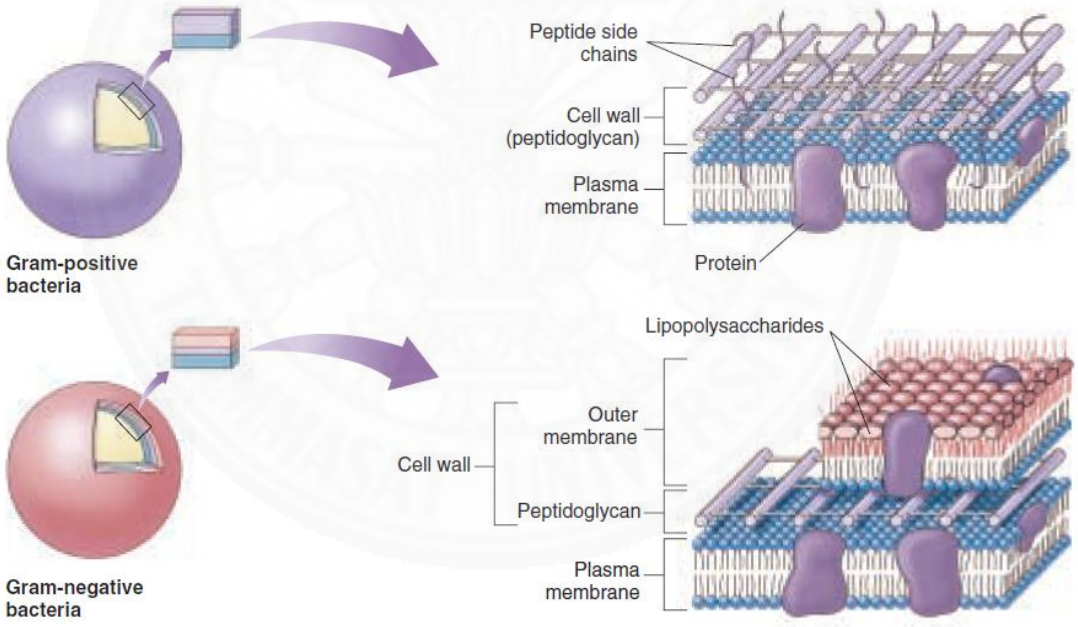

Figure 2.4 Comparison of the gram-positive and gram-negative bacterial cells (7).

Peptidoglycans or murein layer is a structure found only in bacteria cells but not in human cells. This structure is essential to bacteria to maintain shape and withstand change of environmental. Gram-negative bacteria have single layer of peptidoglycans that thinner than multilayer peptidoglycans of gram-positive bacteria (**Figure 2.4**). Teichoic acids are surface fibers of gram-positive bacteria. These structures are able to induce septic shock and mediate the attachment of Staphylococci to mucosal cells.

2.2.1.3 Periplasmic space

Periplasmic space is space between outer membrane and cytoplasmic membrane found only in gram-negative bacteria. This area contains hydrolytic enzymes and gel-like substance to secure nutrient from the environment.

2.2.1.4 Cytoplasmic membrane

Cytoplasmic membrane consisted of lipoprotein bilayer without sterol that different from eukaryote and enzymes, has function of 1- transport of solutes into and out of bacterial cell, 2- synthesis of outer membrane and cell wall, 3- generation of energy, 4- secretion of enzymes and toxins and 5- cell motility.

2.2.1.5 Cell interior

Cell interior include the cytosol, DNA, ribosome- the site of protein synthesis but bacterial ribosomes are 70S with 50S and 30S subunits, nutrient granules, nucleoid, plasmid and endospores.

2.2.1.6 Special structures

Bacteria are also containing special structures such as capsule – a gelatinous layer composed of polysaccharide cover entire bacterium cell to protect phagocytosis, adhere to human tissue and play role as antigen to activate human immunity, flagella or pili the filament assist bacteria movement.

2.2.2 Bacterial diseases

Not all bacteria or bacterial infections cause disease, but most always cause disease. The normal flora bacteria, which are colonized in human body, many of which serve important functions for their hosts; such as helping in the digestion of food, produce vitamins (*e.g.*, vitamin K), protect the host from colonization with pathogenic microbes and activate host innate and immune responses. However, these microbes cause disease if they enter normally sterile sites of the body.

The pathogenic bacteria have mechanisms that promote their growth in the host and cause diseases. While, opportunistic bacteria take advantage of pre-existing conditions, such as immune-suppression, to grow and cause serious disease. Disease results from the damage or loss of tissue or organ function due to the infection or the host inflammatory responses.

Pathogenic bacteria are bacteria that have ability to cause disease (pathogenesis) in host by combination of the two properties:

(1) Invasiveness is the ability to invade tissue by adherence and initial multiplication of tissue, produce of extracellular substances for invasion and ability to overcome host defense mechanisms.

(2) Toxigenesis is the ability of bacteria to produce toxins whether toxins released from bacterial cells calls “exotoxin” and a heat-stable toxin associated with the outer membrane of bacteria calls “endotoxin”. Exotoxin produced and released from bacteria cell specific target to host cell.

2.2.3 Pathogenic actions of bacteria

2.2.3.1 Tissue destruction

The products of bacterial growth are toxic to tissue. Furthermore, many bacteria degrade tissue by bacterial enzymes for their growth and expansion. For instance, *Clostridium perfringens* are opportunistic pathogens that are localized in gastrointestinal tract. These microbes can establish infection in oxygen-depleted tissues and cause gas gangrene. During infection, anaerobic metabolism enzymes (*e.g.*, phospholipase C, collagenase, protease, and hyaluronidase) and products (toxins, acid and gas) destroy the tissue. *Staphylococci* produce numerous enzymes (hyaluronidase, fibrinolysin, and lipases) that modify the tissue environment. *Streptococci* also produce enzymes, including streptolysins S and O, hyaluronidase, DNAases, and streptokinases.

2.2.3.2 Toxin

Toxins are directly harm tissue or trigger destructive biologic activities. Toxins and toxin-like activities are degradative enzymes that cause lysis of cells or specific receptor-binding proteins that initiate toxic reactions in a specific target tissue.

(1) Exotoxins

Exotoxins, produced by gram-positive or gram-negative bacteria, are proteins (including cytolytic enzymes and receptor-binding proteins) that alter a function or kill the cell. In many cases, the toxin gene is encoded on a plasmid or a lysogenic phage.

Cytolytic toxins include membrane-disrupting enzymes, such as the α-toxin, which breaks down sphingomyelin and other membrane phospholipids. Hemolysins disrupt erythrocyte and other cell membrane. Pore-forming toxins, including streptolysin O, can promote leakage of ions and water from the cell and disrupt cellular functions or cell lysis.

Several toxins are dimeric with A and B subunits (A-B toxins). The B portion binds to a specific cell surface receptor, and then the A subunit is transferred into the interior of the cell, where it acts to promote cell injury (B for binding, A for action). The targets of A-B toxins include ribosomes, transport mechanisms, and intracellular signaling, with effects ranging from diarrhea to loss of neuronal function to death.

(2) Endotoxin and other cell wall components

The bacterial cell wall components activate the host's protective systems. The lipid A portion of lipopolysaccharide (LPS) produced by gram-negative bacteria is a powerful activator of acute-phase and inflammatory reactions and is called endotoxin. In gram-positive bacteria, endotoxin-like structures such as teichoic and lipoteichoic acids can have weak immunity responses.

Gram-negative bacteria release endotoxin, which binds to specific receptors (CD14 and TLR4) on macrophages, B cells, and other cells and stimulates the production and release of acute-phase cytokines, such as IL-1, TNF-α, IL-6, and prostaglandins.

2.2.3.3 Pathogenic bacteria by human system

Bacteria can have localized in human body. Identification of pathogenic bacteria should have considered by the site of infection with the quantities of isolates.

(1) Bloodstream

Bloodstream is a close system. Infection in blood circulation or bacteremia is life threatening which differentiate to two categories; intravascular infection and extravascular. Intravascular infection is the infection originated in the vascular system through medical equipment - intravenous catheter or endocarditis. On the other hand extravascular infection is occur from bacterial at infection site enter circulatory system through lymphatic.

(2) Gastrointestinal tract

The gastrointestinal tract has microflora to prevent infection from interfere pathogens. Bacterial cause diarrhea by two mechanisms: enterotoxin-mediated and invasiveness. Enterotoxin-mediated diarrhea cause by *Vibrio cholerae, Aeromonas, Escherichia coli* and *Shigella* which produced enterotoxin. The invasive bacteria include *Salmonella, Shigella, Plesiomonas* and *Campylobater.*

(3) Upper respiratory tract

The upper respiratory tract includes oral cavity, throat, larynx, nasal tissue surrounding. Common pathogens for upper respiratory tract are including Group A *Streptococcus, Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Staphylococcus aureus, Bordetella pertussis, Corynebacterium diphtheriae* and *Burkholderia pseudomallei.*

(4) Genital tract infection

The genital tract infection mostly causes from sexual transmission. In woman, infections are including vaginitis, cervicitis, endometritis and pelvic inflammatory. In man, infections are including urethritis and epididymitis. Major bacterial causes of infection are *Neisseria gonorrhoeae* (Gonococcal), *Chlamydia trachomatis, Haemophilus ducreyi* (chancroid), *Treponema pallidum* (syphilis) and Group B *Streptococcus* which can cause septicemia and meningitis in newborns.

(5) Urinary tract infection (UTI)

The UTI is including cystitis, pyelonephritis, complicated UTI and prostatitis, likely to occur in women more than men because the urethra is located close to anus. Bacteria from intestines can invade from anus to urethra and go up to bladder causing an infection or even by sexual can be transmitted bacteria to urethra

as well. Common pathogens for this system are *E. coli, S. saprophyticus, Klebsiella, Enterobacter, Proteus, Enterococcus, P. aeruginosa* and gram negative bacilli.

2.3 Antimicrobial agents

Antimicrobial agents are substances that attempt to control and manage of infectious diseases. The agent must be in an active form and sufficient to inhibit (Bacteriostatic) or kill (Bactericidal) infecting microorganisms at the target site by their pharmacokinetic properties in the other hand they must have less toxic effects to human or host cell. These agents are powerful and specific to microorganisms due to their target selectivity properties. Antimicrobial agents can classify according to mechanism of action as describe below (6, 8).

2.3.1 Cell wall synthesis inhibitors

The bacterial cell walls are crucial structure for microorganisms' survival and these are not part of humans and animals cell. Therefore, these drugs can selectively kill or inhibit microorganisms, such as penicllins, cephalosporins, bacitracin and vancomycin.

Drugs in this class inhibit transpeptidases or refer as penicillin- binding proteins (PBPs), the enzymes required for cell wall synthesis while bacteria cells are growing. The antimicrobial agent enters the bacterial cells through porin channels in the outer membrane of gram-negative bacteria or diffuses through the cell wall in gram-positive bacteria. β-lactam molecules bind to penicillin- binding proteins (PBPs) of the antimicrobial agent which block function of the PBPs to cross-linking of peptidoglycan and cause weakened or defective cell walls lead to cell lysis.

2.3.2 Cell membrane function inhibitors

Cell membranes are biological barriers that separate and control the substances movement. Damage of permeability function is resulting of cell death. The agent diffuses through outer membrane and cell wall to bind the cytoplasmic membrane, disrupt and destabilize the cytoplasmic membrane. Although this drug class is able to kill microorganisms; it is not selectively and toxic to host. Thus, the drug usage is limited. For examples: polymixin B and colistin.

2.3.3 Protein synthesis inhibitors

Bacteria have 70S ribosomal with 50S and 30S subunit which different from human ribosomal. The antibacterial agents inhibit protein synthesis by binding of 30S, 50S ribosomal subunit or inhibit at the 70S initiation complex, and consequently lead to death or growth inhibition due to disruption of bacterial metabolisms.

Antimicrobial agents act at 30S ribosomal subunit are aminoglycosides (*e.g.* gentamicin, tobramycin and streptomycin) block functioning of initiation complex and cause misreading of mRNA, and tetracyclines (tetracycline and doxycycline) hinder the aminoacyl transferase of transfer RNA (tRNA) to enter the acceptor site on 30S ribosome subunit.

Antimicrobial agents act at 50S ribosomal subunit are chloramphenicol, macrolides (*e.g.* erythromycin, azithromycin and clarithromycin) and lincosamides (*e.g.* clindamycin). Chloramphenicol binds to 50S and inhibits peptidyl transferase to add new amino acids in protein synthesis. Macrolides and lincosamides prevent tRNA released causing termination of the growing protein chain and consequently inhibit of protein synthesis.

2.3.4 DNA/RNA synthesis inhibitors

Nucleic acids (deoxyribonucleic acid; DNA and ribonucleic acid; RNA) are important genetic information of living cells. Interfering of DNA or RNA synthesis is harmful and resulting cell death. The antibiotics such as quinolones (*e.g.* ciprofloxacin, levofloxacin and ofloxacin), metronidazole and rifampin inhibit DNA or RNA synthesis process.

Rifampin inhibits DNA-dependent RNA polymerase. While quinolones interfere DNA synthesis by binding DNA gyrase or DNA topoisomerase II and forming a gyrase-DNA complex that allow unwind DNS strands to be released into bacterial cell lead to cell death.

2.3.5 Other metabolic pathways inhibitors

Bacterial cells consist of many pathways that essential for their survival such as folic acid pathway, which is a precursor of nucleic acid synthesis. Para-aminobenzoic acid (PABA) is metabolite involved in folic acid synthesis. Sulfonamide has similar structural as PABA and competes with PABA bind to

enzyme dihydropteroate synthase (DHPS). Trimethoprim also inhibits the enzyme dihyrofolate reductase (DHFR) of folic acid synthesis that inhibits bacterial DNA synthesis.

2.4 Antimicrobial resistance (AMR)

Pathogenic bacteria have the properties to cause disease by their toxicity and invasiveness through host immunity. These bacterial virulence and antimicrobial resistance have related to bacterial species, virulence and resistance mechanism, ecological niche and host (9). The resistance may cause by intrinsic or acquired resistance.

(1) The intrinsic or natural resistance is an inherent attribute of bacteria such as structure of gram-negative cell wall is the reason of vancomycin resistant.

(2) Acquired resistance is a change of genetic composition to against the antimicrobial agent causing of loss the effectiveness to inhibit or kill microorganism by various resistance mechanisms effective after got pressure from environment: overuse and inappropriate use of antimicrobial or agricultural use in animal food. The mechanisms of antimicrobial resistance are including:

(3) The inactivation or modification of antibiotic (Aminoglycosides, Amphenicols, Antifolates, β-lactams, Glycopeptides and rifamycin) by produced of enzyme to inactivate or destroy antimicrobial agent.

(4) The reduce antibiotic penetrate into bacterial cellular by decreasing permeability at porin channels of cell membrane or developing permeability barriers results to antimicrobial agents cannot entrance and passage through the bacterial cells. (Aminoglycosides, β-lactams)

(5) Increasing active efflux (Aminoglycosides, β-lactams, Macrolides, Quinolones and Tetracyclines) by mediated forming trans-membrane protein channels inserted in the cytoplasmic membrane of gram-positive bacteria and in the outer membrane and the periplasm of gram-negative bacteria, this protein channels can export antimicrobial agent out of the cells.

(6) Alteration in target site of antibiotic to reduces the binding capacity (aminoglycosides, β-lactams, fluoroquinolones, glycopeptides, macrolides, rifamycin and tetracyclines) such as alteration of PBPs then β-lactams can not bind the target.

(7) Alteration of metabolic pathways (Sulfonamides and Trimethroprim) of bacteria that bypasses the reaction.

The antimicrobial resistance is a worldwide public health concerned, resistance to antimicrobial threatening to live, social and economic (10). Several article report of AMR in Asia pacific (11, 12) especially increasing of multidrug resistance but limited report for Lao PDR. The factor influence the multidrug resistance organism due to previous use of antimicrobial therapy, from the hospitalization and whether from the complex disease such as malignancy and diabetes mellitus (13, 14).

2.5 Antimicrobial resistance (AMR) Surveillance

The World Health Organization (WHO) recommends AMR surveillance of the importance bacteria caused infection in hospital and community; *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, Nontyphoidal *Salmonella*, *Shigella* species and *Neisseria gonorrhoeae* (15). *Escherichia coli* and *Klebsiella pneumoniae* are major problem in hospital-associated infection but not limit to Enterobacteriaceae, gram negative glucose non-fermenting like *Pseudomonas aeruginosa* and *Acinetobacter baumannii* also noted as caused of hospital-associated infection. There were the high rate Methicillin Resistant *Staphylococcus aureus* (MRSA), both MRSA infection in community and hospital associated and MRSA carrier especially in healthcare worker. *Salmonella* and *Shigella* species are foodborne pathogen and important for both human and animal sector as One Health concerned. Use of antimicrobial in livestock, resistant bacterial from food animal may result to AMR situation in human (16). *Shigella* isolates were report resistant to ampicillin, tetracycline and trimethoprim-sulfamethoxazole (SXT) in several countries such as Lao PDR, Thai, Vietnam, Isarael, Bangladesh, Hongkong and Brazil (17-20). *Salmonella* isolates were resistant to more than one

antimicrobial(21) especially tetracycline and SXT show high resistance rate. Difference serotypes Salmonella presented slightly different percentage of AMR (22).

Therefore, several reports were published but AMR trend for each region and country may differ. This study will report the trend of antimicrobial resistance from bacterial isolates in Lao PDR collected from January 2012 to December 2015, which might benefit to clinical sector and epidemiology sector to be a baseline for prepare and response the current situation in country.

Specific pathogens and antibiotics resistant for public health concerned are listed below:

2.5.1 Enteropathogenic bacteria are very important and impacts to emergence of antimicrobial resistance **(**AMR**)** through human, food animal and food production. Nontyphoidal *Salmonella* and *Shigella* are common pathogens found arising resistance to fluoroquinolones in most area of the world (15, 23-25) which WHO selected these two bacteria as ones of antibiotic resistance monitoring.

2.5.2 Enterobacteriaceaea Extended Spectrum Beta-lactamase (ESBL) is an Enterobacteriaceae group producing ESBL enzymes that mediate resistance to Beta-lactam agents and third generation cephalosporins organism. More than 150 ESBL-producing strains from gene encode enzymes mutation and/or combination with other resistance mechanisms caused treatment failure. *E.coli*, *Klebsiella* spp and *Proteus* spp are the concerning organism recommended by CLSI to monitoring and testing for ESBL-producing strains.

2.5.3 Methicillin resistant *Staphylococcus aureus* (MRSA) is strain of *S. aureus* that contained gene (*mecA* gene) encodes for penicillin-binding protein (PBP) that express resistant to penicillinase-stable penicillins and Beta-lactam agents. Methicillin is no longer used for treatment *Staphylococcus* but MRSA term is still use to call the group of resistance organism even through oxacillin is the preferred treatment. Cefoxitin is use for surrogate of oxacillin testing. MRSA is one of the concerned pathogen causing health care acquired infection.

2.5.4 Non-Fermenter Gram Negative Bacilli (NFB) is a group of non-catabolize glucose. The main and common known organism are *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Stenotrophomonas maltophilia* (26).

Pseudomonas aeruginosa and *Acinetobacter spp* have intrinsic mechanism to develop resistance to multiple antimicrobial (27).

These group of organisms caused of hospital acquired infection especially in Intensive Care Unit (ICU) ward that a critical concerned of clinical management.

2.6 Laboratory diagnosis and antimicrobial susceptibility test

The laboratory diagnosis of bacterial diseases could be directly detecting bacteria by staining or culturing; or indirect way by immunologic approach. The importance and accuracy of test result requires that the appropriate specimen is collected, delivered expeditiously to the laboratory in the appropriate transport system with information about the clinical diagnosis and choosing appropriate method to test with specimen collected. Appropriated specimen can refer to obtaining specimen at the infection site and avoid contaminated from normal flora placed in appropriate transport medium.

Bacterial detection is including microscopy (wet preparation, gram staining or acid-fast bacilli (AFB) staining), culture on to appropriate media and observing growth and appearance of organism then processing of identification method and antimicrobial susceptibility testing. In other hand, detection nucleic acid by polymerase chain reaction (PCR) or antigen of bacteria and antibodies against bacteria are alternative testing.

2.6.1 Bacterial cultivation

Bacteria cultivation is the process to grow organism present in the infection site that include pathogen cause of infection organism and organism colonized at the site. Specimen will be placed on artificial environment riches with nutrition need of target bacteria including culture media, appropriate temperature and suitable condition required for specific organism (aerobic, anaerobic, microaerophilic or capnophilic). Categorizes of culture media including enrichment media – contains of specific nutrients to enhance growth of particular organism, supportive media – contains of nutrients to support growth of organism, selective and differential media – contains of specific agent to inhibit other organism and support certain organism to

grow. These media help on easily observation of grown bacteria and have sufficient quantities for identification step.

2.6.2 Antimicrobial susceptibility testing

Purpose of antimicrobial susceptibility testing is to determine the susceptible of antimicrobial agents for choosing the appropriate agents to effective treatment for significant bacteria. Assure the susceptibility for drug of choice and detection of the resistance and it reemerge are important goal for laboratory (28). There are several methods for antimicrobial susceptibility testing includes:

2.6.2.1 Disk diffusion method

Disk diffusion method is qualitative measurement, widely use because of its convenient, rapid growth, efficacy and cheap. Interest organism prepares in standard concentration inoculated on growth medium and test with pre-impregnated with a standard concentration of a particular antibiotic dispensed lightly onto the agar surface. The test antibiotic immediately begins to diffuse outward from the disks, creating a gradient of antibiotic concentration in the agar such that the highest concentration is found close to the disk with decreasing concentrations further away from the disk. After an overnight incubation, the bacterial growth around each disc is observed by scaling the clear area of "no growth" (inhibition zone), which refers to the minimum antibiotic concentration sufficient to prevent growth of the test isolate (**Figure 5**). If the test isolate is "susceptible" to a particular antibiotic, an inhibition zone will less than standard interpretation break point chart, and refer to "resistant" when the zone is higher than the cut point (28, 29).

2.6.2.2 Dilution method

Dilution method is quantitative measurement, by testing the isolate to a series of concentrations of antimicrobial agents in a broth or agar to determine the lowest concentration at which the isolate is completely inhibited as evidenced by the absence of visible bacterial growth refer as the minimal inhibitory concentration or MIC. The MIC is thus the minimum concentration of the antibiotic that will inhibit this particular isolate. Culture broths contain different concentration of antimicrobial agent, standard amount of bacterial cell was prepared and inoculate into each antimicrobial agent. Usually agar dilution is test in microwell plate (**Figure 2.6**). Agar dilution prepare by combine antimicrobial agent in different concentration with basal

agar and test with known concentration of organism to see the lowest concentration of antimicrobial combined the can inhibit growth of organism. The dilution method has limitation and suitable for rapid growing organism

2.6.2.3 Epsilometer test (E-test)

Epsilometer test or E-test is a commercially quantitative test that utilizes test strip impregnated with a gradually decreasing concentration of a particular antibiotic. The strip also displays a numerical scale that corresponds to the antibiotic concentration contained therein (**Figure 2.7**).

Interpretation of antimicrobial testing, several standards have been published and use as interpretation standard such as Clinical Laboratory Standard Institute (CLSI), European Committee on Antimicrobial Susceptibility Testing (EUCAST) by show zone diameter or concentration that implies to clinical treatment as the categories below:

(1) Susceptible (S) – isolates are inhibiting in the in vitro testing indicated that the usual concentrations and dose of antimicrobial agents achieve at the site of infection.

(2) Intermediate (I) – the lower response of isolates to the antimicrobial which may need to adjust the concentration and dose to achieve at the site and increase effective of the agent to organism.

(3) Resistant (R) - isolates are not inhibiting in the in vitro testing indicated that the usual concentrations and dose of antimicrobial agent failure to against the organism at the site of infection

(4) Susceptible-Dose Dependent (SDD) – the susceptibility of an isolate is dependent on the dose used in patient.

(5) Nonsusceptible (NS) – is used for isolate which only susceptible interpretive criteria has been designated.

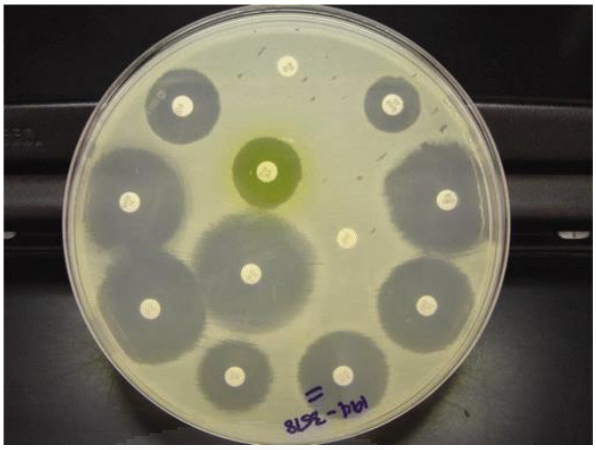

Figure 2.5 Disk diffusion method (30)

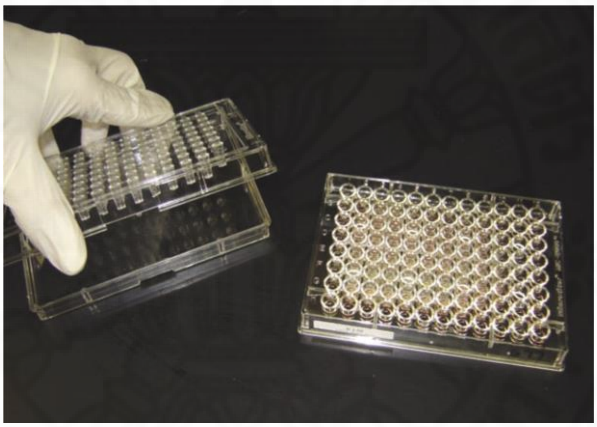

Figure 2.6 *Broth dilution method using microwell plate*

Figure 2.7 Epsilometer-test (E-test)

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Study Design and ethical clearance

This was a retrospective study of antimicrobial susceptibility pattern in the clinical isolates bacteria in Lao PDR between January 2012 to December 2015. Pathogenic bacteria were identified from clinical samples, and then appropriate antimicrobial agents were tested according to bacterial group.

The study proposal was submitted to the Board of Ethics Committee for the Lao People's Democratic Republic (PDR) and was approval on 28 March 2016.

3.2 Specimen collection and handling

The study was conducted at the National Center for Laboratory and Epidemiology (NCLE), Lao PDR. It is the governmental referral health center for disease diagnostic based on the laboratory work.

The specimens were collected from various health services, which then sent to the NCLE for bacterial identification or confirmation. All relevant data concerning socio-demographic factors related to risk factors of bacterial infection and resistance were obtained from hospital or laboratory data registered.

3.2.1 Specimen for clinical diagnostic

Specimens (pus, vaginal discharge, urethral discharge, urine, blood, bodily fluid, upper respiratory tract swab and stool) were collected from patients who have high fever (body temperature was equal or greater than 38°C) or who have sign and symptom of bacterial infection. These specimens were used for clinical diagnostic. The clinician collected specimen onsite and placed in transport media or patients directly came to NCLE with request form for specimen collection.

3.2.2 Specimen for diarrhea surveillance

Diarrhea surveillance was conducted at eight sentinel health facilities. The specimens were collected from patients who had symptom of diarrhea by defecated watery and/or bloody and/or mucous stool equal or greater than 3 times within 24 hours. The fresh stool or rectal swab was placed in Cary Blair transport media. All specimens were kept at 4°C and transported to NCLE within 72 hours after collected.

3.2.3 Specimen for disease outbreak

The disease outbreak specimens (stool/rectal, pus/tissue or throat swab) were collected from outbreak site and transported to NCLE. The stool/rectal swab specimens were collected from diarrhea outbreak suspected cases; these specimens were processed as mention in **3.2.2**. For anthrax suspected cases, pus swab or cut-infected tissues specimens were collected and placed in dried-container. In case of diphtheria suspected, throat swab was collected and placed in amines transport media with charcoal.

3.2.4 Others specimens

Some of specimen were collected and identified at the network laboratories, these specimens were then submitted to NCLE for confirmatory.

3.3 Pathogenic bacteria isolation

The specimens were cultivation via different media according to the standard operating procedures (SOPs) of National Center for Laboratory and Epidemiology. This cultivation was isolated the pathogenic bacteria from normal flora bacteria. The list of media for cultivation are shown below (**Table 3.1**)

Table 3.1 Culture media for bacterial cultivation

Culture media	**Supportive components**	**Purpose**
Blood agar (BA)	5% of sheep blood in basal media	Cultivation of fastidious organism and to determine hemolytic of sheep blood
MacConkey agar (MC)	Lactose	Isolation and differentiation of lactose-fermenting and non lactose-fermenting gram negative bacilli. Inhibit growth of gram positive organisms
Chocolate agar (CH)	2% of hemoglobin and supplement required for certain organisms	Cultivation of *Haemophilus* spp and *Neisseria* spp
Nutrient agar (NA)		Supportive media for facultative organisms
Muller hinton agar (MHT)		Supportive media for antimicrobial susceptibility testing of facultative organisms
Salmonella-Shigella agar (SS)	Lactose, ferric citrate, sodium citrate and have neutral red as indicator	Selective for *Salmonella* spp and *Shigella* spp Inhibit growth of coliform bacterium
Xylose lysine desoxycholate agar (XLD)	Lysine, xylose, lactose, sucrose, ferric ammonium citrate and have phenol red as indicator	Differentiate *Salmonella* spp and *Shigella* spp from others enteric bacterium
Thiosulfate citrate bile salts agar (TCBS)	Contain of bile salt, citrate, sucrose, ferric citrate, sodium thiosulfate and have bromthymol blue as indicator	Selective and differentiate fro *Vibrio* spp
Charcoal ceforazone deoxycholate agar (CCDA)		Selective for *Campylobacter* spp
Modified (MSRV)		Selective for *Salmonella* spp
Selenite broth (SN)	Sodium selenite	Enrichment of *Salmonella* spp
Alkaline peptone water (APW)		Selective and enrichment for *Vibrio* spp
Preston	5% lysed horse blood	Selective and enrichment for *Campylobacter* spp
Heart infusion broth (HIB)		Enrichment of organism in liquid media
Thayer martin agar (TM)	2% hemoglobin with antibiotic	Isolation of *Neisseria gonorrhoeae*
Potassium-tellurite blood agar	5% sheep blood and potassium tellurite	Isolation of *Corynebacterium diphtheriae*

3.3.1 Blood specimen

Two blood collections were obtained from each patient with unknown fever (greater or equal to 38°C for 2-3 days), these specimens were collected from different time or different site to increase the likelihood of detecting pathogens. Whole blood was inoculated in blood culture bottle and submitted to laboratory within 24 hours. This bottle was then inoculated at 35°C, and turbidity of media was observed every day. Subcultivation on selective agars was performed on day 1 and day 7 (**Figure 3.1**).

3.3.2 Stool and rectal swab specimen

The specimen was added into 0.5 ml of 0.85% normal saline solution to obtain working specimen. The pathogenic bacteria were then isolated by culturing the working specimen in different media as indicated in **Figure 3.2**.

3.3.2.1 Isolation of *Salmonella, Shigella, Vibrio, Aeromonas* and *Plesiomonas*

To isolate *Salmonella, Shigella, Vibrio, Aeromonas* and *Plesiomonas***,** the working specimen was inoculated on MacConkey agar, Salmonella-Shigella (SS) agar and Xylose lysine deoxycholate (XLD) agar, at 35°C for 18 to 48 hours.

The hidden samples were pre-enriched in Selenite broth at 35°C for overnight to allow for bacterial repair. Selective plating was performed by streaking the pre- enrichment broth onto SS agar and XLD agar and incubated at 35°C for 18-48 hours.

3.3.2.2 Isolation of *Vibrio* spp

The working specimen was pre-enriched into Alkaline peptone water (APW) at 35°C for 4-6 hours and was streaked on thiosulfate citrate bile salts sucrose (TCBS) agar and incubated at 35°C for 18-48 hours.

3.3.2.3 Isolation of Campylobacter spp

The selective media and filter technique were used to isolate *Campylobacter* spp (31). The working specimen was pre-enriched in Preston broth with 5%lysed horse blood at 35°C for overnight. The pre-enriched sample was transferred to the selective plate. The selective plate, cellulose acetate membrane (pore size 0.45 μM) was placed on the surface of Charcoal ceforazone deoxycholate agar (CCDA) or Blood agar. This membrane allows only slender campylobacters pass

through culture media. The sample was passed through the membrane at 35°C for 30 minutes. The membrane was removed and the sample incubated at 42°C microaerophilic condition for 48-72 hours. Colonies were observed and gram-stained for gram negative bacilli seagull-wing shapes.

3.3.3 Pus, wound and body fluid specimen

Pus, wound and body fluid specimen was cultured on Blood agar, MacConkey agar, Chocolate agar and Heart infusion broth at 35°C for 24 hours. If no bacteria growth was observed, the heart infusion broth was inoculated onto the chocolate agar at 35°C for 24 hours. Pathogen was selected based on site of specimen such as ear, eye, openned wound, or aspirates from sterile site.

3.3.4 Throat and nasal swab specimen

The throat or nasal swab specimen was collected and placed in transport media, then submitted to laboratory. The specimen was inoculated on Blood agar and Chocolate agar at 35°C in capnophilic condition (5% CO_2), and also on MacConkey agar for observing *Burkholderia pseudomallei* (**Figure 3.3**).

For diphtheria suspected case, sample was incubated on Tellurite Blood agar at 35°C for 24-72 hours to differentiate for *Corynebacterium diphtheriae* (**Figure 3.4**).

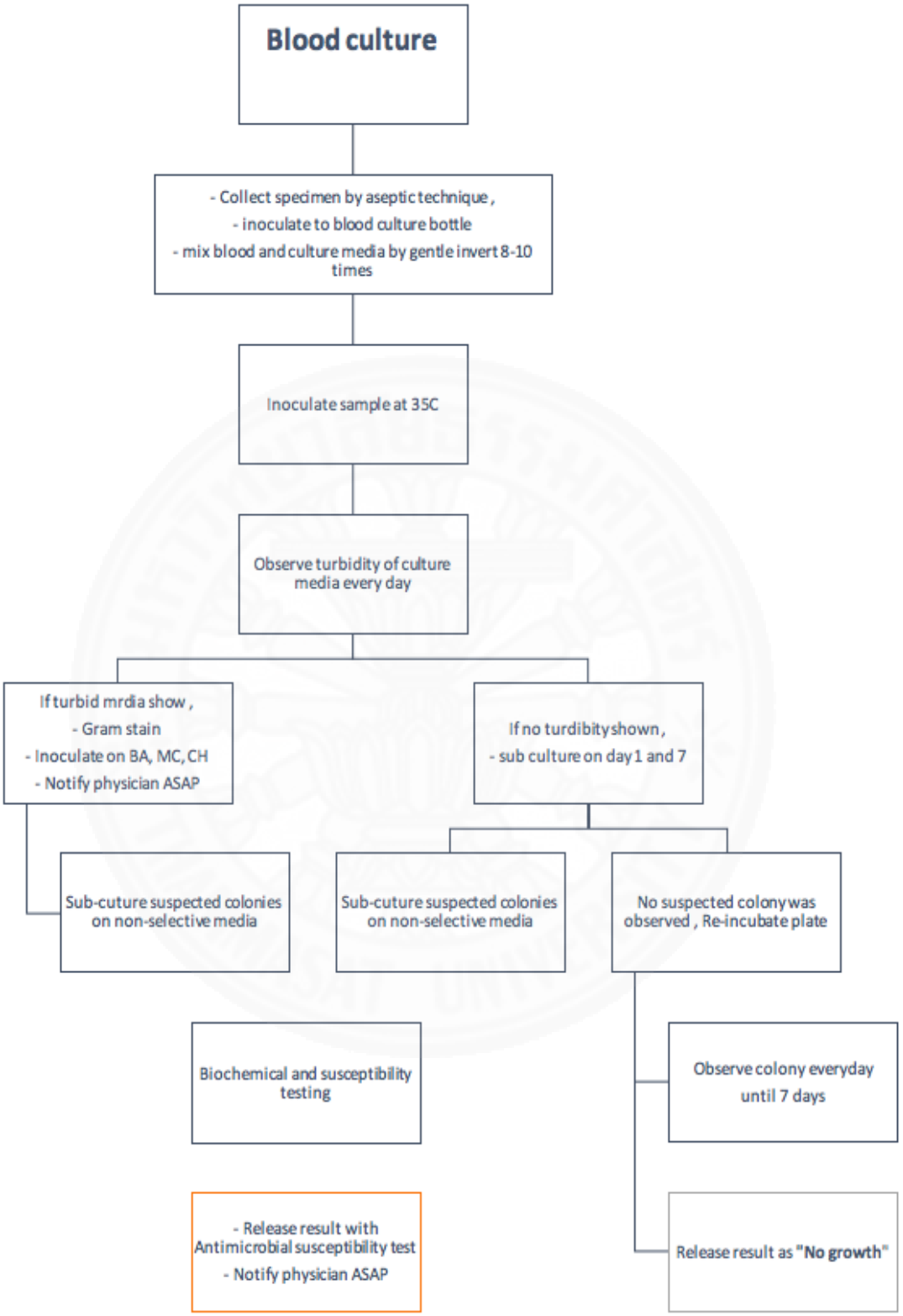
Blood culture
- Collect specimen by aseptic technique ,
- inoculate to blood culture bottle
- mix blood and culture media by gentle invert 8-10 times
Inoculate sample at 35C
Observe turbidity of culture media every day
If turbid mrdia show ,
- Gram stain
- Inoculate on BA, MC, CH
- Notify physician ASAP
If no turdibity shown ,
- sub culture on day 1 and 7
Sub-cuture suspected colonies on non-selective media
Sub-cuture suspected colonies on non-selective media
No suspected colony was observed , Re-incubate plate
Biochemical and susceptibility testing
Observe colony everyday until 7 days
- Release result with Antimicrobial susceptibility test
- Notify physician ASAP
Release result as "No growth"

Figure 3.1 Blood culture process

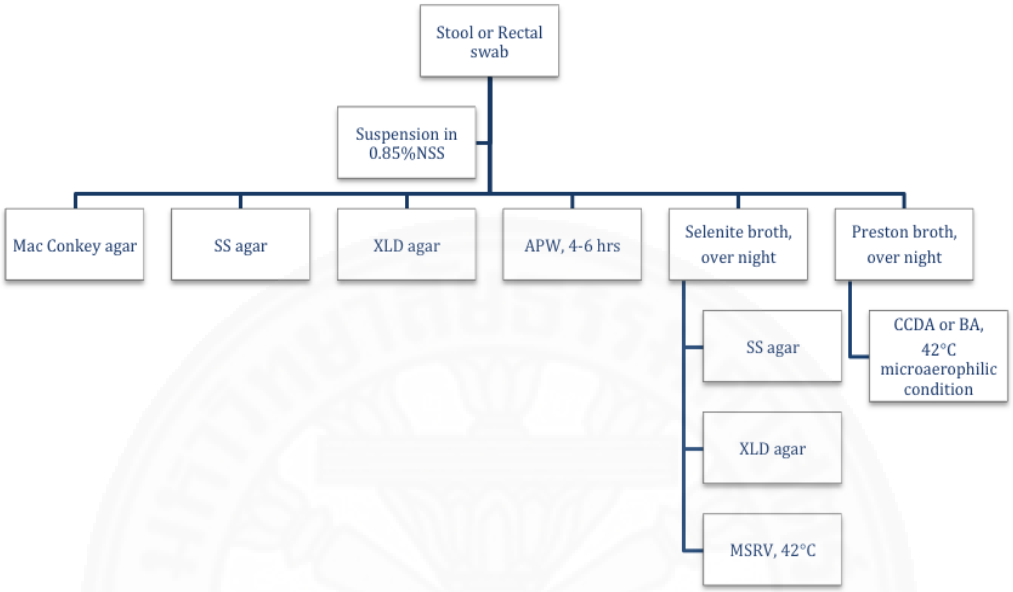

Figure 3.2 Stool and Rectal swab culture process

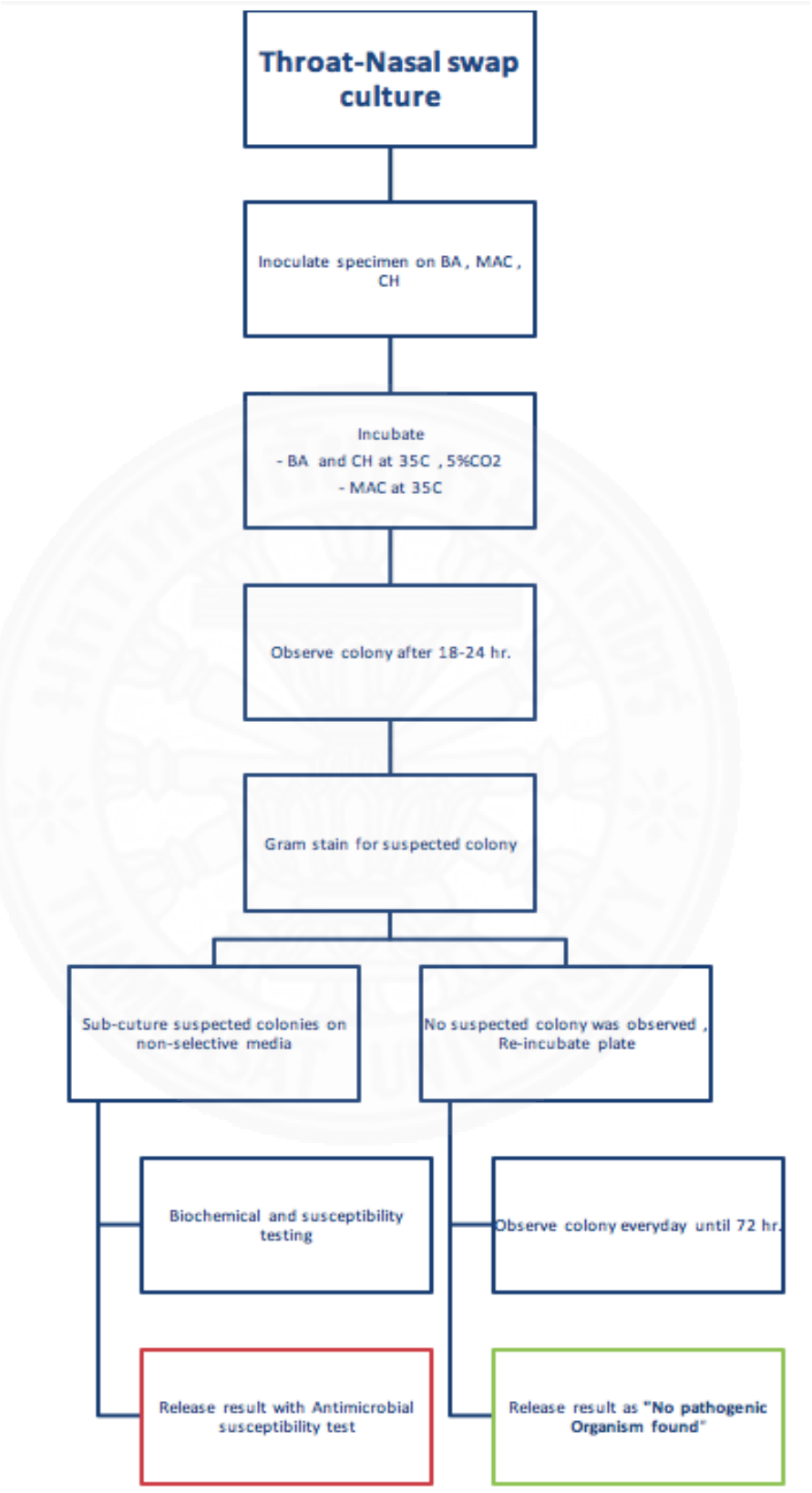

Figure 3.3 Throat or Nasal swab culture process

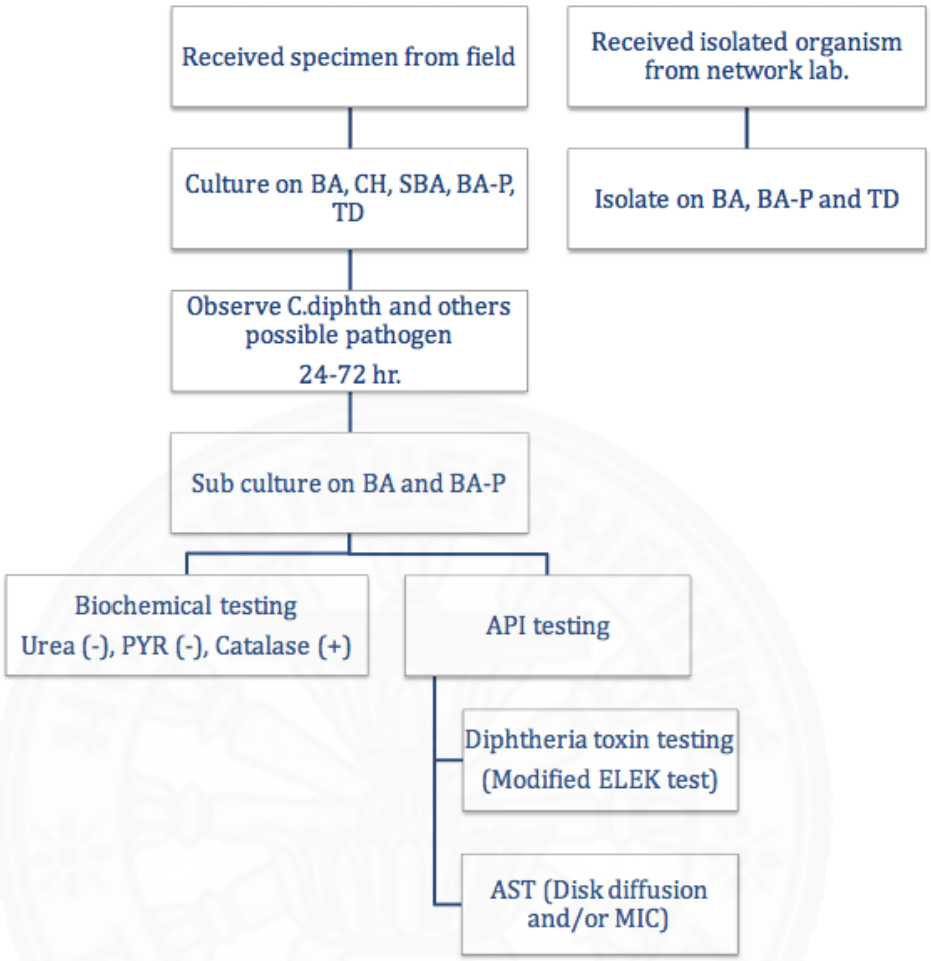

Figure 3.4 Throat or Nasal swab culture for *Corynebacterium diphtheriae*

3.3.5 Genital tract specimen

Genital tract specimens were included urethral discharge, urethral swab and vaginal discharge swab. Specimen was inoculated on Blood agar, Chocolate agar and Thayer Martin (TM) agar. Targeting pathogen are *Neisseria gonorrhoeae*, Group B *Streptococcus* and predominated bacteria.

Additionally, vaginal discharges were preformed microscopic wet preparation for *Trichromonas* spp, microscopic gram stain and bacterial culture. Gram stain was evaluated Nugent score and interpreted for bacterial vaginosis if score was equal or greater than 7 (32).

3.3.6 Urine specimen

A calibrated-loop full (0.001mL) of each urine sample was streaked on Blood agar and MacConkey agar, the culture plate was incubated at 37°C under aerobic condition for 24 to 48 hours. In addition, the sample was centrifuged and streaked pellet on TM agar for gonococcal-suspected case (**Figure 3.5**).

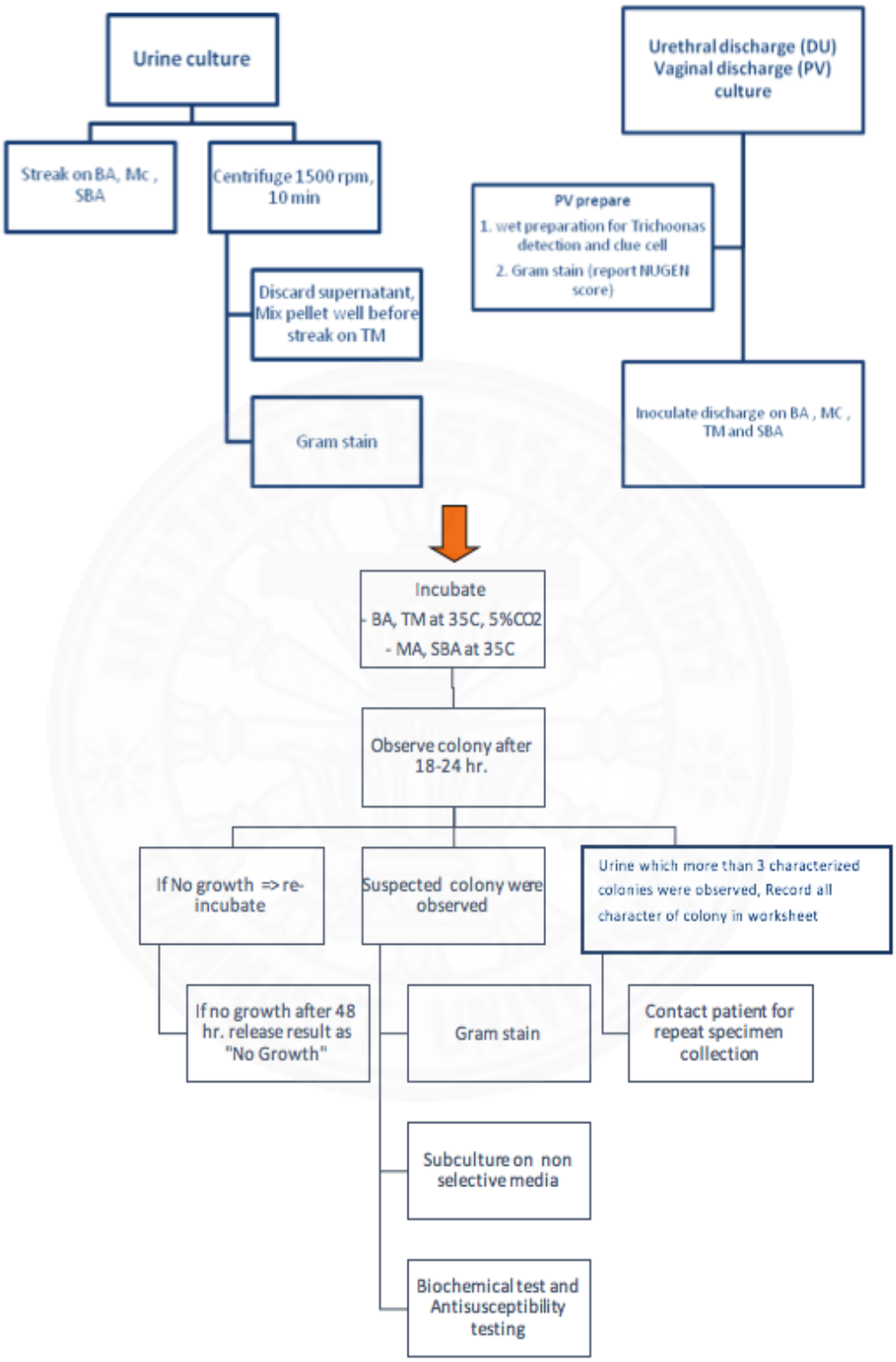

Figure 3.5 Uro-genital tract specimen culture process

3.4 Bacterial identification and serotyping

3.4.1 Gram staining

Gram staining was done for all isolates as per the standard procedures and the smears were examined microscopically, for their morphology.

3.4.2 Bacterial identification by biochemical tests

The suspected organism which grew on culture media was further identified. A single colony was selected and pre-enriched in non-selective media (Nutrient agar). Later on, the pre-enriched bacteria was identified by traditional biochemical test (5). The biochemical test could identify microorganism up to genus level; however, some tests may able to specify species. The traditional biochemical tests are often used for bacterial identification, nevertheless the confirmation testing by commercial reagent- API identification kit (Biomerieux, France) is needed.

3.4.2.1 Gram positive cocci

The identification of gram positive cocci was started with catalase test and then separated to catalase positive (**Figure 3.6**) and catalase negative (**Figure 3.7**). The biochemical tests for gram positive cocci were describe in **Table 3.2**.

3.4.2.2 Gram negative bacilli

Nine biochemical tests including oxidase test were the basic scheme for identification of gram negative bacilli (**Table 3.3)**.

Table 3.2 The biochemical tests for gram positive cocci

Test	**Purpose**
Catalase test	The test will differentiate between catalase positive and catalase negative for gram positive cocci.
Coagulase test	If coagulase positive, identified as *Staphyloccocus aureus*.
Novobiocin susceptibility test	Novobiocin 5μg susceptible test is used to identify *S. saprophyticus* in uro-genital tract specimen.
Bacitracin susceptibility test	Bacitracin (0.04 unit) susceptible test is used to determine Group A *Streptococci* from other beta-hemolytic *Streptococci.*
Pyrolidonylarylamidase (PYR) test	This test is to observe enzyme activity of Group A *Streptococci*.
Pigment production (PPR) test	The test is to identify Group B *Streptococci* from others beta-hemolytic *Streptococci*.
6.5%NaCl tolerance test	This test is to differentiate between enterococci and Group D *Streptococci.*
Esculine hydrolyse test	This test is used to differentiate enterococci and Group D *Streptococci* from others *Streptococci* by observe black color forming.
Optochin susceptible test	This test is used to identify *Streptococcus pneumoniae* by measurement the inhibitory zone size.

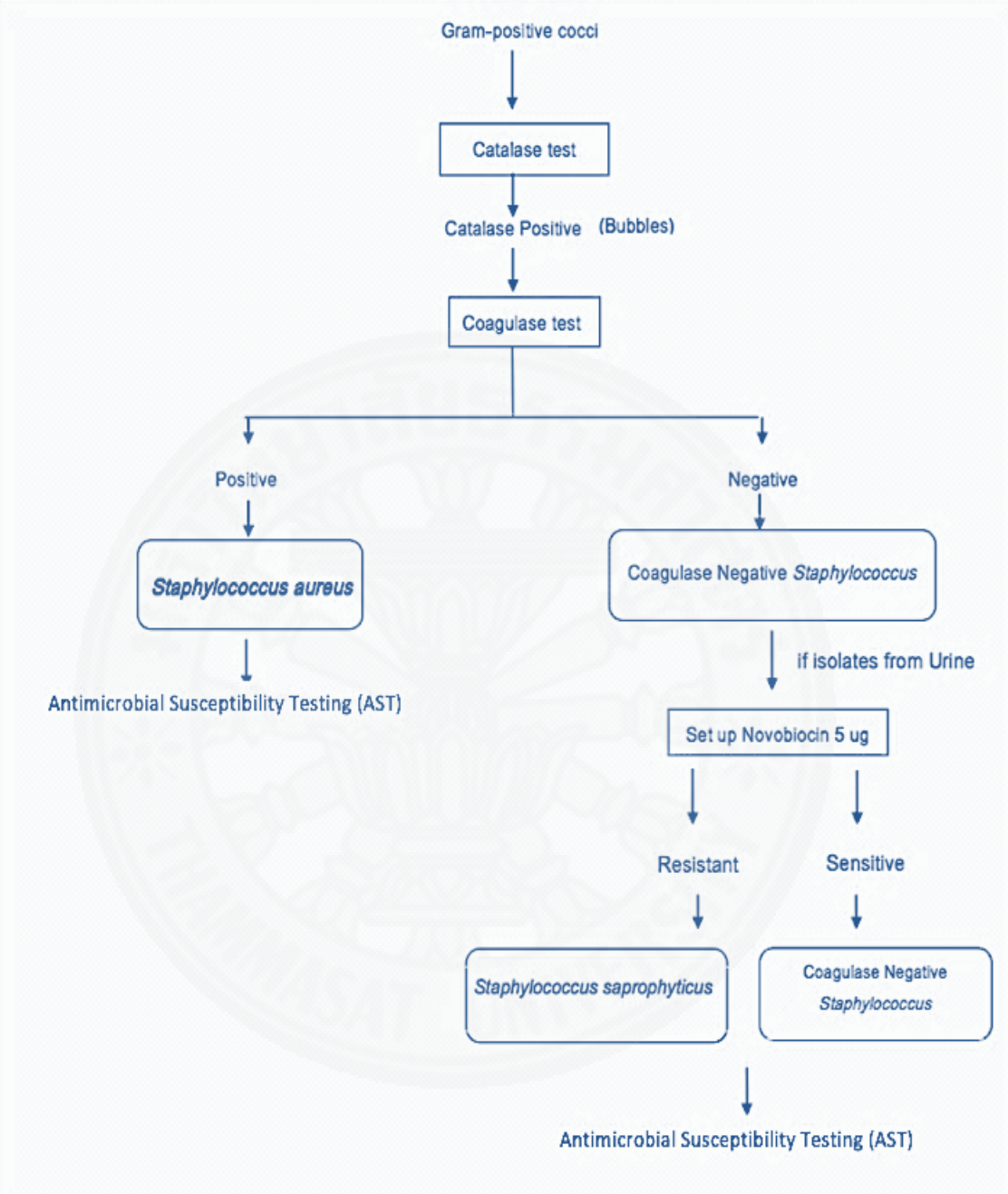

Figure 3.6 Testing algorithm: Catalase positive gram positive cocci

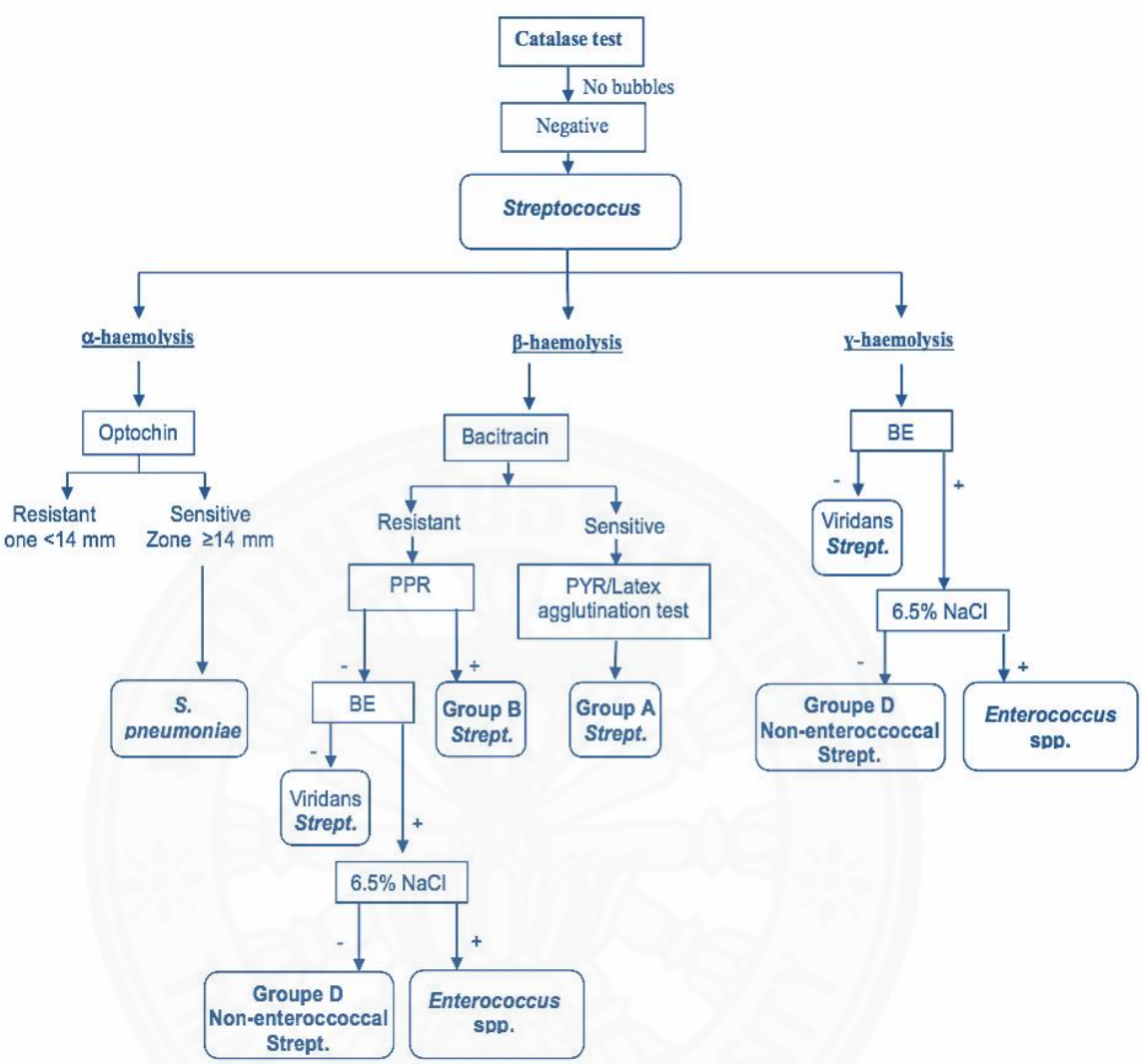

Figure 3.7 Testing algorithm: Catalase negative gram positive cocci

Table 3.3 The biochemical tests for gram negative bacilli

Test	**Purpose**
Oxidase test	The test is used to identify organism that produces cytochrome oxidase which differentiates between major group of oxidase positive and negative.
Triple Sugar Iron agar (TSI)	The test is used to observe four reactions of hydrogen sulfide producing by blackening of agar, gas production by presence of bubbles or cracking of agar and fermentation of sugar by observe color changing of butt and slant part produced of acid (yellow color), alkaline (red color) or no change reaction (remain same color).
Sulfide Indol Motile agar (SIM)	The test is used to observe three reactions of hydrogen sulfide producing by blackening of agar, indol production (red color) after adding Kovac' reagent and motility of organism by observe diffuse growth through agar.
Simmon citrate agar (Citrate)	The test is used to observe citrate utilization changing agar to blue color.
Voges Proskauer (VP)	The test is used to observe reaction after adding of KOH and α-napthol reagent by presence of red color.
Lysine decarboxylation (LDC)	The test is used to o observe reaction change of broth to purple color with evidence of organism growth.
Ornithine decarboxylation (ODC)	The test is used o observe reaction change of broth to purple color with evidence of organism growth.
Adenine dihydrolase (ADH)	The test is used to observe reaction change of broth to purple color with evidence of organism growth.
Urease (Urea)	The test is used to observe ability of organism to produce enzyme urease and hydrolyze urea by changing of broth color to pink.

3.4.3 Serotyping

All presumptive positive samples were confirmed using biochemical tests. The samples were stored in 50% nutrient agar for serotyping test. The serotyping was used for identification of sub-specific species of organisms.

Serotyping of *Salmonella* strains was investigated by using slide agglutination and tube agglutination which identifies surface antigen (lipopolysaccharides, O-antigens) and flagella antigen (proteins, H-antigens), respectively.

The serotypes are tested by Denka-Seiken antisera (Japan) and read based on the antigen combination present according to "Kauffmann-White scheme" (33).

Shigella groups and serotypes were identified by slide agglutination using antisera for surface antigen. The polyvalent antisera were used to identify group or species. The positive sample with polyvalent antisera was further observed with monovalent antisera, with the aim to identify sub-group serotype (34). In addition, *Shigella flexneri* was required to test both monovalent type and group antigens.

3.5 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by using Kirby-Bauer disk diffusion method and interpretation followed the Clinical Laboratory Standard Institute (CLSI) guideline (29).

The colonies from non-selective media were selected and transferred to normal saline, gently mixed until the bacteria suspension was homogeneous. To standardize incoculum size, turbidity of the suspension was measured and adjusted to achieve 0.5 McFarland (approximately 1 to 2 x 10^8 CFU/mL). Within 15 minute after adjusting the turbidity,, 1 ml of the suspension was pipetted to inoculate on dried Muller-Hinton agar (MHT) or the recommended medium.

The suspension was distributed over entire agar surface evenly for three times streaking with rotating plate approximately 60º each time to ensure and evenly distribution and swabbed the rim of the agar at final step.

The antimicrobial disks were placed on agar and pressed each disk to ensure complete contact with the agar surface. The plate was inverted and incubated

at 35°C ±2°C for 18-24 hours. After incubation, the diameters of inhibition zones were measured. The interpretation of inhibition zone followed the CLSI guideline M100-S25 and reported as susceptible, intermediate or resistant to the test antimicrobial agents.

For *Escherichia coli, Klebsiella* spp and *Proteus* spp, which showed extended spectrum beta-lactamese (ESBL) was confirmed by disk diffusion of cephalosporins (**Table 3.4)**.

To examine the Methicillin-resistant *Staphylococcus aureus* (MRSA), cefoxitin was used as a surrogate of *mec*A-mediated oxacillin resistance. Cefoxitin (30 μg) was placed on Muller-Hinton agar and incubated at 35ºC for 16-18 hours. The organism was considered express *mec*A and referred to as MRSA if inhibition zone diameter for *S. aureus* was less than 21 mm.

3.6 Data collection and statistical analysis

The demographic data such as admitted hospital, residence province, age, and gender were collected for data analysis. The clinical samples with no demographic data were excluded from analysis. Statistical analysis was performed using SPSS version 12. The percentages of pathogenic bacteria and antimicrobial resistance were determined. To determine the association between risk factors (sex, age, region, source of collection, and year) and the occurrences of pathogenic bacteria, the Pearson's chi-square was applied. The degree of association between risk factors and the occurrence of pathogenic bacteria were analyzed using odds ratio (OR). All statistical tests were performed at a statistical significance level of $\alpha = 0.05$.

Table 3.4 Screening and Confirmatory test for extended spectrum beta-lactamese (ESBL) –producing in *K. pneumoniae, K. oxytoca, E. coli* and *P. mirabilis* (29)

Resistance bacteria	Screening test by disk diffusion		Confirmation test	
	Antimicrobial agent	Result indicates ESBL production	Antimicrobial agent	Interpretation of ESBL production
K. pneumoniae, K. oxytoca and *E. coli*	Cetriaxone 30 μg	≤ 25 mm	Ceftazidime 30 μg Ceftazidime-clavulanate 30/10 μg And Cefotaxime 30 μg Cefotaxime-clavulanate 30/10 μg	≥ 5 mm zone diameter increase between antimicrobial agent combination with clavulanate vs the antimicrobial agent alone
	Cefotaxime 30 μg	≤ 27 mm		
	Ceftazidime 30 μg	≤ 22 mm		
P. mirabilis	Cefotaxime 30 μg	≤ 27 mm		
	Ceftazidime 30 μg	≤ 22 mm		

CHAPTER 4
RESULTS

4.1 The demographic data of samples collection

A total of retrospective specimens collected during the four years period were 6,944 specimens; 155 specimens with no demographic data were excluded from data analysis. Therefore only 6,789 specimens were included in analysis; 1341, 1873, 1588 and 1987 specimens were collected in 2012, 2013, 2014 and 2015, respectively.

Lao PDR is divided into three regions. The numbers of specimens received from different regions and sources in each year are shown in **Table 4.1**. Sources of specimen collection were categorized into NCLE, nosocomial infection, diarrhea surveillance, referral from network laboratories, and outbreak investigation. The specimens collected from outpatient at NCLE, diarrhea surveillance and nosocomial were conducted in Vientiane capital, central region of Lao PDR. Nosocomial surveillance was conducted from January 2013 to March 2014. Specimens from outbreak investigation were received occasionally; most of specimens were from diarrhea, food poisoning, typhoid fever, and anthrax outbreak.

Specimens were classified into 2 groups, *i.e.,* those with age less than or equal to 5 years, and those with age greater than 5 years as children aged less than 5 years are age of pre-school with lower immunity and several infectious diseases. Numbers of specimens collected from the two age groups of each year are shown in the **Table 4.1**.

Total numbers of specimens collected from male and female were 3,518 (51.8%) and 3,271 (48.2%) cases.

Specimens were categorized to eight types as follows: stool, blood, body fluid, respiratory specimen, pus or wound, urethral discharge, vaginal discharge, and urine. Stool sample was the highest specimen received in 4 years (3,299 out of 6,789 specimens, 48.6%).

Table 4.1 The demographic data during 2012-2015. Data presented as percentage (number)

	Frequency % (n)			
	2012	**2013**	**2014**	**2015**
Region				
• Central	87.1 (1168)	82.5 (1545)	91.4 (1452)	90.5 (1798)
• North	11.2 (150)	10.6 (198)	6.7 (106)	7.4 (148)
• South	1.7 (23)	6.9 (130)	1.9 (30)	2.1 (41)
Source of collection				
• Diarrhea surveillance	41.7 (559)	38.1 (713)	50.0 (794)	39.6 (787)
• NCLE	44.3 (594)	43.3 (811)	40.7 (646)	37.9 (754)
• Nosocomial infection	0.0 (0)	1.2 (23)	4.1 (65)	0.5 (10)
• Referral from network laboratories	1.2 (16)	0.4 (8)	1.3 (20)	2.2 (43)
• Outbreak investigation	12.8 (172)	17.0 (318)	4.0 (63)	19.8 (393)
Age (years)				
• ≤ 5	38.3 (514)	31.1 (583)	30.8 (489)	33.0 (655)
• > 5	61.7 (827)	68.9 (1290)	69.2 (1099)	67.0 (1332)
Sex				
• Male	51.5 (691)	50.5 (945)	51.8 (823)	53.3 (1059)
• Female	48.5 (650)	49.5 (928)	48.2 (765)	46.7 (928)
Type of specimen				
• Stool	53.1 (712)	48.3 (904)	51.6 (819)	43.5 (864)
• Blood	0.1 (1)	3.7 (70)	4.5 (72)	3.0 (59)
• Body fluid	0 (0)	0.1 (2)	0.1 (1)	0.1 (2)
• Respiratory tract	11.1 (149)	8.4 (157)	2.4 (38)	17.1 (340)
• Pus/Wound	7.8 (104)	9.2 (173)	9.6 (152)	8.1 (160)
• Urethral discharge	3.7 (50)	2.8 (52)	3.7 (58)	3.3 (65)
• Vaginal discharge	14.7 (197)	15.3 (287)	13.5 (215)	11.5 (228)
• Urine	9.5 (128)	12.2 (228)	14.7 (233)	3.5 (269)

4.2 Prevalence of bacterial pathogen

Identification methods of bacterial pathogen included bacterial cultivation and identification by traditional biochemical tests and/or commercial biochemical tests. Result interpretations were based on site and type of specimen collected. Stool culture was reported for enteric bacterial pathogen culture, rotavirus immunodiagnostic, molecular technique testing, and microscopic method. Rotavirus was also tested for children age equal or less than 5 years old by rapid diagnostic testing or ELISA. Blood culture showed any growth of microorganism in the culture media. Other specimens were interpreted of pathogen depending on the site of specimen collected.

Results of infection are shown in **Table 4.2.** The prevalence of bacterial infections in 2012, 2013, 2014, and 2015 were 45.1%, 30.8%, 30.6% and 33.8%, respectively. The respective values of infection by other organisms were 0.3%, 8.4%, 10.1% and 8.9%, respectively. Moreover, there were about 0.3% of samples which required additional sample collection.

The factors including sex, age, region, source of collection and year were analyzed in relation with types of infection as shown in **Table 4.3.** Sex, age, source of collection, and year were statistically associated with types of bacterial infection. Females were about 1.80 times at risk of bacterial infection compared with males (95% CI OR: 1.62-2.00, p<0.0001). Patients with age greater than 5 years were at risk of infections compared with younger age (OR = 2.27, p<0.0001). In addition, the samples collected from NCLE, nosocomial infection, referral from network laboratories, and outbreak investigation were 3.53, 4.69, 3.30 and 1.39 times, respectively, more likely to have bacterial infection than those collected from diarrhea surveillance. In contrast to other factors, prevalence of bacterial infections was decreased over time.

Table 4.2 The infection results during 2012-2015. Data presented as percentage (number)

	Frequency % (n)			
	2012	**2013**	**2014**	**2015**
No infection	54.5 (730)	60.5 (1133)	59.3 (941)	57.3 (1138)
Bacterial infection	40.4 (542)	27.9 (522)	28.9 (459)	31.9 (635)
• Mono-infection	30.9 (415)	21.4 (400)	22.3 (354)	24.2 (480)
• Mix-infection	9.5 (127)	6.5 (122)	6.6 (105)	7.8 (155)
Infection with other organisms	5.0 (67)	11.3 (212)	11.6 (184)	10.8 (214)
New sample required	0.1 (2)	0.3 (6)	0.2 (4)	0 (0)
Total	100 (1341)	100 (1873)	100 (1588)	100 (1987)

Table 4.3 The factors contributed to bacterial infection

	No infection (n)	Infection with at least one pathogenic bacteria (n)	Odd Ratio (OR)	95% CI	*P* value
Region					
• Central	3453	1907	1.00	0.92-1.08	1.00
• North	353	191	0.98	0.81-1.18	0.8279
• South	136	60	0.80	0.59-1.09	0.1533
Source of collection					
• Diarrhea surveillance	1875	556	1.00	0.87-1.14	1.00
• NCLE	1362	1424	3.53	3.13-3.98	<0.0001
• Nosocomial infection	41	57	4.69	3.10-7.08	<0.0001
• Referral from network laboratories	44	43	3.30	2.14-5.07	<0.0001
• Outbreak investigation	620	256	1.39	1.17-1.66	0.0002
Age (years)					
• ≤ 5	1322	419	1.00	0.86-1.17	1.00
• > 5	2620	1739	2.09	1.85-2.37	<0.0001
Sex					
• Male	2238	911	1.00	0.90-1.12	1.00
• Female	1704	1247	1.80	1.62-2.00	<0.0001
Year					
• 2012	730	542	1.00	0.85-1.17	1.00
• 2013	1133	522	0.62	0.53-0.72	<0.0001
• 2014	941	459	0.66	0.56-0.77	<0.0001
• 2015	1138	635	0.75	0.65-0.87	<0.0001

The prevalence of each bacterial infection during 2012 and 2015 is presented in **Table 4.4.** The numbers of positive bacterial infections in 2012, 2013, 2014, and 2015 were 542, 522, 459 and 635 samples, respectively**.** Of these, the highest prevalence of bacterial identification was mixed-infections **(**polymicrobial**).** In each year, the common pathogens detected were similar, ***i.e.,*** Bacterial vaginosis, *Salmonella* spp, *Staphylococcus aureus, Escherichia coli* and Coagulase Negative *Staphylococcus.*

Bacterial infection is caused by pathogenic bacteria which might be infected any area of the human body. Thus, different types of specimens selected were based on signs and symptoms. Suitable specimen is an important issue for diagnosis. Therefore, the bacterial identification is categorized based on the specimen as described below.

Table 4.4 Prevalence of bacteria identification among positive samples

	Number of specimen, % (n)			
	2012	**2013**	**2014**	**2015**
Acinetobacter spp	0.0 (0)	1.7 (9)	1.5 (7)	0.3 (2)
Aeromonas spp	2.2 (12)	2.5 (13)	4.8 (22)	3.3 (21)
Bacterial vaginosis	7.9 (43)	15.3 (80)	14.2 (65)	11.3 (72)
Burkholderia pseudomallei	0.2 (1)	0.6 (3)	2.2 (10)	0.2 (1)
Burkholderia spp	0.0 (0)	0.2 (1)	0.0 (0)	0.2 (1)
Campylobacter spp	0.0 (0)	0.4 (2)	0.0 (0)	0.0 (0)
Citrobacter spp	0.2 (1)	0.0 (0)	0.2 (1)	0.0 (0)
Coagulase Negative *Staphylococcus*	6.5 (35)	5.8 (30)	3.5 (16)	3.1 (20)
Corynebacterium diphtheriae	0.5 (3)	1.1 (6)	0.0 (0)	11.0 (70)
Corynebacterium spp	0.0 (0)	0.2 (1)	0.0 (0)	0.2 (1)
Enterobacter spp	0.7 (4)	2.9 (15)	1.3 (6)	1.1 (7)
Enterococcus spp	0.4 (2)	3.4 (18)	4.4 (20)	3.0 (19)
Escherichia coli	4.4 (24)	4.2 (22)	7.4 (34)	2.7 (17)
EHEC	3.7 (20)	0.0 (0)	0.0 (0)	0.0 (0)
EIEC	4.8 (26)	0.0 (0)	0.0 (0)	0.2 (1)
EPEC	10.7 (58)	0.0 (0)	0.0 (0)	2.7 (17)
ETEC.LT	1.7 (9)	0.0 (0)	0.0 (0)	0.9 (6)
ETEC.ST	2.6 (14)	0.0 (0)	0.0 (0)	0.0 (0)
Group A *Streptococcus*	1.3 (7)	1.3 (7)	0.9 (4)	3.8 (24)
Group B *Streptococcus*	0.9 (5)	1.1 (6)	1.3 (6)	1.3 (8)
Group D *Streptococcus*	1.1 (6)	0.4 (2)	0.2 (1)	0.3 (2)
Haemophilus spp	2.8 (15)	0.8 (4)	0.2 (1)	0.3 (2)
Klebsiella spp	0.4 (2)	1.9 (10)	1.5 (7)	1.7 (11)
Morganella spp	0.0 (0)	1.0 (5)	0.2 (1)	0.8 (5)
Neisseria gonorrhoeae	4.1 (22)	2.5 (13)	2.6 (12)	1.3 (8)
Pantoea spp	0.0 (0)	0.0 (0)	0.2 (1)	0.0 (0)
Plesiomonas shigelloides	2.2 (12)	3.4 (18)	3.1 (14)	1.6 (10)
Proteus spp	0.5 (3)	1.5 (8)	0.7 (3)	0.8 (5)
Pseudomonas spp	1.7 (9)	1.9 (10)	1.3 (6)	1.4 (9)
Salmonella spp	6.5 (35)	6.9 (36)	12.0 (55)	10.1 (64)
Serratia marcescens	0.0 (0)	0.2 (1)	0.4 (2)	0.0 (0)
Shigella spp	1.8 (10)	4.4 (23)	2.2 (10)	4.1 (26)
Staphylococcus aureus	5.2 (28)	9.0 (47)	8.1 (37)	6.8 (43)
Staphylococcus saprophyticus	0.0 (0)	0.2 (1)	0.0 (0)	0.2 (1)
Stenotrophomonas maltophilia	0.0 (0)	0.0 (0)	0.4 (2)	0.0 (0)
Streptococcus pneumoniae	0.9 (5)	0.8 (4)	0.2 (1)	0.6 (4)
Vibrio spp	0.7 (4)	1.0 (5)	2.2 (10)	0.5 (3)
Mixed infection	23.4 (127)	23.4 (122)	22.9 (105)	24.4 (155)
Total	100 (542)	100 (522)	100 (459)	100 (635)

The bacterial identification based on the type of specimens

4.2.1 Blood specimen

NCLE has started implementing blood culture since 2013 in the diarrhea surveillance network and expanded to regional laboratories in late of 2013**.** Specimen arriving NCLE were both blood culture bottles and isolates from positive blood culture from the network laboratories**.** Results are summarized in **Table 4.5**.

There was only one referral specimen from network laboratory received in 2012; the specimen was isolated of positive blood culture testing. The identification of referred specimen was *Vibrio cholerae* non O1/O139.

In 2013, seventy specimens were received from acute watery diarrhea surveillance, nosocomial infection, and outbreak investigation. There were 10% (n=7) of bacterial isolated; the most prevalence bacteria was *Salmonella* Typhi (5.8%, n=4). One sample each was identified as *Escherichia coli*, *Burkholderia pseudomallei,* and Coagulase Negative *Staphylococcus*.

In 2014, there were seventy-two specimens received. About 16.7*%* (n=12*)* specimens were bacteria positive, *i.e.*, 6.9% (n=5*)*, 6.9% (n=5*)*, 1.4*%* (n=1*)* and 1.4% (n=1) of *B. pseudomallei*, Coagulase Negative *Staphylococcus*, *Pseudomonas* spp and *S. pneumoniae*, respectively.

In 2015, there were fifty-nine specimens received. About 11.9% (n=7*)* specimens were positive for one of the following pathogens*:* *Salmonella* Typhi (n=5*)*, *Aeromonas* spp (n=1*)* and *E. coli* (n=1*).*

4.2.2 Stool and rectal swab

Stool or rectal swabs were received in transport media. Stool or rectal swab culture was the highest portion of specimens received in all of the four years. 712, 904, 819 and 864 stool and rectal swab specimens were collected in 2012, 2013, 2014 and 2015, respectively, from acute watery diarrhea **(**AWD**)** surveillance program in Vientiane capital, diarrhea and/or food poisoning outbreak investigation and public, and private health facilities**.** Results are summarized in **Table 4.6**.

The highest prevalence of infections with all ages in 2012 were pathogenic *E.coli* **(**n=127, 17.9**%)**, mixed infection with more than 1 species (n=82, 11.5%) and *Salmonella* spp (n=35, 4.9%).

In 2013, the highest prevalence of identified pathogens with all ages were Other infections (n=158, 17.4%), *Salmonella spp* (n= 32, 3.5%), *Shigella spp* (n=23, 2.5%) and *Plesiomonas shigelloides* (n=18, 2.0%). None of pathogenic *E.coli* was detected by PCR.

In 2014, 821 stool/rectal swab specimens were received, only 819 specimens were analyzed and 2 specimens were excluded due to incomplete data collection. Other infections were the highest pathogen (19.6%). *Salmonella* spp was the highest bacterial pathogen (6.7%) of total specimens received.

In 2015, 864 stool/rectal swab specimens were received. Three hundreds and sixty-two specimens (41.9%) contained one of the suspected pathogen. Other infections (n=177, 20.5%) was the highest enteric pathogen. *Salmonella* spp (n=59, 6.8%) was the highest bacterial pathogen of total specimens received.

Table 4.5: Bacterial pathogen identified from blood specimen (Hemoculture)

	Number of specimen % (n)			
	2012	**2013**	**2014**	**2015**
No infection	0 (0)	90 (63)	83.3 (60)	88.1 (52)
Mono-infection	100 (1)	10 (7)	16.7 (12)	11.9 (7)
• *Aeromonas* spp	0 (0)	0 (0)	0 (0)	1.7 (1)
• *B. pseudomallei*	0 (0)	1.4 (1)	6.9 (5)	0 (0)
• Coagulase Negative *Staphylococcus*	0 (0)	1.4 (1)	6.9 (5)	0 (0)
• *E. coli*	0 (0)	1.4 (1)	0 (0)	1.7 (1)
• *Pseudomonas* spp	0 (0)	0 (0)	1.4 (1)	0 (0)
• *Salmonella* spp	0 (0)	5.8 (4)	0 (0)	8.5 (5)
• *S. pneumoniae*	0 (0)	0 (0)	1.4 (1)	0 (0)
• *Vibrio* spp	100 (1)	0 (0)	0 (0)	0 (0)
Total	**100 (1)**	**100 (70)**	**100 (72)**	**100 (59)**

Table 4.6 : Bacterial pathogen identified from stool and rectal swab specimens

	Number of specimen % (n)			
	2012	**2013**	**2014**	**2015**
No infection	60.0 (427)	70.6 (638)	65.7 (538)	58.1 (502)
Mono-infection	28.0 (199)	10.3(93)	13.4 (110)	16.2 (140)
• *Aeromonas* spp	1.7 (12)	1.4 (13)	2.7 (22)	2.2 (19)
• *Campylobacter* spp	0.0 (0)	0.2 (2)	0.0 (0)	0.0 (0)
• EHEC	2.8 (20)	0.0 (0)	0.0 (0)	0.0 (0)
• EIEC	3.7 (26)	0.0 (0)	0.0 (0)	0.1 (1)
• EPEC	8.1 (58)	0.0 (0)	0.0 (0)	1.9 (16)
• ETEC.LT	1.3 (9)	0.0 (0)	0.0 (0)	0.7 (6)
• ETEC.ST	2.0 (14)	0.0 (0)	0.0 (0)	0.0 (0)
• *Plesiomonas shigelloides*	1.7 (12)	2.0 (18)	1.6 (13)	1.2 (10)
• *Salmonella* spp	4.9 (35)	3.5 (32)	6.7 (55)	6.8 (59)
• *Shigella* spp	1.4 (10)	2.5 (23)	1.2 (10)	3.0 (26)
• *Vibrio* spp	0.4 (3)	0.6 (5)	1.2 (10)	0.3 (3)
Mixed infection *	11.5 (82)	1.7 (15)	1.3 (11)	5.2 (45)
Other infections	0.5 (4)	17.4 (158)	19.6 (160)	20.5 (177)
Total	100 (712)	100 (904)	100 (819)	100 (864)

* Mixed infection list of stool culture show in Appendix A

4.2.3 Pus swab

Pus swab specimens were collected from various sites of body such as opened wound from skin whether area of leg, arm, back or swabs from specific system likes ear and eye. Specimen will be culture and identified based on sites of specimen collected. Specimens were received from referral specimens from clinics and hospitals, nosocomial infection and outbreak investigation. Results are summarized in **Table 4.7**.

In 2012, 104 pus specimens were received. There was 1 specimen rejected and requested for new specimen collection. One possible pathogen was detected in pus specimen of the 73 (70.9%) out of 103 specimens. *Staphylococcus aureus*, Coagulase Negative *Staphylococcus,* Group A *Streptococcus* and *Pseudomonas* spp were the highest prevalence with 27 (26.0%), 19 (18.2%), 5 (4.8%) and 5 (4.8%) cases, respectively.

In 2013, there were 172 pus specimens received from outpatient, anthrax outbreak investigation and nosocomial infection surveillance program. One possible pathogen was found in 116 out of 172 specimens (67.4%) of any possible pathogens. The most common pathogen isolated was *S. aureus* (n=43, 25.0%), mixed infection (n=18, 10.4%) and Coagulase Negative *Staphylococcus* (n=9, 5.2%).

In 2014, there were 151 specimens received. *S. aureus*, mixed of pathogens more than 1 organism and *E. coli* were found to be the highest infections (23.2%, 13.2% and 6.6%, respectively).

In 2015, there were 160 specimens received. *S. aureus* (n=41, 25.6%) and mixed infection of pathogens more than 1 organism (n=19, 11.9%) were found to be the highest infection respectively.

Table 4.7: Bacterial pathogen identified from pus specimens

	Number of specimen % (n)			
	2012	**2013**	**2014**	**2015**
No infection	28.8 (30)	32.4 (56)	32.2 (49)	32.5 (52)
Mono-infection	64.5(67)	55.5 (96)	52.0 (79)	55.0 (88)
• *Acinetobacter* spp	0.0 (0)	2.3 (4)	3.3 (5)	0.0 (0)
• *Aeromonas* spp	0.0 (0)	0.0 (0)	0.0 (0)	0.6 (1)
• *B. pseudomallei*	1.0 (1)	1.2 (2)	2.6 (4)	0.6 (1)
• *Burkholderia* spp	0.0 (0)	0.6 (1)	0.0 (0)	0.6 (1)
• *Citrobacter* spp	1.0 (1)	0.0 (0)	0.7 (1)	0.0 (0)
• Coagulase Negative *Staphylococcus*	18.2 (19)	5.2 (9)	2.0 (3)	4.4 (7)
• *Corynebacterium* spp	0.0 (0)	0.6 (1)	0.0 (0)	0.6 (1)
• *Enterobacter* spp	1.0 (1)	4.0 (7)	0.7 (1)	2.5 (4)
• *Enterococcus* spp	0.0 (0)	1.2 (2)	1.3 (2)	2.5 (4)
• *Escherichia coli*	2.8 (3)	2.3 (4)	6.6 (10)	3.8 (6)
• *Group A Streptococcus*	4.8 (5)	2.3 (4)	1.3 (2)	4.4 (7)
• *Group D Streptococcus*	1.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)
• *Haemophilus* spp	0.0 (0)	0.0 (0)	0.0 (0)	0.6 (1)
• *Klebsiella* spp	1.0 (1)	1.2 (2)	3.3 (5)	2.5 (4)
• *Morganella* spp	0.0 (0)	0.6 (1)	0.0 (0)	0.0 (0)
• *Neisseria gonorrhoeae*	0.0 (0)	0.6 (1)	0.0 (0)	0.0 (0)
• *Pantoea* spp	0.0 (0)	0.0 (0)	0.7 (1)	0.0 (0)
• *Plesiomonas shigelloides*	0.0 (0)	0.0 (0)	0.7 (1)	0.0 (0)
• *Proteus* spp	1.0 (1)	3.5 (6)	1.3 (2)	1.9 (3)
• *Pseudomonas* spp	4.8 (5)	4.6 (8)	2.0 (3)	4.4 (7)
• *Serratia marcescens*	0.0 (0)	0.6 (1)	1.3 (2)	0.0 (0)
• *Staphylococcus aureus*	26.0 (27)	24.9 (43)	23.0 (35)	25.6 (41)
• *Staphylococcus saprophyticus*	0.0 (0)	0.0 (0)	1.3 (2)	0.0 (0)
• *Streptococcus pneumoniae*	1.9 (2)	0.0 (0)	0.0 (0)	0.0 (0)
Mixed infection*	3.8 (4)	10.4 (18)	13.1 (20)	11.9 (19)
Other infections	1.9 (2)	1.7 (3)	2.0 (3)	0.6 (1)
New sample required	1.0 (1)	0.0 (0)	0.7 (1)	0.0 (0)
Total	100 (104)	100 (173)	100 (152)	100 (160)

* Mixed infection list of pus culture show in Appendix B

4.2.4 *Throat swab and respiratory specimen*

Respiratory specimen or "Throat swab" specimen was including throat swab, nasal swab, mouth cavity and sputum**.** The prevalence of specimen types and pathogens identified among each year were depended on Lao PDR situation; for exemple; the most prevalence specimen collection in 2012 was sputum and throat swab, where as there was an outbeak of diphtheria in 2015. Results are summarized in **Table 4.8**.

In 2012, sputum **(**lower respiratory tract specimen**)** and throat (upper respiratory tract) specimens were collected**.** The highest prevalence of identified pathogens from sputum specimens were other infections and *Haemophilus* spp. And the highest prevalence of identified pathogens from upper respiratory tract specimens were other infections, *Corynebacterium diphtheriae* and *Haemophilus* spp.

In 2013, 157 specimens were received with 3 specimens rejected due to inappropriate storage condition during transportation. The most common identified pathogens were other infections (n=8, 5.1%), *Corynebacterium diphtheriae* (n=6, 3.8%) and *Haemophilus* spp (n=4, 2.5%).

In 2014, there were 38 specimens received. Only three pathogens were identified as follow: other infections (n=2, 5.3%), Group A *Streptococcus* (n=2, 5.3%) and *Staphylococcus aureus* (n=1, 2.6%).

In 2015, 340 respiratory specimens were received from clinical specimen and outbreak investigation**.** The highest prevalence of identified pathogens were *C***.** *diphtheriae* **(**n=70, 20**.**6**%)**, Group A *Streptococcus* **(**n=17, 5**.**0**%)** and other infections (n=13, 3.8%).

Table 4.8 Bacterial pathogen identified from throat swab, nasal swab, mouth cavity and sputum specimens

	Number of specimen % (n)			
	2012	**2013**	**2014**	**2015**
No infection	64.4 (96)	75.8 (119)	86.8 (33)	67.6 (230)
Mono-infection	17.5 (26)	16.6 (26)	7.9 (3)	27.4 (93)
• *Acinetobacter* spp	0.0 (0)	1.3 (2)	0.0 (0)	0.0 (0)
• Coagulase Negative *Staphylococcus*	0.7 (1)	1.3 (2)	0.0 (0)	0.0 (0)
• *Corynebacterium diphtheriae*	2.0 (3)	3.8 (6)	0.0 (0)	20.6 (70)
• *Enterobacter* spp	0.7 (1)	0.0 (0)	0.0 (0)	0.0 (0)
• *Escherichia coli*	0.7 (1)	0.6 (1)	0.0 (0)	0.0 (0)
• Group A Streptococcus	0.7 (1)	1.9 (3)	5.3 (2)	5.0 (17)
• Group B Streptococcus	0.0 (0)	0.0 (0)	0.0 (0)	0.3 (1)
• *Haemophilus* spp	9.4 (14)	2.5 (4)	0.0 (0)	0.0 (0)
• *Klebsiella* spp	0.0 (0)	0.6 (1)	0.0 (0)	0.3 (1)
• *Pseudomonas* spp	1.4 (2)	1.3 (2)	0.0 (0)	0.0 (0)
• *Staphylococcus aureus*	0.0 (0)	0.6 (1)	2.6 (1)	0.0 (0)
• *Streptococcus pneumoniae*	2.0 (3)	2.5 (4)	0.0 (0)	1.2 (4)
Mixed infection	1.4 (2)	0.6 (1)	0.0 (0)	1.2 (4)
• *Haemophilus spp + Candida* spp	(1)	(0)	(0)	(0)
• *Klebsiella spp + Candida* spp	(1)	(1)	(0)	(0)
• *Klebsiella* spp + Coagulase Negative *Staphylococcus*	(0)	(0)	(0)	(0)
• *Corynebacterium diphtheriae + Candida* spp	(0)	(0)	(0)	(1)
• *Corynebacterium diphtheriae* + Group A *Streptococcus*	(0)	(0)	(0)	(1)
• *Corynebacterium diphtheriae + Haemophilus* spp	(0)	(0)	(0)	(1)
• *Corynebacterium diphtheriae + Staphylococcus aureus*	(0)	(0)	(0)	(1)
Other infections	16.7 (25)	5.1 (8)	5.3 (2)	3.8 (13)
New sample required	0.0 (0)	1.9 (3)	0.0 (0)	0.0 (0)
Total	100 (149)	100 (157)	100 (38)	100 (340)

4.2.5 *Urethral discharge*

Urethral discharge was collected at NCLE and then immediately incoculated on culture media for increasing chance of identified pathogens**.** The pathogen identification was focused on genital tract pathogens and predominant growth organisms**.** *N. gonorrhoeae* was the most prevalence-identified organism rather than Coagulase Negative *Staphylococcus*, which might be contaminated from skin flora during collected the specimen**.** Results are summarized in **Table 4.9**.

In 2012, fifty specimens were collected. About twenty-three specimens were identified pathogens. The highest prevalence of identified pathogens were Coagulase Negative *Staphylococcus* (n=7, 14.0%), *N. gonorrhoeae* (n=6, 12.0%) and other infections (n=5, 10.0%), respectively.

In 2013, fifty-two specimens were collected from refered patients among network clinics and hospitals. There were 55.8% (n=29) of pathogens identified. The most prevalence pathogens were Coagulase Negative *Staphylococcus* (n=10, 19.2%), *N. gonorrhoeae* (n=5, 9.6%), *Escherichia coli* (n=4, 7.7%) and other infections (n=4, 7.7%).

In 2014, there were fifty-eight specimens received. About 26 specimens were at least one pathogen identified. The highest prevalence of idenfied pathogens were *N.gonorrhoeae* (n=7, 12.1%) and *Enterococcus spp* (n=7, 12.1%).

In 2015, 65 specimens were received. About 57.0% (n=37) specimens were bacterial positive; *i.e.*, Coagulase Negative *Staphylococcus* (n=10, 15.4%), *N. gonorrhoeae* (n=7, 10.8%) and *Enterococcus spp* (n=5, 7.7%).

Table 4.9 Bacteria pathogen identified from urethral discharge specimens

	Number of specimen % (n)			
	2012	**2013**	**2014**	**2015**
No infection	54.0 (27)	44.2 (23)	55.2 (32)	43.0 (28)
Mono-infection	36.0 (18)	46.2 (24)	39.7 (23)	50.8 (33)
• *Acinetobacter* spp	0.0 (0)	0.0 (0)	0.0 (0)	3.1 (2)
• Coagulase Negative *Staphylococcus*	14.0 (7)	19.2 (10)	1.7 (1)	15.4 (10)
• *Enterobacter* spp	0.0 (0)	0.0 (0)	5.2 (3)	1.5 (1)
• *Enterococcus* spp	2.0 (1)	1.9 (1)	12.1 (7)	7.7 (5)
• *Escherichia coli*	4.0 (2)	7.7 (4)	3.4 (2)	1.5 (1)
• Group B *Streptococcus*	0.0 (0)	0.0 (0)	0.0 (0)	1.5 (1)
• Group D *Streptococcus*	4.0 (2)	0.0 (0)	0.0 (0)	0.0 (0)
• *Haemophilus* spp	0.0 (0)	0.0 (0)	1.7 (1)	1.5 (1)
• *Klebsiella* spp	0.0 (0)	1.9 (1)	0.0 (0)	3.1 (2)
• *Morganella* spp	0.0 (0)	1.9 (1)	1.7 (1)	1.5 (1)
• *Neisseria gonorrhoeae*	12.0 (6)	7.7 (4)	12.1 (7)	10.8 (7)
• *Proteus* spp	0.0 (0)	1.9 (1)	0.0 (0)	0.0 (0)
• *Staphylococcus aureus*	0.0 (0)	3.8 (2)	1.7 (1)	1.5 (1)
• *Staphylococcus saprophyticus*	0.0 (0)	0.0 (0)	0.0 (0)	1.5 (1)
Mix-infection	0.0 (0)	1.9 (1)	1.7 (1)	3.1 (2)
• *Klebsiella* spp + *Enterobacter* spp + *Haemophilus* spp	(0)	(1)	(0)	(0)
• *Enterobacter* spp + *Candida* spp	(0)	(0)	(1)	(0)
• Coagulase Negative *Staphylococcus* + *Candida* spp	(0)	(0)	(0)	(1)
• *E. coli* + *Klebsiella* spp	(0)	(0)	(0)	(1)
Other infections	10.0 (5)	7.7 (4)	3.4 (2)	3.1 (2)
Total	100 (50)	100 (52)	100 (58)	100 (65)

4.2.6 *Vaginal discharge*

Vaginal discharge was collected at NCLE and then immediately incoculated on culture media as urethral discharge**.** Vaginal discharge specimens were collected from vaginal and cervical areas. The specimens collected from vaginal area were used for gram stain, wet preparation and bacterial culture. Morphology examination under microscropic was interpreted as Nugent score based on types of bacterial morphologies, number of white blood cells and yeast cells**.** Wet preparation was examined for *Trichomonas spp* infection**.** While the specimens collected from cervical area were only tested for bacteria culture. Results are summarized in **Table 4.10.**

In 2012, 197 vaginal discharge specimens were received. One possible pathogen was detected in specimen of the 144 (73.1%) out of 197 specimens. The most common identified pathogens were Bacterial vaginosis (n= 43, 21.8%), mixed infection (n=38, 19.3%) and other infections (n=29, 14.7%). There were 16 (8.2%) of *N. gonorrhoeae* infection.

There were 287 vaginal discharge specimens received in 2013. One possible pathogen was found in 241 out of 287 (74.4%) specimens. The most prevalence identified pathogens were Bacterial vaginosis (n=65, 30.2%), mixed infection (n=61, 28.4%) and other infections (n=37, 12.9%). Eight (2.8%) *N. gonorrhoeae* infections were observed in this year.

In 2014, there were 215 vaginal discharge specimens with 160 (84.0%) specimens found one possible pathogen. The highest prevalence pathogens were mixed infection (n=84, 29.3%), Bacterial vaginosis (n=80, 27.9%) and other infections (n=17, 7.9%). There were five (2.3%) of *N. gonorrhoeae* infections identified in this year.

In 2015, 228 vaginal discharge specimens were received. 178 out of 228 (78.1%) specimens were found one possible pathogen. The most common pathogens were mixed infection (n=73, 32.0%), Bacterial vaginosis (n= 72, 31.6%) and other infections (n=21, 9.2%). There was no *N. gonorrhoeae* infection in this year.

4.2.7 *Urine culture*

Urine specimens were collected at NCLE from patients who were urinary tract infection or genital tract infection**.** If the patient could not provide a specimen within the time requested or patients were admitted in the hospital, then specimen was sent to the laboratory within 4 hours after collection.

There were 128 urine specimens collected from patients who were suspected of urinary tract infection in 2012. There was 1 specimen requested for new specimen collection due to suspected contaminant. All urine specimens were identified pathogens that infected both urinary tract and genital tract (*N. gonorrhoeae*). One possible pathogen was detected in urine specimen of the 31 (23.4%) out of 128 specimens (**Table 4.11**). The highest prevalence isolated for both genders was *E. coli* (n=10, 7.8%). However gonococcal was not found in urine specimen.

In 2013, there were 228 urine specimens collected from patients who were suspected of urinary tract infection and 3 specimens were excluded due to suspected contamination. One or more possible pathogens were found in 38 out of 228 specimens (16.67%). The highest prevalence of identified pathogens with all gender were Coagulase Negative *Staphylococcus* (n=6, 2.6%), *Enterococcus spp* (n=6, 2.6%) and *Enterobacter spp* (n=5, 2.2%).

In 2014, there were 233 urine specimens received and 3 specimens were required for new specimen collection due to suspected contamination. About 56 (24.0%) out of 233 specimens were bacterial positive. The most common pathogens for both genders were *E. coli* (n=19, 8.2%) and mixed infection (n=12, 5.1%).

In 2015, there were 269 specimens received. There were 47 out of 269 specimens (17.5%) found one or more possible pathogens. The most common pathogens with both genders were mixed infection (n= 12, 4.46%), *Enterococcus spp* (n=7, 2.6%) and *E. coli* (n=7, 2.6%).

Table 4.10 Bacterial pathogen identified from vaginal discharge specimens

	Number of specimen % (n)			
	2012	**2013**	**2014**	**2015**
No infection	26.9 (53)	16.0 (46)	25.6 (55)	21.9 (50)
Mono-infection	39.1 (77)	41.5 (119)	38.1 (82)	36.9 (84)
• Bacterial vaginosis	21.8 (43)	27.9 (80)	30.2 (65)	31.6 (72)
• Coagulase Negative *Staphylococcus*	0.5 (1)	0.7 (2)	0.0 (0)	0.0 (0)
• *Enterobacter* spp	0.0 (0)	1.0 (3)	0.0 (0)	0.0 (0)
• *Enterococcus* spp	0.5 (1)	2.8 (8)	1.9 (4)	1.8 (4)
• *Escherichia coli*	4.1 (8)	2.8 (8)	1.4 (3)	0.9 (2)
• Group B *Streptococcus*	2.0 (4)	1.7 (5)	2.3 (5)	1.8 (4)
• Group D *Streptococcus*	0.5 (1)	0.0 (0)	0.0 (0)	0.4 (1)
• *Haemophilus* spp	0.5 (1)	0.0 (0)	0.0 (0)	0.0 (0)
• *Klebsiella* spp	0.5 (1)	1.4 (4)	0.0 (0)	0.4 (1)
• *Neisseria gonorrhoeae*	8.2 (16)	2.8 (8)	2.3 (5)	0.0 (0)
• *Proteus* spp	0.5 (1)	0.0 (0)	0.0 (0)	0.0 (0)
• *Staphylococcus saprophyticus*	0.0 (0)	0.4 (1)	0.0 (0)	0.0 (0)
Mixed infection *	19.3 (38)	29.6 (84)	28.4 (61)	32.0 (73)
Other infections	14.7 (29)	12.9 (37)	7.9 (17)	9.2 (21)
Total	100 (197)	100 (287)	100 (215)	100 (228)

* Mixed infection list of pus culture show in Appendix C

Table 4.11 Bacterial pathogen identified from urine specimens

	Number of specimen % (n)			
	2012	**2013**	**2014**	**2015**
No infection	75.7 (97)	82.0 (187)	74.7 (174)	82.8 (222)
Mono-infection	21.1 (27)	14.9 (34)	18.9 (44)	12.7 (34)
• *Acinetobacter* spp	0.0 (0)	1.3 (3)	0.9 (2)	0.0 (0)
• Coagulase Negative *Staphylococcus*	5.5 (7)	2.6 (6)	3.0 (7)	1.1 (3)
• *Enterobacter* spp	1.6 (2)	2.2 (5)	0.9 (2)	0.7 (2)
• *Enterococcus* spp	0.0 (0)	2.6 (6)	3.0 (7)	2.2 (6)
• *Escherichia coli*	7.8 (10)	1.8 (4)	8.2 (19)	2.6 (7)
• Group A *Streptococcus*	0.8 (1)	0.0 (0)	0.0 (0)	0.0 (0)
• Group B *Streptococcus*	0.8 (1)	0.4 (1)	0.4 (1)	0.7 (2)
• Group D *Streptococcus*	1.6 (2)	0.9 (2)	0.4 (1)	0.4 (1)
• *Klebsiella* spp	0.0 (0)	0.9 (2)	0.9 (2)	1.1 (3)
• *Morganella* spp	0.0 (0)	1.3 (3)	0.0 (0)	1.5 (4)
• *Neisseria gonorrhoeae*	0.0 (0)	0.0 (0)	0.0 (0)	0.4 (1)
• *Proteus* spp	0.8 (1)	0.4 (1)	0.4 (1)	0.7 (2)
• *Pseudomonas* spp	1.6 (2)	0.0 (0)	0.9 (2)	0.7 (2)
• *Staphylococcus aureus*	0.8 (1)	0.4 (1)	0.0 (0)	0.4 (1)
Mixed infection *	0.8 (1)	0.9 (2)	5.1 (12)	4.5 (12)
Other infections	1.6 (2)	0.9 (2)	0.0 (0)	0.0 (0)
New sample required	0.8 (1)	1.3 (3)	1.3 (3)	0.0 (0)
Total	100 (128)	100 (228)	100 (233)	100 (269)

* Mixed infection list of pus culture show in Appendix D

4.3 Antimicrobial susceptibility

Susceptibility testing of the 11 selected pathogens to appropriate antimicrobial agents; such as ampicillin (AM) 10 μg, amoxicillin-clavulanate (AMC) 20/10 μg, amikacin (AN) 30 μg, chloramphenicol (C) 30 μg, ceftazidime (CAZ) 30 μg, ciprofloxacin (CIP) 5 μg, ceftriaxone (CRO) 30 μg, erythromycin (E) 15 μg, cefoxitin (FOX) 30 μg, gentamicin (GM) 10 μg, nalidixic acid (NAL) 30 μg, ofloxacin (OFX) 5 μg, penicillin (PE) 10 units, trimethoprim-sulfamethoxazole (SXT) 1.25/23.75 μg and tetracycline (TE) 30 μg was carried out by the Kirby-Bauer disk diffusion method. The susceptibility results were categorized to resistance (R), susceptible (S) and intermediate (I) by using the cut off values of each drug according to the CLSI guideline. The percentage of resistant to each antimicrobial agent was calculated which classified among year, and the degree year contributed to resistance was evaluated by using an odd ratio (OR).

4.3.1 ***Aeromonas* spp**

There were twenty-one, fourteen, twenty-seven and twenty-nine of *Aeromonas* spp isolates selected to perform antimicrobial susceptibility in 2012, 2013, 2014 and 2015, respectively. Seven antimicrobial agents were tested against *Aeromonas* spp *i.e.* AMC, C, CIP, CRO, GM, SXT and TE. The susceptibility results are summarized in **Table 4.12** and **Figure 4.1**. The resistant prevalence of AMC in 2015 was the highest with 67.9%, thus year 2015 was statistically significantly 15.2 times associated with resistant prevalence compared to 2012 (p=0.004). Other antimicrobial susceptibility results were showed the low resistant prevalence.

4.3.2 **Coagulase Negative *Staphylococcus***

The susceptibility test of Coagulase Negative *Staphylococcus* was performed in 2012 to 2015, which were 32, 30, 16 and 20 isolates, respectively. There were eight antimicrobial agents tested with this pathogen; AMC, AM, C, CIP, E, GM, SXT and TE. The results are summerized in **Table 4.13** and **Figure 4.2.**

In 2013, there were statistically significant increased odd ratio of AM (OR=12.5, p=0.026) and TE (OR=2.9, p=0.015) compared to 2012. Whereas in 2015, there were statistically significant higher odd ratio of C (OR=3.7; p=0.037) and GM (OR=3.7; p=0.039) drugs than 2012**.** The prevalence of resistance to C, CIP and E in

2015 were increased compare to 2013, which were statistically significant higher odd ratio about 4.1 (p = 0.027), 6.5 (p = 0.022) and 8.5 (p = 0.03), respectively. There were increasing trend of most antibiotic showed in **Figure 4.2.**

4.3.3 ***Enterococcus* spp**

About 26, 26 and 32 bacterial isolates were successful susceptibility testing in 2013, 2014 and 2015, respectively**.** Six antimicrobial agents; *i.e.* AM, C, CIP, E, PE and TE were tested against *Enterococcus* spp**.** The results are summarized in **Table 4.14** and **Figure 4.3**. The prevalence of C was increased from 37.5% in 2013 to 65.2% in 2015. And the resistance rate of E was remained stable with higher than 70%. The prevalence of resistance of AM and PE was showed decreasing trend with 0% in 2015. In year 2014, the PE resistance was 0.2 times lower than 2013, which was statistically significant (p=0.012)**.** Although the odd ratio of PE in 2015 could not calculated, but there was statistically signification of resistant prevalence when compared to 2013 (p <0.001).

4.3.4 ***Escherichia coli***

There were 29, 35, 49 and 32 bacterial isolates were successful drug susceptibility testing in 2012, 2013, 2014 and 2015, respectively**.** There were nine antimicrobrial agents used in drug susceptibility tests; AMC, AM, C, CIP, CRO, GM, NAL, SXT and TE. The results of pecent resistace to each drug are summarized in **Table 4.15** and **Figure 4.4**.

The overall susceptibility profiles of this organism were varied from 30 to 90%. The hightest resistant prevalence with all years were AM (78-90%), SXT (64-80%) and TE (68-89%).

Resistant prevalence of NAL with all years were approximately 50%. In 2013, the rate was increased to 70% which the odd of resistance in 2013 was twice that of resistance in 2012 (OR=2.8), and was statistically significant (p= 0.049).

The resistant prevalence of CRO, which was one of the screening indicators for ESBL-producing ***E. coli*** were varied from 32 to 54%.

Table 4.12 The percent resistance of *Aeromonas* spp to antimicrobial drugs in 2012 to 2015

Drug	Year	Percent Resistance	Odd ratio[a] (95% CI)	Odd ratio[b] (95% CI)	Odd ratio[c] (95% CI)
AMC	2012	35.7	-	-	-
	2013	50.0	5.6 (0.8-38.5)	-	-
	2014	46.2	2.7 (0.6-11.8)	0.9 (0.2-3.2)	-
	2015	67.9	**15.2 (2.4-95.3)***	2.1 (0.6-7.7)	2.5 (0.8-7.4)
C	2012	9.5	-	-	-
	2013	7.1	0.8 (0.1-9.7)	-	-
	2014	8.0	0.9 (0.1-7.1)	1.1 (0.1-13.7)	-
	2015	13.8	2.2 (0.4-13.2)	2.1 (0.2-20.6)	1.8 (0.3-11.0)
CIP	2012	9.5	-	-	-
	2013	7.1	0.70.06-8.92	-	-
	2014	0.0	-	-	-
	2015	6.9	0.7 (0.1-5.4)	1.0 (0.1-11.6)	-
CRO	2012	0.0	-	-	-
	2013	7.7	-	-	-
	2014	7.7	-	1.0 (0.1-12.2)	-
	2015	14.8	-	1.9 (0.2-19.1)	1.9 (0.3-11.5)
GM	2012	0.0	-	-	-
	2013	7.1	-	-	-
	2014	0.0	-	-	-
	2015	7.7	-	1.1 (0.1-13.1)	-
SXT	2012	16.7	-	-	-
	2013	21.4	1.1 (0.2-6.6)	-	-
	2014	18.5	0.9 (0.2-4.5)	0.8 (0.2-4.1)	-
	2015	29.6	1.7 (0.4-7.6)	1.5 (0.3-7.1)	1.9 (0.5-6.6)
TE	2012	26.3	-	-	-
	2013	28.6	1.0 (0.2-4.9)	-	-
	2014	11.1	0.4 (0.1-2.0)	0.3 (0.1-1.7)	-
	2015	10.7	0.5 (0.1-2.6)	0.3 (0.1-1.6)	1.0 (0.2-5.2)

[a]Odd ratio of drug resistance compared to year 2012, [b]compared to year 2013 and [c] compared to year 2014

[*]In year 2015, the resistance of AMC was statistically significant ($p = 0.004$) compared to 2012

Table 4.13 The percent resistance of Coagulase Negative *Staphylococcus* to antimicrobial drugs in 2012 to 2015

Drug	Year	Percent Resistance	Odd ratio[a] (95% CI)	Odd ratio[b] (95% CI)	Odd ratio[c] (95% CI)
AMC	2012	6.3	-	-	-
	2013	3.4	0.5 (0.1-6.2)	-	-
	2014	6.3	1.0 (0.1-11.9)	1.9 (0.1-32.0)	-
	2015	21.1	4.0 (0.7-24.4)	7.5 (0.8-72.9)	4.0 (0.4-40.1)
AM	2012	66.7	-	-	-
	2013	96.2	**12.5 (0.9-172.1)***	-	-
	2014	86.7	3.3 (0.3-31.1)	0.3 (0.0-3.1)	-
	2015	94.7	9.0 (0.7-125.3)	0.7 (0.0-12.3)	2.8 (0.2-33.9)
C	2012	43.3	-	-	-
	2013	40.7	0.9 (0.3-2.6)	-	-
	2014	46.7	1.1 (0.3-4.0)	1.3 (0.4-4.5)	-
	2015	73.7	**3.7 (1.1-12.8)***	**4.1 (1.1-14.6)****	3.2 (0.8-13.5)
CIP	2012	32.3	-	-	-
	2013	7.1	**0.2 (0.0-0.9)***	-	-
	2014	18.8	0.6 (0.1-2.9)	3.0 (0.4-20.2)	-
	2015	33.3	1.1 (0.3-4.1)	**6.5 (1.1-37.1)****	2.2 (0.4-10.7)
E	2012	22.0	-	-	-
	2013	16.0	0.7 (0.2-2.4)	-	-
	2014	9.0	0.8 (0.2-3.4)	1.1 (0.3-4.8)	-
	2015	17.0	6.2 (0.7-54.3)	**8.5 (1.0-75.8)****	7.6 (0.7-78.1)
GM	2012	22.6	-	-	-
	2013	20.7	0.9 (0.3-2.9)	-	-
	2014	33.3	1.8 (0.5-7.3)	1.9 (0.5-7.8)	-
	2015	47.4	**3.7 (1.0-13.2)***	3.5 (1.0-12.3)	1.8 (0.4-7.3)
SXT	2012	47.4	-	-	-
	2013	39.3	0.8 (0.2-2.5)	-	-
	2014	33.3	0.6 (0.1-2.5)	0.8 (0.2-3.2)	-
	2015	31.6	0.5 (0.1-1.9)	0.7 (0.2-2.4)	0.9 (0.2-4.3)
TE	2012	60.0	-	-	-
	2013	81.5	**2.9 (0.9-9.9)***	-	-
	2014	68.8	1.5 (0.4-5.3)	0.5 (0.1-2.1)	-
	2015	73.7	1.9 (0.5-6.6)	0.6 (0.2-2.6)	1.3 (0.3-5.5)

[a]Odd ratio of drug resistance compared to year 2012, [b]compared to year 2013 and [c] compared to year 2014

*Compare to year 2012: the resistance of AM in 2013 (p = 0.026), C in 2015 (p = 0.037), CIP in 2013 (p = 0.019), GM in 2015 (p = 0.039) and TE in 2013 (p = 0.015) were statistically significant.

**Compare to year 2013: the resistance of C in 2015 (p = 0.027), CIP in 2015 (p = 0.022) and E in 2015 (p = 0.03) were statistically significant.

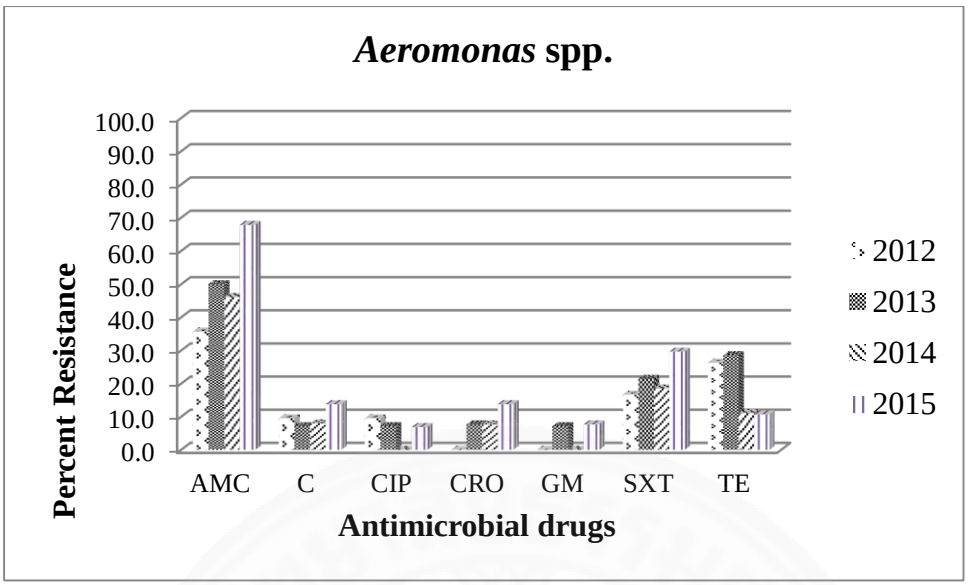

Figure 4.1 The percent resistance of *Aeromonas* spp to antimicrobial drugs in 2012 to 2015

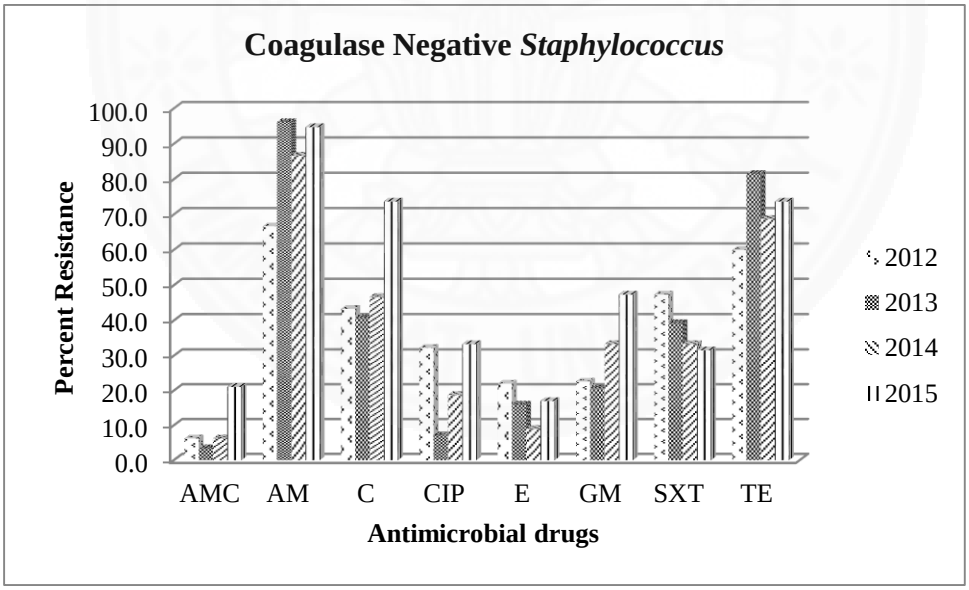

Figure 4.2 The percent resistance of Coagulase Negative *Staphylococcus* to antimicrobial drugs in 2012 to 2015

Table 4.14 The percent resistance of *Enterococcus* spp to antimicrobial drugs in 2013 to 2015

Drug	Year	Percent Resistance	Odd ratio[a] (95% CI)	Odd ratio[b] (95% CI)
AM	2013	11.5	-	-
	2014	4.2	0.3 (0.0-3.5)	-
	2015	0.0	-	-
C	2013	37.5	-	-
	2014	47.8	1.3 (0.4-4.4)	-
	2015	65.2	2.9 (0.8-9.9)	2.0 (0.6-6.7)
CIP	2013	35.1	-	-
	2014	35.7	5.0 (0.6-51.8)	-
	2015	30.0	3.0 (0.36-34.6)	0.8 (0.1-4.4)
E	2013	76.2	-	-
	2014	71.4	3.8 (0.4-37.5)	-
	2015	87.0	5.0 (0.5-49.3)	2.7 (0.6-12.4)
PE	2013	47.6	-	-
	2014	13.0	**0.2 (0.0-0.7)***	-
	2015	0.0	-	-
TE	2013	90.5	-	-
	2014	77.3	0.4 (0.1-2.1)	-
	2015	80.0	0.4 (0.1-2.9)	1.2 (0.2-5.9)

[a]Odd ratio of drug resistance compared to year 2013, and [b]compared to year 2014

*Compare to year 2013: the resistance of PE in 2014 was statistically significant (p = 0.012)

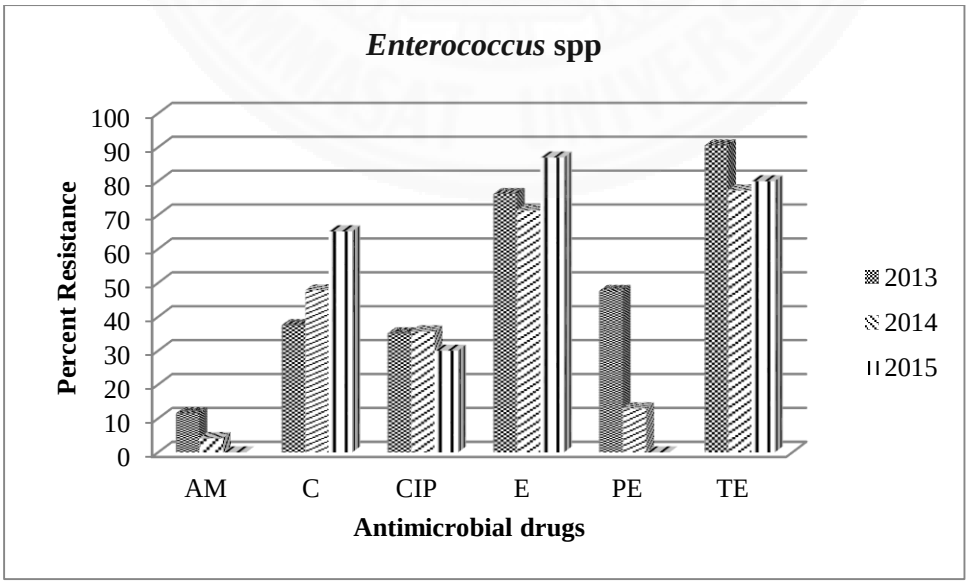

Figure 4.3 The percent resistance of *Enterococcus* spp to antimicrobial drugs in 2013 to 2015

4.3.5 ***Klebsiella* spp**

The susceptibility tests of 18, 22 and 22 *Klebsiella* spp isolates to AMC, AM, C, CIP, CRO, GM, NAL, SXT and TE were successful performed in 2013, 2014 and 2015, respectively**.** The susceptibility profiles were varied (**Table 4.16** and **Figure 4.5**) with statistically significant increased of resistance to AMC, SXT and TE. The AMC resistace was increased from 0% in 2013 to 22.7% in 2014 (p=0.017) and 40.9% in 2015 (p=0.006). SXT resistace was increased from 22% in 2013 to 61.9% in 2014 (p=0.013) and 59.1% in 2015 (p=0.019). And the TE resistance was increased from 17.8% in 2013 to 68.2% (p=0.011) and 65.0% (p=0.022), in 2014 and 2015 respectively. Other antimicrobial susceptibility showed temporary resistace in 2014 but the prevalace was decreased in 2015.

4.3.6 ***Neisseria gonorrhoeae***

The susceptibility of CIP, CRO, PE, TE and OFX against *N. gonorrhoeae* isolates were performed in 2012, 2013 and 2014 with 21, 13, and 14 isolates, respectively**.** Results are summarized in **Table 4.17** and **Figure 4.6**. With all years, the highest resistace rates up to 100% were CIP, PE, TE and OFX. CRO was only antimicrobial agent with remained susceptible in all years.

4.3.7 ***Plesiomonas shigelloides***

There were 21, 21, 21 and 19 of *Plesiomonas shigelloides* isolates that were successful performed drug susceptibility testing in 2012, 2013, 2014 and 2015 respectively**.** The susceptibility profile of AMC, C, CIP, CRO, GM, SXT and TE are summarized in **Table 4.18** and **Figure 4.7**. This organism was remained susceptible to all tested antibiotic agents except TE. The resistant prevalence of TE was statistically significant increased in 2013 (50%, p<0.001), 2014 (20%, p=0.035) and 2015 (42%, p=0.001) when compared to 2012 (0%).

4.3.8 ***Pseudomonas spp***

There were 10, 13, 21 and 13 of *Pseudomonas* spp isolates that were successful susceptibility tested to AN, CAZ CIP and GM in 2012, 2013, 2014 and 2015, respectively**.** The susceptibility profiles of AN, CAZ and CIP were similar trend with temporary increase resistance rates in 2014 and then declined (**Table 4.19** and **Figure 4.8**). In 2014, AN resistace rate was statistically significant increased to 60% (p=0.027) when compared to 0% of resistance in 2013. There was statistically

significant decreased of CAZ (0%, p=0.016) in 2015 compare to baseline year 2012 (28.6%). The resistance to CIP was also statistically significant decreased in 2015 (0%, p=0.029) which compared to resistance rate in 2014 (30.0%)

4.3.9 ***Salmonella* spp**

There were 49, 44, 56 and 78 of *Salmonella* spp isolates that were successful susceptibility tested in 2012, 2013, 2014 and 2015 respectively**.** The susceptibility results of AM, C, CIP, CRO, GM, NAL, SXT and TE against *Salmonella* spp are summarized in **Table 4.20** and **Figure 4.9**. There were statistically significant decreased of AM resistant prevalence in 2013 (45.5%, p<0.001) and 2014 (61.1%, p=0.026) from 80.0% in 2012. Whereas other antimicrobial agents, bacterial isolates were remained susceptible.

4.3.10 ***Shigella* spp**

There were 17, 28, 12 and 26 of *Shigella* spp isolates that were successful performed susceptibility testing in 2012, 2013, 2014 and 2015 respectively**.** The antimicrobial agents against *Shigella* spp were AM, C, CIP, CRO, NAL, SXT and TE. Results are demonstrated in **Table 4.21** and **Figure 4.10.** The highest resistant prevalence with all years were SXT and TE (80 to 100%). NAL was statistically significant incrased resistance rate in 2014 (70.0%, p=0.034) and 2015 (63.6%, p=0.001) when compared with 18.5% resistace rate in 2013. The CIP resistance rate was also increased form 0% in 2012 and 2013 to 19.2% resistance rate in 2015 (p=0.015). The susceptibility of AM and C were also showed increasing resistance trend but no statistically significant were observed. In contrast to other agents, CRO susceptibility was statistically significant decreased from 20.0% of resistance rate in 2012 to 0% in later years.

4.3.11 ***Staphylococcus aureus***

The antimicrobial agents againsted *Staphylococcus aureus* isolates were AMC, AM, C, CIP, E, FOX, GM, SXT and TE with 27, 48, 40 and 56 of isolates were tested in 2012, 2013, 2014 and 2015, respectively**.** Results are summarized in **Table 4.22** and **Figure 4.11**. The susceptibility profiles of all drugs except AM were remained susceptible. The AM resistance rate was statistically significant increased from 80% in 2012 to more than 97% in later years.

Table 4.15 The percent resistance of *E. coli* to antimicrobial drugs in 2012 to 2015

Drug	Year	Percent Resistance	Odd ratio[a] (95% CI)	Odd ratio[b] (95% CI)	Odd ratio[c] (95% CI)
AMC	2012	21.4	-	-	-
	2013	11.1	0.4 (0.1-2.6)	-	-
	2014	27.3	1.3 (0.3-6.1)	3.0 (0.8-11.8)	-
	2015	21.9	1.0 (0.2-5.2)	2.2 (0.5-9.7)	0.7 (0.3-2.2)
AM	2012	78.3	-	-	-
	2013	90.0	3.8 (0.7-21.5)	-	-
	2014	81.8	1.3 (0.4-4.4)	0.5 (0.1-2.1)	-
	2015	90.3	2.6 (0.6-12.2)	1.0 (0.2-5.6)	2.1 (0.5-8.6)
C	2012	51.9	-	-	-
	2013	51.6	0.9 (0.3-2.6)	-	-
	2014	38.6	0.5 (0.2-1.4)	0.6 (0.2-1.5)	-
	2015	35.5	0.5 (0.2-1.5)	0.5 (0.2-1.4)	0.9 (0.3-2.3)
CIP	2012	55.6	-	-	-
	2013	39.0	0.9 (0.3-2.6)	-	-
	2014	52.3	0.9 (0.4-2.4)	1.0 (0.4-2.6)	-
	2015	35.7	0.5 (0.2-1.5)	0.5 (0.2-1.5)	0.5 (0.2-1.3)
CRO	2012	53.8	-	-	-
	2013	40.6	0.6 (0.2-2.2)	-	-
	2014	42.2	0.6 (0.2-2.2)	1.0 (0.4-2.6)	-
	2015	32.1	0.4 (0.1-1.6)	0.7 (0.2-2.0)	0.7 (0.3-1.8)
GM	2012	35.7	-	-	-
	2013	43.8	1.4 (0.5-4.0)	-	-
	2014	38.6	1.1 (0.4-3.0)	0.8 (0.3-2.0)	-
	2015	29.6	0.8 (0.2-2.4)	0.5 (0.2-1.6)	0.7 (0.2-1.9)
NAL	2012	57.7	-	-	-
	2013	70.0	**2.8 (1.0-7.9)***	-	-
	2014	63.4	2.4 (0.9-6.2)	0.7 (0.3-2.0)	-
	2015	47.8	1.2 (0.4-3.5)	0.4 (0.1-1.2)	0.5 (0.2-1.5)
SXT	2012	64.7	-	-	-
	2013	80.8	2.3 (0.6-9.2)	-	-
	2014	53.5	0.7 (0.2-2.1)	**0.3 (0.1-0.9)****	-
	2015	70.0	1.2 (0.3-4.3)	**0.5 (0.2-1.9)****	1.9 (0.7-5.2)
TE	2012	88.9	-	-	-
	2013	76.7	0.3 (0.1-1.8)	-	-
	2014	68.9	**0.2 (0.0-0.9)***	0.7 (0.2-1.9)	-
	2015	70.0	0.2 (0.0-1.2)	0.7 (0.2-2.3)	1.1 (0.4-2.9)

[a]Odd ratio of drug resistance compared to year 2012, [b]compared to year 2013 and [c] compared to year 2014

*Compare to year 2012: the resistance of NAL in 2013 (p = 0.049), and TE in 2014 (p = 0.023) were statistically significant.

**Compare to year 2013: the resistance of SXT in 2014 (p = 0.022), and in 2015 (p = 0.012) were statistically significant.

Table 4.16 The percent resistance of *Klebsiella* spp to antimicrobial drugs in 2013 to 2015

Drug	Year	Percent Resistance	Odd ratio[a] (95% CI)	Odd ratio[b] (95% CI)
AMC	2013	0.0	-	-
	2014	22.7	**-***	-
	2015	40.9	**-***	3.7 (1.0-13.4)
AM	2013	94.1	-	-
	2014	100	-	-
	2015	95.2	1.3 (0.1-21.6)	-
C	2013	23.5	-	-
	2014	40.0	2.2 (0.5-9.1)	
	2015	45.0	2.7 (0.6-11.1)	1.2 (0.4-4.3)
CIP	2013	16.7	-	-
	2014	28.6	2.7 (0.6-13.4)	-
	2015	38.1	4.0 (0.7-18.8)	1.5 (0.4-5.6)
CRO	2013	0.0	-	-
	2014	57.1	**-***	-
	2015	19.0	-	**0.2 (0.0-0.7)****
GM	2013	11.8	-	-
	2014	50.0	**7.5 (1.4-40.9)***	-
	2015	23.8	2.7 (0.5-16.1)	0.3 (0.1-1.2)
NAL	2013	17.6	-	-
	2014	31.6	3.7 (0.7-19.6)	
	2015	33.3	3.1 (0.6-17.0)	1.1 (0.3-4.6)
SXT	2013	22.2	-	-
	2014	61.9	**5.7 (1.4-23.5)***	
	2015	59.1	**5.1 (1.3-20.5)***	0.9 (0.3-3.0)
TE	2013	17.8	-	-
	2014	68.2	**5.6 (1.4-21.9)***	-
	2015	65.0	**4.8 (1.2-19.2)***	0.9 (0.2-3.1)

[a]Odd ratio of drug resistance compared to year 2013, and [b]compared to year 2014

[*]Compare to year 2013: the resistance of AMC in 2014 ($p = 0.017$) and in 2015 ($p = 0.006$), CRO in 2014 ($p < 0.001$), GM in 2014 ($p = 0.012$), SXT in 2014 ($p = 0.013$) and in 2015 ($p = 0.019$), TE in 2014 ($p = 0.011$) and 2015 ($p = 0.022$) was statistically significant

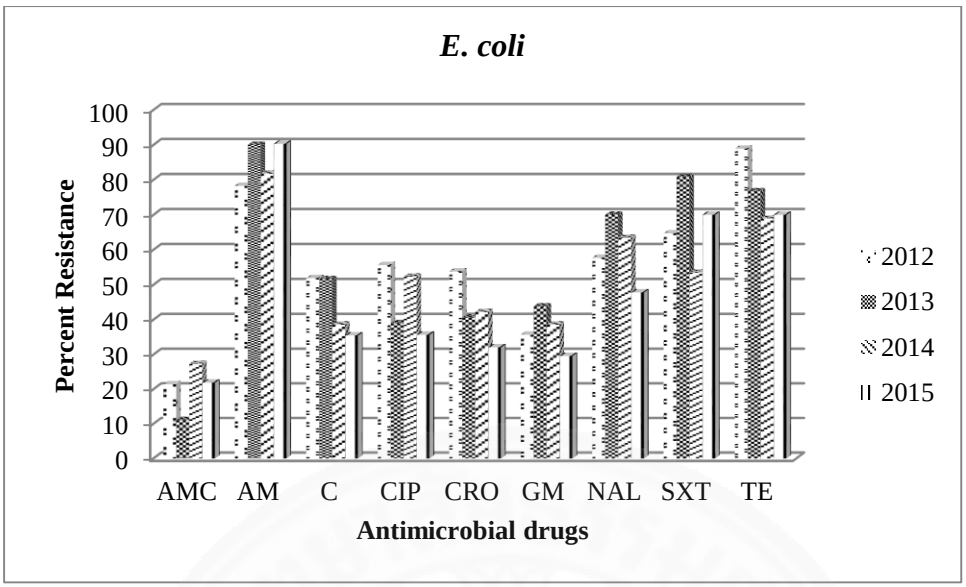

Figure 4.4 The percent resistance of *E. coli* to antimicrobial drugs in 2012 to 2015

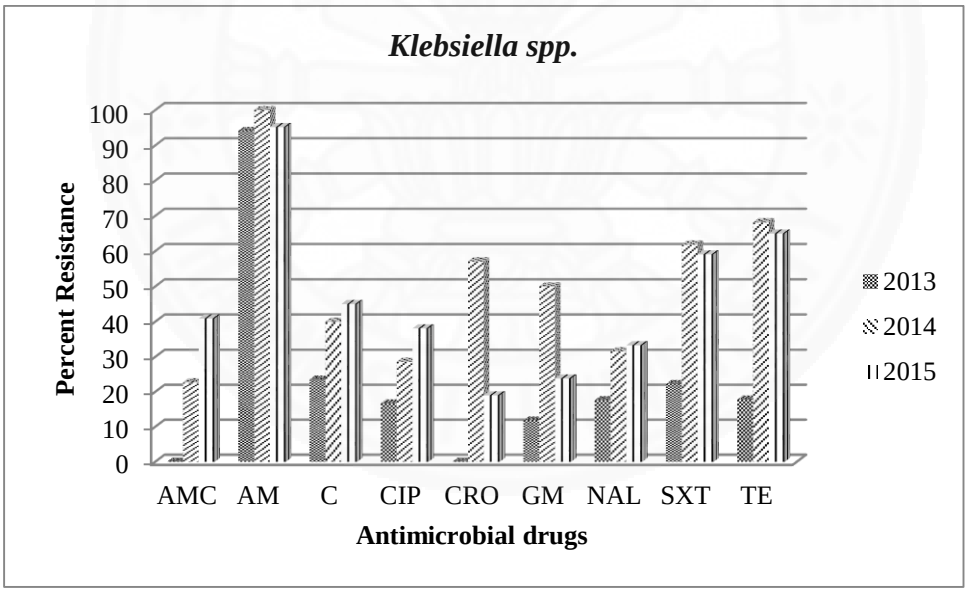

Figure 4.5 The percent resistance of *Klebsiella* spp to antimicrobial drugs in 2013 to 2015

Table 4.17 The percent resistance of *Neisseria gonorrhoeae* to antimicrobial drugs in 2012 to 2014

Drug	Year	Percent Resistance	Odd ratio[a] (95% CI)	Odd ratio[b] (95% CI)
CIP	2012	85.7	-	-
	2013	70.0	0.4 (0.1-2.4)	-
	2014	100.0	-	**-***
CRO	2012	0.0	-	-
	2013	0.0	-	-
	2014	0.0	-	-
PE	2012	77.8	-	-
	2013	100.0	-	-
	2014	100.0	-	-
TE	2012	100.0	-	-
	2013	100.0	-	-
	2014	100.0	-	-
OFX	2012	100.0	-	-
	2013	100.0	-	-
	2014	100.0	-	-

[a]Odd ratio of drug resistance compared to year 2012, and [b]compared to year 2013

*Compare to year 2013: the resistance of CIP in 2014 was statistically significant (p = 0.034)

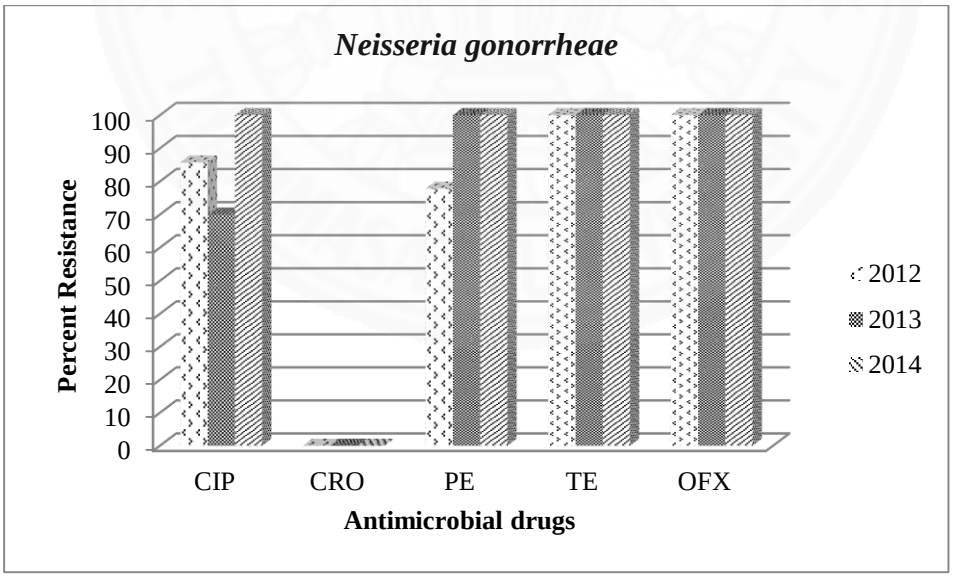

Figure 4.6 The percent resistance of *Neisseria gonorrhoeae* to antimicrobial drugs in 2012 to 2014

Table 4.18 The percent resistance of *Plesiomonas shigelloides* to antimicrobial drugs in 2012 to 2015

Drug	Year	Percent Resistance	Odd ratio[a] (95% CI)	Odd ratio[b] (95% CI)	Odd ratio[c] (95% CI)
AMC	2012	0.0	-	-	-
	2013	10.5	-	-	-
	2014	0.0	-	-	-
	2015	10.5	-	1.0 (0.1-7.9)	-
C	2012	9.5	-	-	-
	2013	4.8	0.5 (0.0-5.7)	-	-
	2014	0.0	-	-	-
	2015	5.3	0.5 (0.0-6.3)	1.1 (0.1-19.1)	-
CIP	2012	4.8	-	-	-
	2013	0.0	-	-	-
	2014	10.5	2.4 (0.2-28.3)	-	-
	2015	10.5	2.4 (0.2-28.3)	-	1.0 (0.1-7.9)
CRO	2012	0.0	-	-	-
	2013	4.8	-	-	-
	2014	0.0	-	-	-
	2015	6.7	-	1.4 (0.1-24.8)	-
GM	2012	0.0	-	-	-
	2013	5.3	-	-	-
	2014	4.8	-	0.9 (0.1-15.5)	-
	2015	5.6	-	1.1 (0.1-18.3)	1.2 (0.1-20.3)
SXT	2012	28.6	-	-	
	2013	20.0	0.6 (0.1-4.5)	-	
	2014	20.0	0.6 (0.1-4.5)	1.0 (0.2-4.7)	
	2015	15.8	0.5 (0.1-3.7)	0.8 (0.1-3.9)	0.8 (0.1-3.9)
TE	2012	0.0	-	-	-
	2013	50.0	-*	-	-
	2014	20.0	-*	**0.3 (0.1-1.0)****	-
	2015	42.1	-*	0.7 (0.2-2.6)	2.9 (0.7-12.1)

[a]Odd ratio of drug resistance compared to year 2012, [b]compared to year 2013 and [c] compared to year 2014

*Compare to year 2012: the resistance of TE in 2013 ($p < 0.001$), in 2014 ($p = 0.035$) and in 2015 ($p = 0.001$) were statistical significant.

**Compare to year 2013: the resistance of TE in 2014 was statistically significant ($p = 0.047$)

Table 4.19 The percent resistance of *Pseudomonas* spp to antimicrobial drugs in 2012 to 2015

Drug	Year	Percent Resistance	Odd ratio[a] (95% CI)	Odd ratio[b] (95% CI)	Odd ratio[c] (95% CI)
AN	2012	12.5	-	-	-
	2013	0.0	-	-	-
	2014	60.0	4.8 (0.5-48.5)	-**	-
	2015	25.0	2.7 (0.2-31.1)	-	0.6 (0.1-2.9)
CAZ	2012	28.6	-	-	-
	2013	10.0	0.2 (0.0-2.6)	-	-
	2014	21.1	0.4 (0.1-3.3)	2.4 (0.2-25.0)	-
	2015	0.0	-*	-	-
CIP	2012	10.0	-	-	-
	2013	15.4	1.6 (0.1-21.1)	-	-
	2014	30.0	3.9 (0.4-37.6)	2.4 (0.4-14.0)	-
	2015	0.0	-	-	-***
GM	2012	11.1	-	-	-
	2013	15.4	1.5 (0.1-19.0)	-	-
	2014	42.9	6.0 (0.6-57.0)	4.1 (0.7-23.4)	-
	2015	38.5	5.0 (0.5-53.0)	3.4 (0.5-22.4)	0.8 (0.2-3.4)

[a]Odd ratio of drug resistance compared to year 2012, [b]compared to year 2013 and [c] compared to year 2014

[*]Compare to year 2012: the resistance of CAZ in 2015 was statistically significant ($p = 0.016$)

[**]Compare to year 2013: the resistance of AN in 2014 was statistically significant ($p = 0.027$)

[***]Compare to year 2014: the resistance of CIP in 2015 was statistically significant ($p = 0.029$)

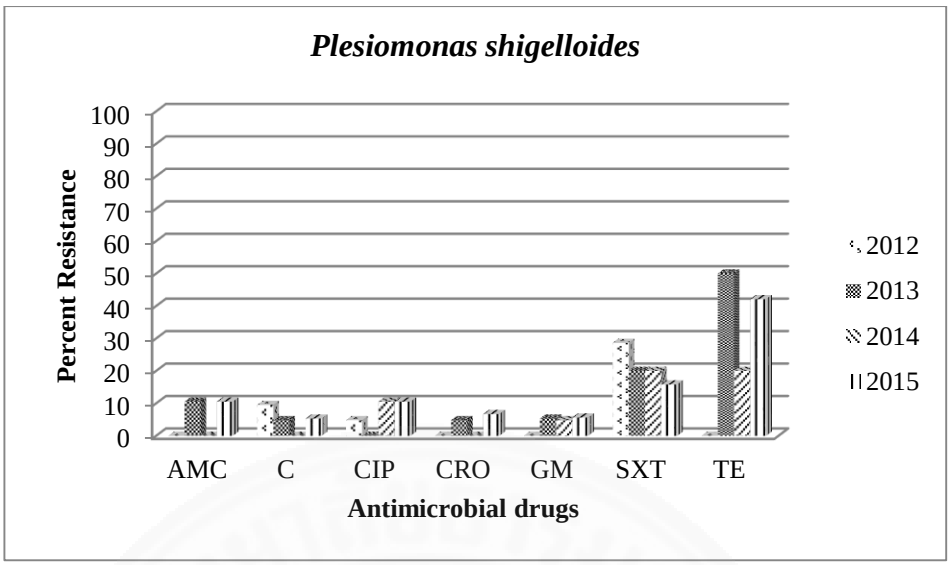

Figure 4.7 The percent resistance of *Plesiomonas shigelloides* to antimicrobial drugs in 2012 to 2015

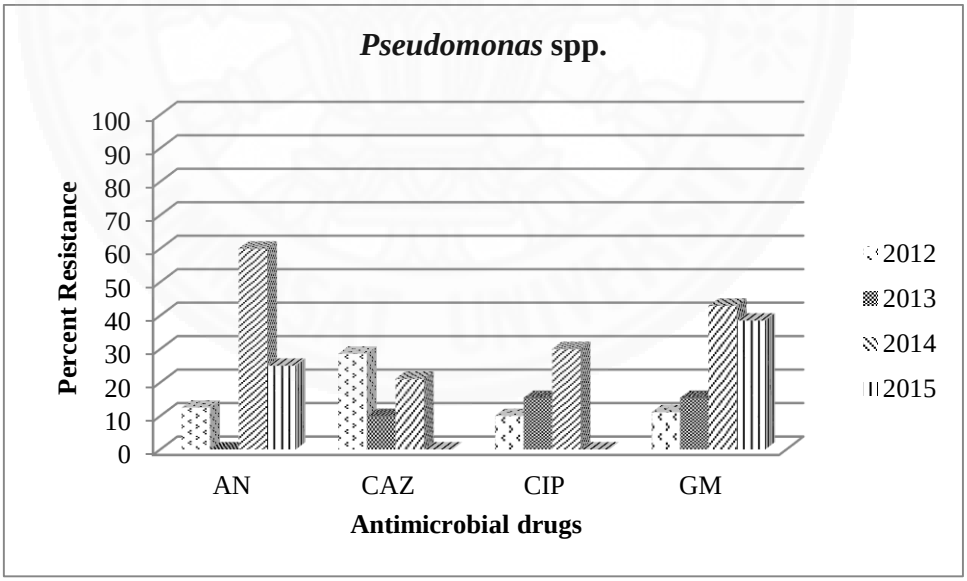

Figure 4.8 The percent resistance of *Pseudomonas* spp to antimicrobial drugs in 2012 to 2015

Table 4.20 The percent resistance of *Salmonella* spp to antimicrobial drugs in 2012 to 2015

Drug	Year	Percent Resistance	Odd ratio[a] (95% CI)	Odd ratio[b] (95% CI)	Odd ratio[c] (95% CI)
AM	2012	80.0	-	-	-
	2013	45.5	**0.2 (0.1-0.5)***	-	-
	2014	61.1	**0.4 (0.1-0.9)***	1.9 (0.8-4.2)	-
	2015	65.8	0.4 (0.2-1.1)	**2.3 (1.1-4.9)****	1.2 (0.6-2.5)
C	2012	24.5	-	-	-
	2013	23.8	1.0 (0.5-2.6)	-	-
	2014	7.4	**0.3 (0.1-0.8)***	**0.3 (0.1-0.9)****	-
	2015	12.8	0.5 (0.2-1.2)	0.5 (0.2-1.2)	1.8 (0.6-6.2)
CIP	2012	4.1	-	-	-
	2013	2.3	0.7 (0.1-8.4)	-	-
	2014	3.7	1.1 (0.2-8.4)	1.6 (0.1-18.4)	-
	2015	7.7	3.0 (0.6-16.1)	3.5 (0.4-30.1)	2.7 (0.5-14.5)
CRO	2012	14.6	-	-	-
	2013	6.8	0.4 (0.1-1.8)	-	-
	2014	0.0	-*	-	-
	2015	1.3	**0.1 (0.0-0.7)***	0.2 (0.0-1.8)	-
GM	2012	ND	-	-	-
	2013	8.3	-	-	-
	2014	4.9	-	0.6 (0.1-4.3)	-
	2015	7.9	-	1.0 (0. 2-6.3)	1.7 (0.3-10.9)
NAL	2012	8.3	-	-	-
	2013	0.0	-	-	-
	2014	14.5	1.8 (0.5-6.3)	-**	-
	2015	12.0	1.5 (0.4-5.1)	-**	0.8 (0.3-2.3)
SXT	2012	36.4	-	-	-
	2013	20.5	0.5 (0.2-1.3)	-	-
	2014	25.9	0.6 (0.2-1.6)	1.4 (0.5-3.5)	-
	2015	23.1	0.5 (0.2-1.3)	1.2 (0.5-2.9)	0.9 (0.4-1.9)
TE	2012	67.3	-	-	-
	2013	48.8	0.5 (0.2-1.1)	-	-
	2014	59.3	0.7 (0.3-1.6)	1.5 (0.7-3.4)	-
	2015	62.8	0.8 (0.4-1.7)	1.8 (0.8-3.8)	1.2 (0.6-2.4)

ND: Not done; [a]Odd ratio of drug resistance compared to year 2012, [b]compared to year 2013 and [c] compared to year 2014

[*]Compare to year 2012: the resistance of AM in 2013 ($p < 0.001$) and in 2014 ($p = 0.026$), C in 2014 ($p = 0.019$), CRO in 2014 ($p = 0.004$) and in 2015 ($p = 0.003$) were statistically significant.

[**]Compare to year 2013: the resistance of AM in 2015 ($p = 0.029$), C in 2014 ($p = 0.024$), NAL in 2014 ($p = 0.01$) and in 2015 ($p = 0.019$) were statistically significant.

Table 4.21 The percent resistance of *Shigella* spp to antimicrobial drugs in 2012 to 2015

Drug	Year	Percent Resistance	Odd ratio[a] (95% CI)	Odd ratio[b] (95% CI)	Odd ratio[c] (95% CI)
AM	2012	68.75	-	-	-
	2013	46.4	0.3 (0.1-1.2)	-	-
	2014	54.5	0.4 (0.1-2.3)	1.4 (0.3-5.6)	-
	2015	61.5	0.6 (0.1-2.3)	1.8 (0.6-5.5)	1.3 (0.3-5.6)
C	2012	64.7	-	-	-
	2013	51.9	0.5 (0.1-2.0)	-	-
	2014	54.5	0.7 (0.1-3.6)	1.1 (0.3-4.6)	-
	2015	73.1	1.2 (0.3-4.8)	2.5 (0.8-8.0)	2.3 (0.5-9.8)
CIP	2012	0.0	-	-	-
	2013	0.0	-	-	-
	2014	8.3	-	-	-
	2015	19.2	-	**-****	2.6 (0.3-25.3)
CRO	2012	20.0	-	-	-
	2013	0.0	**-***	-	-
	2014	0.0	-	-	-
	2015	0.0	**-***	-	-
NAL	2012	35.3	-	-	-
	2013	18.5	0.4 (0.1-1.5)	-	-
	2014	70	5.8 (0.9-37.8)	**10.3 (1.9-54.3)****	-
	2015	63.6	2.9 (0.87-11.1)	**7.7 (2.1-28.3)****	0.8 (0.2-3.7)
SXT	2012	93.3	-	-	-
	2013	88.9	0.6 (0.1-6.0)	-	-
	2014	83.3	0.4 (0.0-4.5)	0.6 (0.1-4.3)	-
	2015	92.3	0.9 (0.1-10.3)	1.5 (0.2-9.8)	2.4 (0.3-19.5)
TE	2012	82.4	-	-	-
	2013	85.2	1.2 (0.2-6.3)	-	-
	2014	91.7	2.4 (0.2-25.9)	1.9 (0.2-19.2)	-
	2015	100.0	-*	-**	-

[a]Odd ratio of drug resistance compared to year 2012, [b]compared to year 2013 and [c] compared to year 2014

[*]Compare to year 2012: the resistance of CRO in 2013 (p = 0.013) and in 2015 (p = 0.018), TE in 2015 (p = 0.029) were statistically significant.

[**]Compare to year 2013: the resistance of CIP in 2015 (p = 0.015), NAL in 2014 (p = 0.0034) and in 2015 (p = 0.001), TE in 2015 (p = 0.045) were statistically significant.

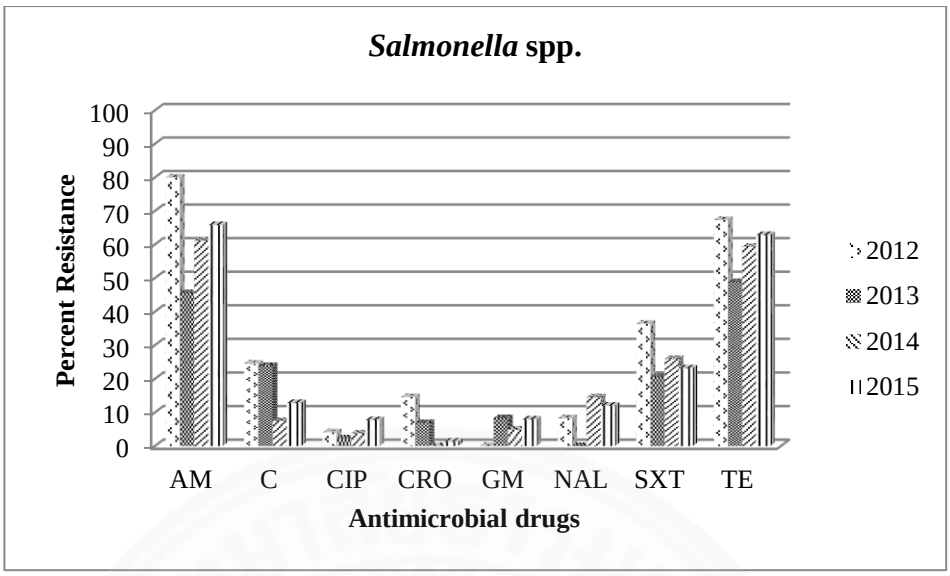

Figure 4.9 The percent resistance of *Salmonella* spp to antimicrobial drugs in 2012 to 2015

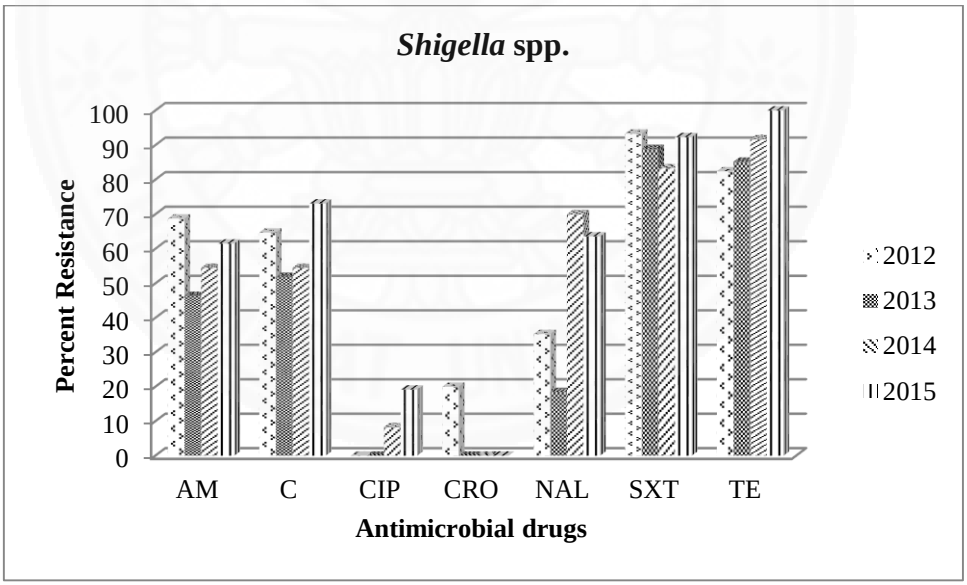

Figure 4.10 The percent resistance of *Shigella* spp to antimicrobial drugs in 2012 to 2015

Table 4.22 The percent resistance of *Staphylococcus aureus* to antimicrobial drugs in 2012 to 2015

Drug	Year	Percent Resistance	Odd ratio[a] (95% CI)	Odd ratio[b] (95% CI)	Odd ratio[c] (95% CI)
AMC	2012	9.1	-	-	-
	2013	15.6	2.0 (0.4-10.6)	-	-
	2014	12.8	1.6 (0.3-9.1)	0.8 (0.2-2.8)	-
	2015	9.1	1.1 (0.2-6.1)	0.5 (0.2-1.8)	0.7 (0.2-2.5)
AM	2012	80.0	-	-	-
	2013	97.9	**11.8 (1.1-123.2)***	-	-
	2014	97.4	**9.3 (0.9-97.5)***	0.8 (0.1-13.0)	-
	2015	98.0	**12.0 (1.1-125.8)***	1.0 (0.1-16.8)	1.3 (0.1-21.4)
C	2012	22.2	-	-	-
	2013	23.3	1.0 (0.3-3.3)	-	-
	2014	13.9	0.6 (0.2-2.1)	0.5 (0.2-1.7)	-
	2015	9.3	0.4 (0.1-1.3)	0.3 (0.1-1.1)	0.6 (0.2-2.4)
CIP	2012	3.8	-	-	-
	2013	10.6	3.0 (0.3-27.0)	-	-
	2014	12.8	3.7 (0.4-33.5)	1.2 (0.3-4.6)	-
	2015	5.4	1.5 (0.2-15.2)	0.5 (0.1-2.1)	0.4 (0.1-1.7)
E	2012	23.1	-	-	-
	2013	22.5	0.9 (0.3-2.9)	-	-
	2014	33.3	1.5 (0.5-4.6)	1.7 (0.6-4.7)	-
	2015	18.2	0.8 (0.2-2.4)	0.8 (0.3-2.1)	0.4 (0.2-1.2)
FOX	2012	ND	-	-	-
	2013	9.5	-	-	-
	2014	20.5	-	2.5 (0.5-12.8)	-
	2015	8.0	-	0.8 (0.1-4.9)	0.3 (0.1-1.2)
GM	2012	7.4	-	-	-
	2013	4.4	0.6 (0.1-4.6)	-	-
	2014	10.5	1.5 (0.3-8.7)	2.5 (0.4-14.6)	-
	2015	9.3	1.3 (0.2-7.1)	2.2 (0.4-11.9)	0.9 (0.2-3.5)
SXT	2012	8.3		-	-
	2013	16.3	2.2 (0.2-19.9)	-	-
	2014	11.1	1.4 (0.1-13.7)	0.6 (0.2-2.4)	-
	2015	5.4	0.7 (0.1-6.8)	0.3 (0.1-1.2)	0.5 (0.1-2.2)
TE	2012	38.5	-	-	-
	2013	56.5	2.1 (0.8-5.6)	-	-
	2014	47.2	1.5 (0.5-4.2)	0.7 (0.3-1.7)	-
	2015	30.4	0.7 (0.3-1.9)	**0.3 (0.2-0.8)****	0.5 (0.2-1.2)

ND: Not done; [a]Odd ratio of drug resistance compared to year 2012, [b]compared to year 2013 and [c] compared to year 2014

*Compare to year 2012: the resistance of AM in 2013 (p = 0.013), in 2014 (p =0.031) and in 2015 (p = 0.012) were statistically significant.

**Compare to year 2013: the resistance of TE in 2015 was statistically significant (p = 0.008)

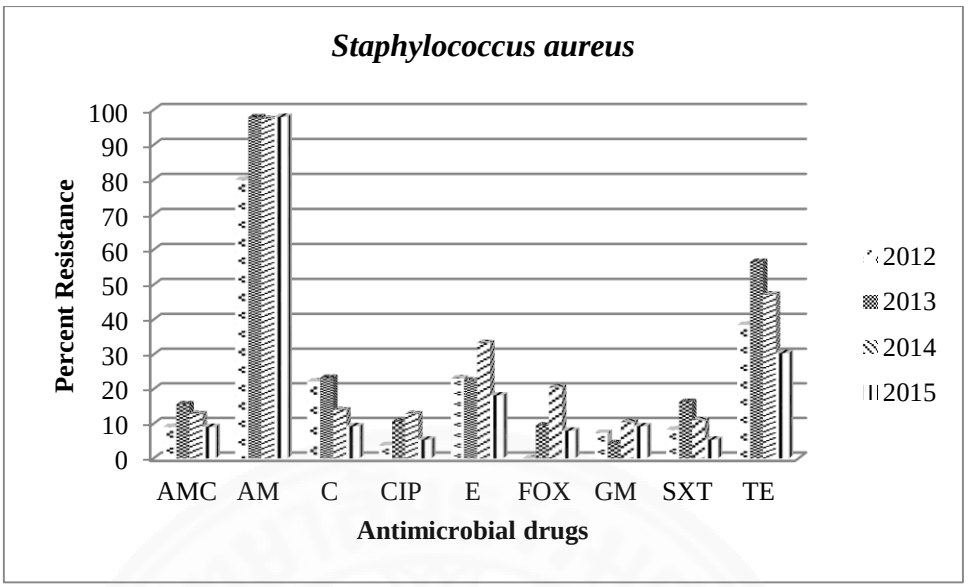

Figure 4.11 The percent resistance of *Staphylococcus aureus* to antimicrobial drugs in 2012 to 2015

CHAPTER 5
DISCUSSION

5.1 The prevalence of pathogenic bacteria from clinical samples

Pathogenic bacteria are bacteria that can cause infection. Although most bacteria are harmless or often beneficial, some are pathogenic, with the number of species are seen to cause infectious diseases in humans. The diseases of a bacterial infection depend on the area of the body that is affected. Thus the identified pathogens were categorized according to specimen types which related to the disease infection. In blood culture, *Salmonella Typhi, B. pseudomallei* and Coagulase Negative *Staphylococcus* were the predominant identified pathogens which similar to previous report in Lao PDR (35) that found the highest prevalence of *Salmonella* Typhi caused the bacteremia.

The most common pathogens that identified in stool/rectal swab specimens were pathogenic *E. coli* and *Salmonella* spp in 2012 and *Salmonella* spp in 2013 to 2015 following with *Shigella* spp, *Aeromonas* spp and *P. shigelloides* respectively. There was no reported of *Aeromonas spp* infection in Lao PDR from previous study of Yamashiro and colleague (19). This study was the first reported of *Aeromonas* spp identified in stool/rectal swab specimens from 2012 to 2015. These results indicate that the changing of pathogen discovered in the country.

S. aureus was the predominant pathogens found in pus swab from all areas of body with all over 4 years following by Coagulase Negative *Staphylococcus* and *E. coli,* respectively. *S. aureus* tend to infect the skin, often causing abscesses.

The reports of pneumonia cases from Cambodia, Thailand and Vietnam (36) showed the most common community-acquired pneumonia pathogen were *S. pneumoniae* and *H. influenzae* which similar to the present results in 2012. Nevertheless finding of respiratory pathogen was depended on sources and occurrences in each year. In 2012, the most prevalence pathogen in specimens from both upper and lower respiratory tracts was *Haemophilus spp*. In 2014, Group A *Streptococcus* was the highest identified pathogen from upper respiratory swab

specimens. In 2013 and 2015, there were outbreaks of diphtheria suspected and the most identified pathogen from specimens collected in the suspected group was *Corynebcterium diphtheriae*.

Coagulase Negative *Staphylococcus* and *N. gonorrhoeae* were the most common organisms identified from urethral discharge. Coagulase Negative *Staphylococcus*, the opportunistic bacteria usually considered as normal skin flora and contaminants organism, but also caused infection in urine (37) and it was possible to find as genital tract for both male and female. However Bacterial vaginosis and mixed infection were the highest pathogen found from vaginal discharge. And predominant of infection in urine culture each year was different. In this study, Enterobacteriaceae such as *Escherichia coli, Klebsiella* spp, *Morganella morganii* were the highest caused of urinary tract infection.

5.2 The correlation of infection with demographic information

A total of 6789 specimens were collected during 2012 to 2015. Theses samples were collected from various sources and sent to National Center for Laboratory and Epidemiology (NCLE), Lao PDR for isolating and identifying the pathogenic bacteria. Of these, the bacterial infection rate was randomly from year 2012 to 2015, which were 40.4%, 27.9%, 28.9% and 31.9%, respectively. These results indicated decreasing trend of bacterial infection. This data might be affected from the implement of national policy of Lao PDR to survey and control the bacterial infection. This program has been supported by United State Agency for International Development (USAID) - Emerging Pandemic Threats Program (EPT) and the European Union (EU) through the World Health Organization (WHO). Thus, the nation policy should be continuing for successful controlling bacterial infections.

The pathogenic infections were similar among the region of samples collection, this might be indicated that no different of health knowledge among people of Lao PDR. In contrast, the source of collection was significant to infection rate. Comparing to diarrhea surveillance, the out patient to NCLE, nosocomial infection, outbreak investigation and referral from network laboratories showed the higher rate of infection. Because the diarrhea surveillance was collected only the stool samples

where as others could be any types of samples. Thus the chance to found infection in stool samples was lower than others.

The bacteria infection in female was 1.80 times higher than male (p<0.0001) which similar to study in Italy (38) on UTI infection that found higher prevalence of bacterial infection in UTI female cases. Vaginal discharge was one of the highest three samples of this study. The occurrence of coliform bacteria could be more likely to infected through the shorter urogenital-physiology of female than male (39) supported these result.

Age groups were one of the studied factor influence of infection, in this study the older population (> 5 years) showed significant higher risk to get infection than the younger population (p<0.0001).

The result comparison between this study and others may defined that, prevalence of infection in different gender is depending on pathogen, sign and symptom, sites of infection and types of specimen collected, including sample size of each specimen types resulted to statistic calculation.

5.3 The antimicrobial susceptibility and the trend over 4-year

Antimicrobial resistance is current interests of global both in health and animal sector. There were eleven bacterial types successfully completed in drug susceptibility testing. Among these, three bacteria including *E. coli*, *K. pneumoniae*, *S. aureus* causing infectious in hospital and community infection; and four bacteria types comprising of nontyphoidal *Salmonella*, *N. gonorrhoeae S. pneumoniae* and *Shigella spp*. causing community infection. These bacteria are concerned bacterial required surveillance and monitoring according global action plan (15) in global and also in Asian countries such as Thailand (40). Overall resistance rate of bacterial isolates in Lao PDR from this study were not high as neighboring country or global report from Thailand, Vietnam, China (11) but a trend of increasing the resistance rate could be observed such as MRSA, ESBL-producing organism and *Shigella* spp.

***Aeromonas* spp**: the gram-negative, which are water and food-borne pathogens. The common species that could cause human disease including *A. hydrophila*, *A. dhakensis*, *A. veronii biovar sobria* and *A caviae*. This organism causes

diarrhea, wound infection and bacteremia. The antimicrobial susceptibility was different among species. Most *Aeromonas* strains are resistant to penicillin, ampicillin, carbenicillin, and ticarcillin; most are susceptible to trimethoprim-sulfamethoxazole, fluoroquinolones, second and third generation cephalosporins, aminoglycosides, carbapenems, chloramphenicol, and tetracyclines (41-45). In the present study, only amoxicillin clavulanate was increasing resistance rates from 35.7 to 67.9% over four years. This increasing of resistance was comparable to the report of Maluping (2005) which was 89% resistance of *Aeromonas* spp, isolated from different sources in the Philippines and Thailand (46).

Coagulase-negative *Staphylococcus*: are a group of gram-positive bacteria which can divide to coagulase-negative and coagulase-positive. The coagulase-negative *Staphylococcus* are more virulence than the positive group, they could produce the virulence factor that used to invading tissue. These pathogens are the normal skin flora which can cause nosocomial infection and sepsis (4, 47). The species that were mostly found in clinical samples are *Staphylococcus haemolyticus* and *Staphylococcus epidermidis* (48, 49). In this study the NCLE was only identified the genus group which were significant for treatment. The treatment of coagulase-negative *Staphylococcus* was difficult due to the high prevalence of multidrug resistance. The widely used antimicrobial agents; penicillins, cephalosporins, macrolides, aminoglycosides, and tetracyclines have been resistance reported (50, 51). In contrast to other reports, the present study showed low resistance rate of chloramphenical, ciprofloxacin, ceftriaxone, gentamicin, trimethoprim-sulfamethoxazole and tetracycline. The amoxicillin clavulanate was only antimicrobial that found increasing of resistance from 35.7% to 67.9% over four years. The amoxicillin is the drug in the same family which penicillin. Therefore, this study was showed the evidence of penicillins resistance in Lao PDR which similar to previously reported. However, the penicillin resistance of coagulase-negative Staphylococcus in Lao PDR was showed the lower percentage (67.9%) when compared to neighboring countries; *i.e.,* Thailand (100% resistance) (49) and Cambodia (85% resistance) (52).

Enterococcus spp: are the gram-positive cocci that can produce lactic acid. The enterocci are common organisms in the intestines of humans, which are

unlikely to cause infection. But there are some types such as *E. faecalis* and *E. faecium* that cause nosocomial infection (53). Enterococci are resistant to a large number of antimicrobial agents including, aminoglycosides, clindamycin, antistaphylococcal penicillins (oxacillin, methicillin, and nafcillin), cephalosporins, and most fluoroquinones (54). In recent study, the enterococcus showed high resistance rate of erythromycin (>70%) and tetracycline (> 77%) since 2013. These results were similar to report from from neighboring countries such as Thailand (45-76% resistance to erythromycin; and 75% resistance to tetracycline) (55, 56). The result of chloramphenicol was showed the increasing resistance trend from 37% to 65% during 2013 to 2015. But the report from Chaiwong (2014) showed 26% of resistance to chloramphenicol. Surprising, the resistance rates of ampicillin and penicillin were declined which 0% in 2015 in both drugs. This susceptibility pattern was similar to report in Thailand. Several studies in Thailand since 2004 showed various result of ampicillin (24-43%) and penicillin (~51%) resistance rate (57, 58)(Srifuengfung et al., 2004; Thapa et al, 2007) and the recent report showed the 0.8% resistance of ampilcillin (55).

***E.coli**:* are the normal flora in humans gut, which were originally susceptible to many antimicrobial agents. However, the resistance has been developed due to selective pressure by repeated exposure to antimicrobial agent. In Southeast Asia, many studies had been conducted to observe the resistance of this organism. The ampicillin resistance (≥ 50%) had been reported in Southeast Asian countries including Thailand, Singapore and Malaysia (59), which similar to the present study in Lao PDR (> 78%). The overall ampicillin resistance prevalence in the Southeast Asia was much higher than other countries of the world (59).

The fluoroquinolone resistance has been worldwide. The result in this study showed the resistance rate 35-51% against ciprofloxacin. In Asia-Pacific region, ciprofloxacin resistance was increased from 0 to 57.5% in 1992 to 2013 (60-62). Now the WHO have been established the network to monitoring *E. coli* resistant to third-generation cephalosporins, which are widely used for intravenous treatment of severe infections in hospitals, and to fluoroquinolones, which are among the most widely used oral antibacterial drugs in the community. In Lao PDR, the 3rd generation of

cephalosporins have not been used but the NCLE will be tested this drug to monitor the resistance according to WHO recommendation.

The high resistance rate (68-90%) of tetracycline against *E.coli* was observed since 2012 until 2015. The similar resistance rate also reported from Malaysia (62%) and the Phillippines (92%). The persistence of high tetracycline resistance over 4-years in Lao PDR and in neighboring countries confirmed the widespread of tetracycline resistance *E. coli* in the Southeast Asia region (12).

The result of trimethoprim-sulfamethoxazole showed the persistence resistance with 53-80% over 4-years. The results from Thailand and the Phillippines also showed high resistance rate (64-100%) (12, 62) and 92% (63), respectively.

The overall results of *E.coli* drug susceptibility profile showed high resistance to ampicillin, tetracycline and trimethoprim-sulphamethoxazole. Thus the *E. coli* pathogen was multidrug resistance. The serious situation is urgently needed health policy to control the spread of multidrug throughout the nationwide.

***Klebsiella* spp**: is a member of the family Enterobacteriaceae. *Klebsiella* are nonmotile, rod-shaped, gram-negative bacteria, with are routinely found in the human nose, mouth, and gastrointestinal tract as normal flora; however, they can also behave as opportunistic human pathogens (64). This pathogen can produce a prominent polysaccharide capsule with encases the entire cell surface, and provides resistance against many host defense mechanisms. Similar to *E. coli*, *Klebsiella* spp acquires resistance to multiple antibacterial drugs mainly through horizontal transfer of mobile genetic elements such as transposons or plasmids. Resistance to other widely used and available oral antibacterial drugs such as cotrimoxazole and fluoroquinolones (*e.g.* ciprofloxacin) has emerged and spread globally (15).

The results of ampicillin showd high resistance rate (94-100%) which similar to previous study from Lao PDR (35) with 94% resistance rate. This data indicate that ampicillin could not be used in Lao PDR. Amoxicillin-clavulanate was showed the significant increasing resistance rate from 0% in 2013 to 22.7% in 2014 and 40.9% in 2015. The similar resistance rate of 66% was reported from Thailand (62).

The ciprofloxacin resistance was prevalent in the Philippines (62%), Thailand (29-43%) and Singapore (22%) (12, 62). Similar to the present report, the

ciprofloxacin resistance rate against *Klebseilla* spp was approximately 38%. From recommendation of WHO, the third-generation cephalosporins against *Klebseilla* spp should be monitored. One of the third-generation cephalosporins; ceftriaxone was tested in this study. The results showed significant increasing the resistance from 0% in 2013 to 57% in 2014, but the resistance rate was deceasing to 19% in 2015. The deceasing resistance trend might be the controlling of using the third-generation cephalosporins in Lao PDR. The resistance to ceftriaxone was also reported from neighbor countries; Thailand (40%-100%) and Myanmar (60%) (12, 15).

The resistance to tetracycline was significant increasing from 17.8% in 2013 to more than 65% in 2015-2015. The high tetracycline resistance prevalence was also reported from the Phillippines (53%) (12).

The result of trimethoprim-sulphamethoxazole showed the increasing resistance with 22% in 2013 to more than 60% in 2014-2015. This result was also increasing from previous report (29%) in Lao PDR (35). In addition, the resistance of this regiment was also observed in the Phillippines (89%), Singapore (48%) and Thailand (54%) (62, 63, 65).

From the results of present study, the *Klebsiella* spp showed three-drug resistance. This indicated that there is an emerging of multidrug resistance *Klebsiella* spp in Lao PDR. This situation should be closely monitored and should revise the health policy to control this multidrug resistance.

Neisseria gonorrhoeae: is the bacterium that causes gonorrhoea, which is a sexually transmitted diseases. The pathogen can cause acute infection of the reproductive tract that may be symptomatic or asymptomatic. If untreated, or inappropriately treated, it can develop the severe; such as genital and reproductive tract inflammation and damage, and infertility. The major problem of this pathogen is multidrug resistance including penicillins, tetracycline, sulfonamides, spectinomycin, quinolones, macrolides and cephalosporins (66). The only effective antimicrobial agent is the third generation cephalosporins, which are the last remaining options for treatment.

In the present study was tested with five antimicrobial agents including the 3rd generation cephalosporins; ceftriaxone. The results showed four drugs; penicillin, tetracycline, ofloxacin and ciprofloxacin resistance as high rate (100%).

The only one drug; ceftriaxone was remained susceptible among 3 years, which no any resistance found. There was no resistance of ceftriaxone against *Neisseria gonorrhoeae* in Thailand but 18% of resistance prevalence found in Myanmar (15). As mention above, the 3rd generation of cephalosporin is the last hope for gonorrhoea treatment, therefore the WHO was launched the Gonococcal Antimicrobial Surveillance Programme (GASP) to coordinate gonococcal antimicrobial resistance surveillance, monitor longitudinal trends in antimicrobial resistance and provide data to inform treatment guidelines (15).

Plesiomonas shigelloides: is a gram-negative, rod-shaped bacterium which has been isolated from freshwater, freshwater fish, and shellfish and from many types of animals including humans. *P. shigelloides* could be isolated from feces of humans both with and without diarrhea (intra-intestinal). This pathogen is also found in extra-intestinal specimens in patients who are an immune deficiency.

P. shigelloides is usually susceptible to chloramphenicol, the quinolones, cephalosporins, aztreonam and imipenem (67-77). Tetracycline, trimethoprim-sulphamethoxazole and aminoglycoside susceptibility is variable. The results of present study showed the low resistance prevalence to all tested drugs except tetracycline. The resistance rate of tetracycline was significant increasing from 20% of intermediate result in 2012 to 40-50% resistance over four years. Unfortunately, there is limited susceptibility data of this pathogen in Asian countries, due to the low ability to cause human diseases.

***Pseudomonas* spp**: is a gram-negative, aerobic gammaproteobacteria, belonging to the family Pseudomonadaceae. These pathogens are able to cause variety of infections including bacteremia (78), chronic suppurative otitis media (79), respiratory tract infection (80), cystic fibrosis (81) and pneumonia. The common species that found in immune-compromised patients and in hospitalized patients is *Pseudomonas aeruginosa*. *Pseudomonas* spp infections are a significant global concern due to its ability to infect all body tissues and wide variety of virulence factors such as the production of biofilm that is protect the bacteria from host defense.

The antimicrobial agents used for *Pseudomonas* spp treatment are aminoglycosides, fluroquinolones, cephalosporins and carbapenems (78, 82). The decline susceptibility of beta-lacams, carbapenems, quinolones and aminoglycosides

against *Pseudomonas* spp have been reported (79, 83). The recently used antimicrobial agent in many countries is cephalosporins.

In the present study, only four antimicrobial agents (amikacin, gentamicin, ciprofloxacin and ceftaxidime) were tested. The resistance rate of amikacin was increasing in 2014 (60%) then the rate was decreasing in 2015 (25%). Ciprofloxacin and ceftaxidime were low resistance rate with found 0% resistance rate in 2015. The study of Chang and colleague (2017), which survey the antibiotic profiles in Asia-Pacific region during 2010-2013. The results from this report showed low resistance rate of amikacin (10%), ciprofloxacin (22%) and ceftaxidime (21%) (84). This similar susceptibility profile was also reported from Thailand with 27%, 23% and 38%, respectively (62). These two reports were shown the 3rd cepharosporin resistance. Nevertheless, in Lao PDR, the different resistance pattern of cepharosporins; ciprofloxacin and ceftaxidime was different from other parts in this region. Thus, the resistance of *Pseudomonas* spp has not yet spread to Lao PDR.

***Salmonella* spp**: is a gram negative bacteria with containing more than 2600 different serotypes or serovars that differentiated by their antigenic presentation. Nontyphoidal *Salmonella* is the primarily cause gastroenteritis, bacteremia, and focal infection which is common in developing countries (85). The symptom of nontyphoidal *Salmonella* infection or salmonellosis, may be fever, abdominal pain, diarrhoea, nausea and vomiting. Salmonella bacteria are widely distributed in domestic and wild animals. Humans are infected by contacted through the consumption of contaminated food of animal origin (mainly eggs, meat, poultry, and milk).

For mild and moderate cases, the antimicrobial therapy is not recommended. Because, the antimicrobials may not completely eliminate the bacteria and may select for resistant strains, which subsequently can lead to the drug becoming ineffective. However, the treatment is needed in infants, the elderly, and immunocompromised patients. The antimicrobial agents for salmonellosis treatment are trimethoprim/sulfamethoxazole, ciprofloxacin, azithromycin, or ceftriaxone.

The susceptibility profile in this study showed that pathogens were susceptible to various types antimicrobial agents; chloramphenicol, ciprofloxacin, ceftriaxone, gentamicin, nalidixic acid and trimethoprim/sulfamethoxazole. The

Salmonella spp (86)was highly resistance to ampicillin (~45 to 80%) and tetracycline (~48 to 67%).

Resistance to tetracycline among *Salmonella* spp in Thailand was 69% in 2009 (87) to 90% in 2011 (88). In Malaysia, Vietnam and Cambodia tetracycline resistance rates were high with 47-70% (89, 90), 40-59% (86, 91) and 42% (92), respectively. While the tetracycline in Indonesia and the Phillippines was much lower with 29% (93) and 20% (87), respectively.

In Thailand, ampicillin resistance increased from 46% in 2009 (87) to 100% in 2011 (88). In Indonesia, the rates of resistance remained stable in the late 1990s: 19% in 1998 and 23% in 2007 (93). This data was contrast to our results, the resistance of ampicillin in Lao PDR was much higher than other countries.

***Shigella* spp**: is a gram-negative, facultative anaerobic, nonspore-forming, nonmotile, rod-shaped bacterium. Shigella is one of the leading bacterial causes of diarrhea worldwide. The pathogens are transmitted by ingestion of contaminated food or water, or through person-to-person contact. Infection of *Shigella* spp or Shigellosis, causes severe inflammation and death of the cells lining the colon. This inflammation results in the diarrhea and even dysentery. The disease is usually self-limiting but may become life-threatening if patients are immunocompromised or adequate medical care. The *Shigella* spp. includes four species: *S. dysenteriae* (serogroup A), *S. flexneri* (serogroup B), *S. boydii* (serogroup C), and *S. sonnei* (serogroup D). *S. flexneri* and *S. nonnei* are the common *Shigella* species in developing countries.

Antimicrobial treatment can reduce morbidity, mortality and transmission. The antibiotics commonly used are trimethoprim-sulfamethoxazole, tetracycline, ciprofloxacin, chloramphenicol, and ampicillin. However, increasing antimicrobial resistance in *Shigella* spp has been reported worldwide (24, 94, 95).

In this study, the antimicrobial resistance against *Shigella* spp was observed in five agents; ampicillin, choramphenicol, nalidixic acid, trimethoprim-sulfamethoxazole and tetracycline. This indicated the emerging of multidrug resistance *Shigella* spp in Lao PDR. Fortunately, this pathogen was susceptible to ciprofloxacin and ceftriaxome (cepharosporins). These resistances indicate the need

for continuous monitoring of antibiotic resistance in order to update the recommendations for empirical antibiotic therapy of suspected shigellosis.

The previous reports showed resistance to tetracycline, trimethoprim-sulfamethoxazole and ampicillin in Thailand (20), Indonesia (96) and Vietnam (97). Whereas, there was no nalidixic acid resistance found among other Southeast Asia region except in Thailand (12, 98).

Staphylococcus aureus: is a gram-positive bacterium that commonly found on the skin and in the nose. *S. aureus* can cause a variety of infections, most notably skin, soft tissue, bone and bloodstream infections. It is the major cause of both hospital- and community-acquired infection.

Among all the antibiotic resistance achieved by *Staphylococcus aureus*, two most remarkable ones are methicillin and vancomycin resistance. The study in Lao PDR found *S. aureus* was the second most common cause of bacteremia, and was associated with a mortality rate of 17% (35).

The resistance to antibiotic especially Methicillin resistance *S. aureus* (MRSA), concerned organism in both communities-associated infection and hospital-associated infection were reported worldwide (99). In this study cefoxitin (FOX) was use as a surrogate for oxacillin and methicillin since 2013. Significant increasing resistances to penicillin group (ampicillin) from 2012 to 2015 were observed as well as increasing number of Fox-resistance in 2014 was remarked even though number of resistance was not high as report from other countries (12, 99).

CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

This is retrospective review of laboratory data on prevalence of bacterial infection from 8 types of specimen collected from 2012 to 2015. Specimens were processed according bacteriology disciplines: bacteriology culture, identification and antimicrobial susceptibility testing. Identified pathogens were tested for antimicrobial susceptibility testing aligned with CLSI standards. This study provided information of infection rate, factors contribution to infection, and drug susceptibility profile among four-year. The results can concluded that

1. The prevalence of at least one bacterial infection from 2012 to 2015 were 45.1%, 30.8%, 30.6% and 33.8% respectively. The decreasing prevalence was statistical significant. The results were represented Lao PDR situation, and could be used as reference value for future study. In addition, this might be the most successful of health policy in Lao PDR.
2. To analyze the factors that influenced to infection rate, the odd ratio was calculated. The factors that contributed to the infection prevalence were sex, age, region and source of collection. The female was 1.80 times more likely to have bacterial infection than male. The older population (> 5 years) was a significant higher risk to get infected than the younger population (OR = 2.27). In addition, the samples collected from NCLE, nosocomial infection, referral from network laboratories and outbreak investigation were 3.53, 4.69, 3.30 and 1.39 times more likely to have bacterial infection than diarrhea surveillance, respectively.
3. The antimicrobial susceptibility test was followed the procedure of NCLE and CLSI guideline. There were eleven bacterium selected to assess the susceptibility. Overall results, antimicrobial susceptibility profile were in satisfication level (susceptible). Except *E. coli*, *Klebsiella* spp, *Nisseria gonorrhoeae* and *Shigella* spp showed high

resistance rate to various antimicrobial agents. Moreover, these pathogens were defined as multidrug resistance (MDR), due to resistance at least three antimicrobial agents. Monitoring of AMR trend in the country is one of key supporting information for planning and control measure of AMR. Expanding site of specimen collection is required to representativeness of the country data.

REFERENCES

1. Hays J. FACTS AND DETAILS, Southeast Asia, Laos 2008 [updated May 2014. Available from: http://factsanddetails.com/southeast-asia/Laos/sub5_3d/entry-2982.html.

2. Agency CI. The World Factbook 2013-14. Washington, DC: Central Intelligence Agency, 2013 . Washington, DC2013-14 [Available from: https://www.cia.gov/library/publications/the-world-factbook/geos/la.html.

3. WHO and Ministry of Health LP. Health Service Delivery Profile, Lao PDR 2012 [Available from: http://www.wpro.who.int/health_services/service_delivery_profile_laopdr.pdf.

4. Murray PR, Rosenthal KS, Pfaller MA. Medical Microbiology: Elsevier Health Sciences; 2015.

5. Forbes BA, Sahm DF, Weissfeld AS, Bailey WR. Bailey & Scott's diagnostic microbiology: Elsevier Mosby; 2007.

6. Levinson W. Review of Medical Microbiology and Immunology, Eleventh Edition: McGraw-Hill Companies,Incorporated; 2010.

7. Raven PH, Mason KA, Johnson GB, Losos JB, Singer SR. Biology: McGraw-Hill; 2011.

8. Betty A. Forbes DFSaASW. Bailey & Scott's Diagnostic Microbiology. 12th ed: Mosby; 2007.

9. Beceiro A, Tomas M, Bou G. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clinical microbiology reviews. 2013;26(2):185-230.

10. Bhatia R, & Narain, J. P. The growing challenge of antimicrobial resistance in the South-East Asia Region - Are we losing the battle? Indian J Med Res, 132(5), . . The Indian journal of medical research. 2010;132(5):482-6.

11. Kang CI, Song JH. Antimicrobial resistance in Asia: current epidemiology and clinical implications. Infection & chemotherapy. 2013;45(1):22-31.

12. Lestari ES, Severin JA, Verbrugh HA. Antimicrobial resistance among pathogenic bacteria in Southeast Asia. The Southeast Asian journal of tropical medicine and public health. 2012;43(2):385-422.

13. Delgado-Valverde M, Sojo-Dorado J, Pascual A, Rodriguez-Bano J. Clinical management of infections caused by multidrug-resistant Enterobacteriaceae. Ther Adv Infect Dis. 2013;1(2):49-69.

14. Khennavong M, Davone V, Vongsouvath M, Phetsouvanh R, Silisouk J, Rattana O, et al. Urine antibiotic activity in patients presenting to hospitals in Laos: implications for worsening antibiotic resistance. The American journal of tropical medicine and hygiene. 2011;85(2):295-302.

15. World Health Organization., World Health Organization. Antimicrobial resistance : global report on surveillance.(In IRIS). Geneva: World Health Organization; 2014. xxii, 232 p. p.

16. Archawakulathep A, Ta Thi Kim C, Meunsene D, Handijatno D, Hassim HA, Rovira HRG, et al. Perspectives on Antimicrobial Resistance in Livestock and Livestock Products in ASEAN Countries. The Thai Journal of Veterinary Medicine. 2014;44(1):9.

17. Ashkenazi S. Shigella infections in children: new insights. Seminars in pediatric infectious diseases. 2004;15(4):246-52.

18. Shiferaw B, Solghan S, Palmer A, Joyce K, Barzilay EJ, Krueger A, et al. Antimicrobial susceptibility patterns of Shigella isolates in Foodborne Diseases Active Surveillance Network (FoodNet) sites, 2000-2010. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;54 Suppl 5:S458-63.

19. Yamashiro T, Nakasone N, Higa N, Iwanaga M, Insisiengmay S, Phounane T, et al. Etiological study of diarrheal patients in Vientiane, Lao People's Democratic Republic. Journal of clinical microbiology. 1998;36(8):2195-9.

20. Isenbarger DW, Hoge CW, Srijan A, Pitarangsi C, Vithayasai N, Bodhidatta L, et al. Comparative antibiotic resistance of diarrheal pathogens from Vietnam and Thailand, 1996-1999. Emerg Infect Dis. 2002;8(2):175-80.

21. Li Y, Xie X, Xu X, Wang X, Chang H, Wang C, et al. Nontyphoidal salmonella infection in children with acute gastroenteritis: prevalence, serotypes, and antimicrobial resistance in Shanghai, China. Foodborne pathogens and disease. 2014;11(3):200-6.

22. Sirichote P, Bangtrakulnonth A, Tianmanee K, Unahalekhaka A, Oulai A, Chittaphithakchai P, et al. Serotypes and antimicrobial resistance of Salmonella enterica ssp in central Thailand, 2001-2006. The Southeast Asian journal of tropical medicine and public health. 2010;41(6):1405-15.

23. Gebrekidan A, Dejene TA, Kahsay G, Wasihun AG. Prevalence and antimicrobial susceptibility patterns of Shigella among acute diarrheal outpatients in Mekelle hospital, Northern Ethiopia. BMC Res Notes. 2015;8:611.

24. Haifei Yang GC, Yulin Zhu, Yanyan Liu, Jun Cheng, Lifen Hu, Ying Ye, Jiabin Li. Surveillance of Antimicrobial Susceptibility Patterns among Shigella Species Isolated in China during the 7-Year Period of 2005-2011. Ann Lab Med. 2013;33(2)(Mar):111-5.

25. Lee WS, Puthucheary SD, Parasakthi N, Choo KE. Antimicrobial susceptibility and distribution of non-typhoidal Salmonella serovars isolated in Malaysian children. Journal of tropical pediatrics. 2003;49(1):37-41.

26. Ruppé É, Woerther PL, Barbier F. Mechanisms of antimicrobial resistance in Gram-negative bacilli. Ann Intensive Care. 2015;5(1):61.

27. Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clinical microbiology reviews. 2009;22(4):582-610.

28. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2009;49(11):1749-55.

29. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. CLSI document. 2015;35(3).

30. L. Barth Reller MW, James H. Jorgensen, Mary Jane Ferraro. Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2009;49(11):1749-55.

31. LESLIE SPEEGLE MEM, STEFFEN BACKERT, OMAR A. OYARZABAL. Use of Cellulose Filters To Isolate Campylobacter spp. from Naturally Contaminated Retail Broiler Meat. Journal of food protection. 2009;72(12):2592–6.

32. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. Journal of clinical microbiology. 1991;29(2):297-301.

33. Patrick A.D. Grimont F-XW. ANTIGENIC FORMULAE OF THE SALMONELLA SEROVARS. 9th ed. Paris, France: WHO Collaborating Center for Reference and Research on Salmonella; 2007.

34. Van der Ploeg CA, Viñas, María R, Terragno, Raquel, Bruno, Susana B,, Binsztein N. Laboratory Protocol: "Serotyping of Shigella spp.". WHO Global Foodborne Infections Network; 2010. p. 24.

35. Phetsouvanh R, Phongmany S, Soukaloun D, Rasachak B, Soukhaseum V, Soukhaseum S, et al. Causes of community-acquired bacteremia and patterns of antimicrobial resistance in Vientiane, Laos. Am J Trop Med Hyg. 2006;75(5):978-85.

36. Goyet S, Vlieghe E, Kumar V, Newell S, Moore CE, Bousfield R, et al. Etiologies and resistance profiles of bacterial community-acquired pneumonia in Cambodian and neighboring countries' health care settings: a systematic review (1995 to 2012). PLoS One. 2014;9(3):e89637.

37. Kumari N, Rai A, Jaiswal CP, Xess A, Shahi SK. Coagulase negative Staphylococci as causative agents of urinary tract infections-prevalence and resistance status in IGIMS, Patna. Indian J Pathol Microbiol. 2001;44(4):415-9.

38. Magliano E, Grazioli V, Deflorio L, Leuci AI, Mattina R, Romano P, et al. Gender and age-dependent etiology of community-acquired urinary tract infections. ScientificWorldJournal. 2012;2012:349597.

39. Simon J. Tsiouris MaFDL, MD. Gender and Bacterial Infections. 2004 [cited 05/05/2017]. In: Principles of Gender-Specific Medicine [Internet]. Academic Press, [cited 05/05/2017]; [1396].

40. Sangkitporn S. Antimicrobial Resistance: Global and National Status http://narst.dmsc.moph.go.th/: National Antimicrobial Resistance Surveillance Center, Thailand; 2017 [cited 2017 June 2017]. Available from: http://narst.dmsc.moph.go.th/data/AMR_Global and National Status.pdf.

41. Janda JM, Abbott SL. Evolving concepts regarding the genus Aeromonas: an expanding Panorama of species, disease presentations, and unanswered questions. Clin Infect Dis. 1998;27(2):332-44.

42. Motyl MR, McKinley G, Janda JM. In vitro susceptibilities of Aeromonas hydrophila, Aeromonas sobria, and Aeromonas caviae to 22 antimicrobial agents. Antimicrob Agents Chemother. 1985;28(1):151-3.

43. Overman TL, Janda JM. Antimicrobial susceptibility patterns of Aeromonas jandaei, A. schubertii, A. trota, and A. veronii biotype veronii. J Clin Microbiol. 1999;37(3):706-8.

44. Vila J, Marco F, Soler L, Chacon M, Figueras MJ. In vitro antimicrobial susceptibility of clinical isolates of Aeromonas caviae, Aeromonas hydrophila and Aeromonas veronii biotype sobria. J Antimicrob Chemother. 2002;49(4):701-2.

45. Aravena-Román M, Inglis TJ, Henderson B, Riley TV, Chang BJ. Antimicrobial susceptibilities of Aeromonas strains isolated from clinical and environmental sources to 26 antimicrobial agents. Antimicrob Agents Chemother. 2012;56(2):1110-2.

46. Maluping RP, Lavilla-Pitogo CR, DePaola A, Janda JM, Krovacek K, Greek C. Antimicrobial susceptibility of Aeromonas spp., Vibrio spp. and Plesiomonas shigelloides isolated in the Philippines and Thailand. International Journal of Antimicrobial Agents 2005;25 345–53

47. Bennett JE, Dolin R, Blaser MJ. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 197 – Staphylococcus epidermidis and Other Coagulase-Negative Staphylococci. Eighth Edition ed. Canada: ELSEVIER 2015.

48. Koksal F, Yasar H, Samasti M. Antibiotic resistance patterns of coagulase-negative staphylococcus strains isolated from blood cultures of septicemic patients in Turkey. Microbiol Res. 2009;164(4):404-10.

49. Seng R, Leungtongkam U, Thummeepak R, Chatdumrong W, Sitthisak S. High prevalence of methicillin-resistant coagulase-negative staphylococci isolated from a university environment in Thailand. Int Microbiol. 2017;20(2):65-73.

50. de Allori MC, Jure MA, Romero C, de Castillo ME. Antimicrobial resistance and production of biofilms in clinical isolates of coagulase-negative Staphylococcus strains. Biol Pharm Bull. 2006;29(8):1592-6.

51. Bartlett JG. Changing trends in bacterial infections: Staphylococcus aureus, bacterial pneumonia, Clostridium difficile. Top HIV Med. 2007;15(3):94-8.

52. Hout B. Drug resistance in bacteria isolated from patients presenting with wounds at a non-profit Surgical Center in Phnom Penh, Cambodia from 2011–2013. Tropical Diseases,
Travel Medicine and Vaccines. 2015;1(4).

53. Jett BD, Huycke MM, Gilmore MS. Virulence of enterococci. Clin Microbiol Rev. 1994;7(4):462-78.

54. French GL. Enterococci and vancomycin resistance. Clin Infect Dis. 1998;27 Suppl 1:S75-83.

55. Chaiwong T, Srivoramas T, Panya M, Wanram S, Panomket P. Antibiotic resistance patterns of Enterococcus spp. isolated from Musca domestica and Chrysomya megacephala in ubon Ratchathani. J Med Assoc Thai. 2014;97 Suppl 4:S1-6.

56. Danchaivijitr S, Dhiraputra C, Rongrungruang Y, Worajitr M, Jintanothaitavorn D. Antimicrobial susceptibility of community and hospital acquired bacteria. J Med Assoc Thai. 2005;88 Suppl 10:S14-25.

57. Thapa B, Tattawasart U, Manjai A, Chantarasuk Y. Antimicrobial Resistance and Species Prevalence of Enterococcal
Isolates in Srinagarind Hospital, Northeastern Thailand. KKU Res J (GS) 2007;7 (4)(October - December):97-108.

58. Srifuengfung S, Techachaiwiwat W, Sanpanya, S, Dhiraputra C. The antibiotic susceptibility of Enterococcus species in Siriraj Hospital during 1994 and 2001. J Infect Dis Antimicrob Agents. 2004;May- August(2):47-52.

59. Erb A, Stürmer T, Marre R, Brenner H. Prevalence of antibiotic resistance in Escherichia coli: overview of geographical, temporal, and methodological variations. Eur J Clin Microbiol Infect Dis. 2007;26(2):83-90.

60. Cheong YM, Fairuz A, Jegathesan M. Antimicrobial resistance pattern of bacteria isolated from patients seen by private practitioners in the Klang Valley. Singapore Med J. 1995;36(1):43-6.

61. Jean SS, Coombs G, Ling T, Balaji V, Rodrigues C, Mikamo H, et al. Epidemiology and antimicrobial susceptibility profiles of pathogens causing urinary tract infections in the Asia-Pacific region: Results from the Study for Monitoring

Antimicrobial Resistance Trends (SMART), 2010-2013. Int J Antimicrob Agents. 2016;47(4):328-34.

62. Lim C, Takahashi E, Hongsuwan M, Wuthiekanun V, Thaalikitkul V, Hinjoy S, et al. Epidemiology and burden of multidrug-resistant bacterial infection in a developing country. eLife. 2016;5: e18082.

63. Bell JM, Turnidge JD, Gales AC, Pfaller MA, Jones RN, Group SAS. Prevalence of extended spectrum beta-lactamase (ESBL)-producing clinical isolates in the Asia-Pacific region and South Africa: regional results from SENTRY Antimicrobial Surveillance Program (1998-99). Diagn Microbiol Infect Dis. 2002;42(3):193-8.

64. Ristuccia PA, Cunha BA. Klebsiella. Infection Control & Hospital Epidemiology. 1984;5 (7):343-7.

65. Kusum M, Wongwanich S, Dhiraputra C, Pongpech P, Naenna P. Occurrence of extended-spectrum beta-lactamase in clinical isolates of Klebsiella pneumoniae in a University Hospital, Thailand. J Med Assoc Thai. 2004;87(9):1029-33.

66. Unemo M, Shafer WM. Antimicrobial resistance in Neisseria gonorrhoeae in the 21st century: past, evolution, and future. Clin Microbiol Rev. 2014;27(3):587-613.

67. Clark RB, Lister PD, Arneson-Rotert L, Janda JM. In vitro susceptibilities of Plesiomonas shigelloides to 24 antibiotics and antibiotic-beta-lactamase-inhibitor combinations. Antimicrob Agents Chemother. 1990;34(1):159-60.

68. Holmberg SD, Wachsmuth IK, Hickman-Brenner FW, Blake PA, Farmer JJ. Plesiomonas enteric infections in the United States. Ann Intern Med. 1986;105(5):690-4.

69. Auckenthaler R, Michéa-Hamzehpour M, Pechère JC. In-vitro activity of newer quinolones against aerobic bacteria. J Antimicrob Chemother. 1986;17 Suppl B:29-39.

70. Brenden RA, Miller MA, Janda JM. Clinical disease spectrum and pathogenic factors associated with Plesiomonas shigelloides infections in humans. Rev Infect Dis. 1988;10(2):303-16.

71. Hossain MS, Hossain A. Effect of antibiotics on serum bactericidal action on Plesiomonas shigelloides by normal human serum. Bangladesh Med Res Counc Bull. 1994;20(3):117-22.

72. Kain KC, Kelly MT. Antimicrobial susceptibility of Plesiomonas shigelloides from patients with diarrhea. Antimicrob Agents Chemother. 1989;33(9):1609-10.

73. Reinhardt JF, George WL. Plesiomonas shigelloides-associated diarrhea. JAMA. 1985;253(22):3294-5.

74. Stock I, Wiedemann B. Natural antimicrobial susceptibilities of Plesiomonas shigelloides strains. J Antimicrob Chemother. 2001;48(6):803-11.

75. Visitsunthorn N, Komolpis P. Antimicrobial therapy in Plesiomonas shigelloides-associated diarrhea in Thai children. Southeast Asian J Trop Med Public Health. 1995;26(1):86-90.

76. von Graevenitz A. Manual of clinical microbiology. 4th ed. Washington, DC: American Society for Microbiology; 1985.

77. Wong TY, Tsui HY, So MK, Lai JY, Lai ST, Tse CW, et al. Plesiomonas shigelloides infection in Hong Kong: retrospective study of 167 laboratory-confirmed cases. Hong Kong Med J. 2000;6(4):375-80.

78. Moniri R, Mosayebi Z, Movahedian A. Increasing Trend of Antimicrobial Drug-Resistance in Pseudomonas aeruginosa Causing Septicemia. Iranian Journal of Public Health 2006;35(1):58-62.

79. Mansoor T, Musani MA, Khalid G, Kamal M. Pseudomonas aeruginosa in chronic suppurative otitis media: sensitivity spectrum against various antibiotics in Karachi. J Ayub Med Coll Abbottabad. 2009;21(2):120-3.

80. Tripathi P, Banerjee G, Saxena S, Gupta MK, Ramteke PW. Antibiotic resistance pattern of Pseudomonas aeruginosa isolated from patients of lower respiratory tract infection African Journal of Microbiology Research. 2011;5(19) (September 2011):2955-9.

81. Syrmis MW, O'Carroll MR, Sloots TP, Coulter C, Wainwright CE, Bell SC, et al. Rapid genotyping of Pseudomonas aeruginosa isolates harboured by adult and paediatric patients with cystic fibrosis using repetitive-element-based PCR assays. J Med Microbiol. 2004;53(Pt 11):1089-96.

82. Szabó D, Szentandrássy J, Juhász Z, Katona K, Nagy K, Rókusz L. Imported PER-1 producing Pseudomonas aeruginosa, PER-1 producing Acinetobacter baumanii and VIM-2-producing Pseudomonas aeruginosa strains in Hungary. Ann Clin Microbiol Antimicrob. 2008;7:12.

83. Hirsch EB, Tam VH. Impact of multidrug-resistant Pseudomonas aeruginosa infection on patient outcomes. Expert Rev Pharmacoecon Outcomes Res. 2010;10(4):441-51.

84. Chang Y-T, Coombs G, Ling T, Balaji V, Rodrigues C, Mikamo H, et al. Epidemiology and trends in the antibiotic susceptibilities of Gram-negative bacilli isolated from patients with intra-abdominal infections in the Asia-Pacific region, 2010–2013. International Journal of Antimicrobial Agents 2017;49 (2017) 734–9.

85. Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, et al. The global burden of nontyphoidal Salmonella gastroenteritis. Clin Infect Dis. 2010;50(6):882-9.

86. Trung NV, Carrique-Mas JJ, Nghia NH, Tu LT, Mai HH, Tuyen HT, et al. Non-Typhoidal Salmonella Colonization in Chickens and Humans in the Mekong Delta of Vietnam. Zoonoses Public Health. 2017;64(2):94-9.

87. Lee HY, Su LH, Tsai MH, Kim SW, Chang HH, Jung SI, et al. High rate of reduced susceptibility to ciprofloxacin and ceftriaxone among nontyphoid Salmonella clinical isolates in Asia. Antimicrob Agents Chemother. 2009;53(6):2696-9.

88. Wannaprasat W, Padungtod P, Chuanchuen R. Class 1 integrons and virulence genes in Salmonella enterica isolates from pork and humans. Int J Antimicrob Agents. 2011;37(5):457-61.

89. Tiong V, Thong KL, Yusof MY, Hanifah YA, Sam JI, Hassan H. Macrorestriction analysis and antimicrobial susceptibility profiling of Salmonella enterica at a University Teaching Hospital, Kuala Lumpur. Jpn J Infect Dis. 2010;63(5):317-22.

90. Benacer D, Thong KL, Watanabe H, Puthucheary SD. Characterization of drug resistant Salmonella enterica serotype Typhimurium by antibiograms, plasmids, integrons, resistance genes and PFGE. J Microbiol Biotechnol. 2010;20(6):1042-52.

91. Ta YT, Nguyen TT, To PB, Pham dX, Le HT, Thi GN, et al. Quantification, serovars, and antibiotic resistance of salmonella isolated from retail raw chicken meat in Vietnam. J Food Prot. 2014;77(1):57-66.

92. Meng CY, Smith BL, Bodhidatta L, Richard SA, Vansith K, Thy B, et al. Etiology of diarrhea in young children and patterns of antibiotic resistance in Cambodia. Pediatr Infect Dis J. 2011;30(4):331-5.

93. Bukitwetan P, Suryawidjaja JE, Salim OCh, Hidayat A, Herwana E, Lesmana M. Serovar distribution and antibiotic susceptibility of nontyphoidal Salmonella isolated from pediatric patients in Jakarta, Indonesia. Southeast Asian J Trop Med Public Health. 2007;38(6):1088-94.

94. Bhattacharya D, Sugunan AP, Bhattacharjee H, Thamizhmani R, Sayi DS, Thanasekaran K, et al. Antimicrobial resistance in Shigella--rapid increase & widening of spectrum in Andaman Islands, India. Indian J Med Res. 2012;135:365-70.

95. Ashkenazi S, Levy I, Kazaronovski V, Samra Z. Growing antimicrobial resistance of Shigella isolates. J Antimicrob Chemother. 2003;51(2):427-9.

96. Tjaniadi P, Lesmana M, Subekti D, Machpud N, Komalarini S, Santoso W, et al. Antimicrobial resistance of bacterial pathogens associated with diarrheal patients in Indonesia. Am J Trop Med Hyg. 2003;68(6):666-70.

97. Nguyen TV, Le PV, Le CH, Weintraub A. Antibiotic resistance in diarrheagenic Escherichia coli and Shigella strains isolated from children in Hanoi, Vietnam. Antimicrob Agents Chemother. 2005;49(2):816-9.

98. Oyofo BA, Lesmana M, Subekti D, Tjaniadi P, Larasati W, Putri M, et al. Surveillance of bacterial pathogens of diarrhea disease in Indonesia. Diagn Microbiol Infect Dis. 2002;44(3):227-34.

99. Chen CJ, Huang YC. New epidemiology of Staphylococcus aureus infection in Asia. Clin Microbiol Infect. 2014;20(7):605-23.

APPENDICES

APPENDIX A

STOOL CULTURE MIXED INFECTION

Table A1. Mixed infection of organism more than 1 in Stool culture from 2012 to 2015

2012		2013		2014		2015	
Pathogens	**(n)**	**Pathogens**	**(n)**	**Pathogens**	**(n)**	**Pathogens**	**(n)**
Aeromonas spp + P. shigelliodes	1	*Aeromonas spp + P. shigelliodes*	1	*Aeromonas spp + P. shigelloides*	2	*Aeromonas spp + P. shigelliodes*	2
Aeromonas spp + Vibrio spp	1	*P. shigelloides + Vibrio spp*	1	*Aeromonas spp + Vibrio spp*	2	EHEC + Rotavirus	1
EHEC + EIEC	2	Rotavirus + *Campylobacter spp*	1	EPEC + Rotavirus	1	EIEC + Rotavirus	2
EHEC + ETEC.LT	4	Rotavirus + *Salmonella spp*	5	*P. shigelloides + Vibrio spp*	2	EPEC + ETEC.LT	2
EHEC + ETEC.LT + ETEC.ST	2	Rotavirus + *Shigella spp*	3	*Salmonella spp + P. shigelloides*	1	EPEC + ETEC.LT + Rotavirus	1
EHEC + ETEC.ST	3	*Salmonella spp + P. shigelloides*	2	*Shigella spp + Aeromonas spp*	1	EPEC + Rotavirus	16
EHEC + ETEC.ST + *Salmonella spp*	1	*Salmonella spp + Shigella spp*	1	*Shigella spp + P. shigelloides*	2	ETEC.LT + Rotavirus	3
EHEC + *P. shigelloides*	2	*Shigella spp + P. shigelloides*	1			*P. shigelloides + Vibrio spp*	1
EHEC + *Salmonella spp*	1					Rotavirus + *Aeromonas spp*	2
EIEC + *Shigella spp*	1					Rotavirus + *Salmonella spp*	2
EIEC + *P. shigelloides*	1					*Rotavirus + Salmonella spp + Aeromonas spp*	1
EIEC + ETEC.LT	2					*Salmonella spp + Aeromonas spp*	3
EIEC + ETEC.ST	1					*Salmonella spp + P. shigelloides*	4
EIEC + Rotavirus	1					*Salmonella spp + Vibrio spp*	3

2012		2013		2014		2015	
Pathogens	**(n)**	**Pathogens**	**(n)**	**Pathogens**	**(n)**	**Pathogens**	**(n)**
EIEC + *Samonella spp + Shigella spp*	1					*Salmonella spp + Vibrio spp + P. shigelloides*	1
EIEC + *Shigella spp*	5					*Shigella spp + Aeromonas spp*	1
EIEC + *Shigella spp + Aeromonas spp*	1						
EPEC + *Aeromonas spp*	1						
EPEC + *Campylobacter spp*	1						
EPEC + EIEC	3						
EPEC + ETEC.LT	9						
EPEC + ETEC.LT + ETEC.ST	4						
EPEC + ETEC.ST	5						
EPEC + *P. shigelloides*	1						
EPEC + Rotavirus	1						
EPEC + *Salmonella spp*	5						
ETEC.LT + *Aeromonas spp*	2						
ETEC.LT + ETEC.ST	8						
ETEC.LT + *P. shigelloides*	3						
ETEC.LT + *Salmonella spp*	2						
ETEC.ST + *P. shigelloides*	1						
ETEC.ST + *Salmonella spp*	1						
Rotavirus + *Salmonella spp*	1						
Salmonella spp + Aeromonas spp	2						
Salmonella spp + Vibrio spp	1						
Shigella spp + Aeromonas spp	1						
Total	**82**		**15**		**11**		**45**

APPENDIX B

PUS CULTURE MIXED INFECTION

Table B1. Mixed infection of organism more than 1 in pus culture from 2012 to 2015

2012		2013		2014		2015	
Pathogens	**(n)**	**Pathogens**	**(n)**	**Pathogens**	**(n)**	**Pathogens**	**(n)**
Klebsiella spp + Pseudomonas spp	2	*Acinetobacter spp + Enterobacter spp*	1	*Acinetobacter spp + Pseudomonas spp*	1	Citrobacter spp + Pseudomonas spp	1
Staphylococcus aureus + Group A *Streptococcus*	1	*Acinetobacter spp + Enterococcus spp*	1	*Enterobacter spp + Acinetobacter spp*	1	Enterobacter spp + Enterococcus spp + Staphylococcus aureus	1
Vibrio fluvialis + Acinetobactor spp	1	*Acinetobacter spp + Pseudomonas spp*	1	*Enterococcus spp + Klebsiella spp + Pseudomonas spp*	1	Enterobacter spp + Serratia spp	1
		Enterobacter spp+ Burkholderia pseudomallei	1	*Escherichia coli + Citrobacter spp + Pseudomonase s*	1	Escherichia coli + Enterococcus spp	1
		Escherichia coli + Pseudomonas spp	1	*Escherichia coli + Enterococcus spp*	1	Escherichia coli + Klebsiella spp	2
		Escherichia coli + Acinetobacter spp + Pseudomonas spp	1	*Escherichia coli + Klebsiella spp*	1	Escherichia coli + Proteus spp	1
		Klebsiella spp + Aeromonas spp	1	*Escherichia coli + Klebsiella spp + Proteus spp*	1	Escherichia coli + Pseudomonas spp	1
		Klebsiella spp + Coagulase Negative *Staphylococcus*	1	*Klebsiella spp + Acinetobacter spp*	1	Escherichia coli + Staphylococcus aureus	1
		Klebsiella spp + Enterobacter spp	2	*Klebsiella spp + Acinetobacter spp + Pseudomonas sp*	1	Escherichia coli + Staphylococcus aureus + Proteus spp	1
		Klebsiella spp + Group D *Streptococcus*	1	*Klebsiella spp + Citrobacter spp*	1	Klebsiella spp + Morganella spp	1

2012		2013		2014		2015	
Pathogens	**(n)**	**Pathogens**	**(n)**	**Pathogens**	**(n)**	**Pathogens**	**(n)**
		Klebsiella spp + Serratia marcescens	1	*Klebsiella spp* + Group A *Streptococcus*	1	Staphylococcus aureus + Enterobacter spp	2
		Proteus spp + Citrobacter spp	1	*Klebsiella spp + Morganella spp*	1	Staphylococcus aureus + Group A Streptococcus	4
		Proteus spp + Enterobacter spp	1	*Klebsiella spp + Pseudomonas spp*	1	Staphylococcus aureus + Morganella spp	1
		Pseudomonas spp+ Coagulase Negative Staphylococcus	1	*Klebsiella spp + Staphylococcus aureus*	1	Staphylococcus aureus + Pseudomonas spp + Enterobacter spp.	1
		Staphylococcus aureus + Group A Streptococcus	3	*Proteus spp + Enterococcus spp*	1		
				Proteus spp + Pseudomonas spp	1		
				Staphyloccocus aureus + Psuedomonas spp	1		
				Staphylococcus aureus + Enterobacter spp	1		
				Staphylococcus aureus + Group A *Streptococcus*	1		
				Staphylococcus aureus + Pseudomonas spp + Enterobacter spp	1		
Total	**4**		**18**		**20**		**19**

APPENDIX C

VAGINAL DISCHARGE CULTURE MIXED INFECTION

Table C1. Mixed infection of organism more than 1 in Vaginal discharge culture from 2012 to 2015

2012		2013		2014		2015	
Pathogens	**(n)**	**Pathogens**	**(n)**	**Pathogens**	**(n)**	**Pathogens**	**(n)**
Candida spp + Bacterial vaginosis	28	*Candida spp* + Bacterial vaginosis	49	*Candida spp* + Bacterial vaginosis	39	*Candida spp* + Bacterial vaginosis	40
Enterobacter spp + *Citrobacter spp*	1	*Citrobacter spp* + *Candida spp*	1	*Citrobacter spp* + Bacterial vaginosis	1	Coagulase Negative *Staphylococcus* + Bacterial vaginosis	1
Escherichia coli + Bacterial vaginosis	2	Coagulase Negative *Staphylococcus* + *Candida spp*	1	*Enterococcus spp* + Bacterial vaginosis	1	*Enterobacter spp* + Bacterial vaginosis	2
Escherichia coli + *Candida spp*	2	*Enterobacter spp* + Bacterial vaginosis	1	Enterococcus spp + Candida spp	1	*Enterococcus spp* + Bacterial vaginosis	2
Escherichia coli + *Candida spp* + Bacterial vaginosis	1	*Enterobacter spp* + Group D *Streptococcus*	1	*Escherichia col*i + Bacterial vaginosis	3	*Enterococcus spp* + *Candida spp*	2
Group B *Streptococcus* + *Candida spp* + Bacterial vaginosis	2	Enterococcus spp + Bacterial vaginosis	7	*Escherichia coli* + *Candida spp* + Bacterial vaginosis	1	*Enterococcus spp* + *Candida spp* + Bacterial vaginosis	1
Group D *Streptococcus* + 2 Bacterial vaginosis	1	*Enterococcus spp* + *Candida spp* + Bacterial vaginosis	1	*Escherichia coli* + *Enterococcus spp* + Bacterial vaginosis	1	*Escherichia coli* + Bacterial vaginosis	2
Neisseria gonorrhoeae + Bacterial vaginosis	1	*Escherichia coli* + Bacterial vaginosis	5	Group B *Streptococcus* + Bacterial vaginosis	2	*Escherichia coli* + *Candida spp*	1
		Escherichia coli + *Candida spp*	3	Group B *Streptococcus* + *Candida spp* + Bacterial vaginosis	1	*Escherichia coli* + *Enterococcus spp* + Bacterial vaginosis	1

2012	2013		2014		2015	
Pathogens	**(n)**	**Pathogens**	**(n)**	**Pathogens**	**(n)**	**Pathogens**	**(n)**
		Escherichia coli + *Candida spp* + Bacterial vaginosis	3	Group B *Streptococcus* + *Escherichia coli* + Bacterial vaginosis	2	*Escherichia coli* + Group B *Streptococcus* + Bacterial vaginosis	1
		Escherichia coli + *Enterococcus spp* + *Candida spp*	1	*Haemophilus spp* + Bacterial vaginosis	2	Group B *Streptococcus* + Bacterial vaginosis	6
		Group B *Streptococcus* + *Candida spp*	1	*Klebsiella spp* + *Candida spp*	2	Group B *Streptococcus* + *Candida spp*	1
		Group D *Streptococcus* + Bacterial vaginosis	5	*Neisseria gonorrhoeae* + Bacterial vaginosis	3	Group B *Streptococcus* + *Candida spp* + Bacterial vaginosis	4
		Haemophilus spp + *Candida spp* + Bacterial vaginosis	1	*Pseudomonas spp* + *Candida albicans* + Bacterial vaginosis	1	Group D *Streptococcus* + Bacterial vaginosis	1
		Klebsiella spp + Bacterial vaginosis	1	*Stephylococcus aureus* + *Candida spp*	1	*Klebsiella spp* + Bacterial vaginosis	1
		Klebsiella spp + *Candida spp*	1			*Klebsiella spp* + Group B *Streptococcus*	1
		Neisseria gonorrhoeae + Bacterial vaginosis	1			*Klebsiella spp* + Group B *Streptococcus* + Bacterial vaginosis	1
		Staphylococcus aureus + *Candida spp*	1			*Neisseria gonorrhoeae* + Bacterial vaginosis	1
						Niesseria gonorrhoeae + Group B *Streptococcus*	1
						Staphylococcus aureus + Bacterial vaginosis	1
						Trichomonas vaginalis + Bacterial vaginosis	2
Total	**38**		**84**		**61**		**73**

APPENDIX D

URINE CULTURE MIXED INFECTION

Table D1. Mixed infection of organism more than 1 in urine culture from 2012 to 2015

2012		2013		2014		2015	
Pathogens	**(n)**	**Pathogens**	**(n)**	**Pathogens**	**(n)**	**Pathogens**	**(n)**
Klebsiella spp + Proteus spp	*1*	Group D *Streptococcus + Enterobacter spp*	1	*Acinetobacter spp + Pseudomonas spp*	1	*Acinetobacter spp + Chromobacterium violaceum*	1
		Klebsiella spp + Morganella spp	1	*Citrobacter spp + Morganella spp*	1	*Citrobacter spp + Enterobacter spp*	1
				Enterobacter spp + Citrobacter spp	1	*Enterococcus spp + Candida spp*	1
				Escherichia coli + Klebsiella spp	1	*Enterococcus spp + Klebsiella spp + Pseudomonas spp*	1
				Escherichia coli + Klebsiella spp + Pantoea spp	1	*Escherichia coli* + Coagulase Negative *Staphylococcus*	1
				Escherichia coli + Klebsiella spp + Pseudomonas spp	1	*Escherichia coli + Morganella morganii*	1
				Escherichia coli + Pseudomonas spp	3	*Klebsiella spp + Enterococcus spp*	1
				Group B Streptococcus + *Escherichia coli*	1	*Klebsiella spp + Proteus spp*	2
				Klebsiella spp + Pseudomonas spp	1	*Morganella spp* + Coagulase Negative *Staphylococcus*	1
				Proteus spp + Pseudomonas spp	1	*Enterobacter spp + Proteus spp*	2
Total	**1**		**2**		**12**		**12**

BIOGRAPHY

Name	Miss Phanthaneeya Teepruksa
Date of Birth	July 08, 1979
Educational Attainment	1997 - 2000: Bachelor of Sciences (Medical Technology) 2014 - Present: Master of Sciences (Bioclinical Sciences)
Work Position	Laboratory (Microbiology) World Health Organization, Lao PDR
Work Experiences	September 2011 - Present: Laboratory (Bacteriology Expert) World Health Organization, Lao PDR PDR October 2006-2011: Laboratory Supervisor (Immunodiagnostics) US Naval Medical Research Unit no. 2 (US NAMRU-2), Phnom penh, Cambodia August 2003-2006: Medical Technologist Bumrungrad Internatoinal Hospital, Bangkok November 2002- July 2003: Research assistant Center of Vaccine Development, Institute of Science and Technology for Development, Mahidol University, Salaya, Nakornpathom July 2001-2002: Research assistant Shoklo Malaria Research Unit (SMRU) part of Wellcome-Mahidol University-Oxford Tropical Medicine Research Program, Mae sot, Tak