Antimicrobial Resistance (AMR)

Tricycle Program

"Cambodia, Lao P.D.R and Myanmar"

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Importance of Tricycle Program

The World Health Organization (WHO), through its Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) program, has initiated recently the One Health monitoring and surveillance of Extended Spectrum Beta-Lactamase producing E. coli (ESBL Ec) in humans clinical, the food-chain (animal, agricultural) and environmental samples through its so-called Tricycle Project [1] or Tricycle Program. ESBL-Ec were responsible for severe morbidity and mortality in humans, with significant associated health costs and disease burdens. Therefore, this project supports the concept that a simplified, integrated, trans-sectoral surveillance system of bacterial resistance to antibiotics could be implemented on a global [2] and regional basis.

The Tricycle Project aims to strengthen understanding of the impacts and risks of antimicrobial use; and to inform effective response strategies to reduce the impact of Antimicrobial Resistance (AMR) at the human-animal-plant-food-environment interface [3]. This project not only seeks to address the lack of data on the contribution of the environment, but also seeks to overcome the barriers to integrated surveillance in low- and middle-income countries (LMICs) by focusing on a single indicator "ESBL Ec" [4]. Therefore, the surveillance protocol on the Tricycle Project aims to understand the magnitude of AMR along the food chain in different countries and regions [5]. It would bring critical, timely and invaluable information to assess the efficacy of the various control programs of AMR overtime [2].

Global and Regional Perspective

Global Perspective

As part of AGISAR meeting, the first expert meeting for surveillance protocol development for the WHO Integrated Global Survey on ESBL Ec Tricycle Project, has been conducted. The WHO Integrated Global Survey protocol has been developed, with the support of AGISAR members and experts from WHO Collaborating Centres, to support the establishment and implementation of multi-sectoral integrated surveillance in a simple and doable way. This Tricycle Project with One Health approach in the Member States is based on one indicator, ESBL producing E. coli. Some work packages (WP) that have been discussed are: surveillance in humans, surveillance in the food chain, surveillance in the environment, molecular characterization, epidemiology design and analysis, antimicrobial usage (AMU), and management coordination [5,6].

Tricycle Project was carried out and facilitated by enhanced collaboration across the Tripartite and United Nations Environment Programme (UNEP). Currently, 8 countries (i.e. Ghana, Indonesia, Madagascar, Malaysia, Pakistan, Senegal, Bangladesh, and Sri Lanka) in WHO Regions were piloting and participating in this ESBL Ec Tricycle Project [3,6,7]. On the other hand, The Fleming Fund also will provide ongoing support to Tricycle coordination and training, and providing regional technical assistance and quality assurance in 22 countries across regions (i.e. Eswatini, Kenya, Malawi, Tanzania, Uganda, Zambia, Zimbabwe, Burkina Faso, Ghana, Nigeria, Senegal, Sierra Leone, Bangladesh, Bhutan, Indonesia, Nepal, Sri Lanka, Myanmar, Laos, Pakistan, Timor-Leste, Papua New Guinea) [8].

ESBL-Ec are diverse and essentially ubiquitous microorganisms that can readily cross barriers between humans, animals and the environment in a cyclic and reciprocating manner [2]. Therefore, method for surveillance protocol on the Tricycle Project [Table 1] uses the frequency comparative of a single and highly representative indicator, ESBL Ec in humans, the food chain, and the environment [5]. This work would link to existing data collection activities and sources [3]. However, updates of further developments will be re-evaluated after the pandemic outbreak of the Corona virus-2019 novel.

Component	Details
Sample sources	Specimens from humans, foods (retail meats), animals (intended for foods), environment
Target bacteria	Escherichia coli (E. coli)
Sampling design	Source, information, design, frequency
Laboratory testing methodology	Bacterial culture, isolate identification, standardized AST, quality control, recommended antimicrobials for surveillance, characterization
Data management, analysis, reporting	Sample information, culture results, AST results, additional lab tests, software (WHONET)

Table 1. Component of Tricycle Program [9]

Some research and innovation objectives and activities that have been set up, such as: (a) improve and standardize AMR surveillance systems, from sampling to data analysis including sampling frame, tools, methodology and reporting; (b) strengthen the use of surveillance data to identify human and non-human reservoirs of AMR; (c) optimize the use of surveillance data to estimate burden and assess the impact of interventions; (d) develop novel techniques to supplement and promote the exchange of surveillance data Research; (e) improve and standardize the surveillance system of antibiotic use Information; (f) determine and model the contribution of contamination sources, environmental reservoirs and exposure routes on the emergence and spread of AMR; (g) evaluate the relationship between AMR and the environment, climate change, and pollution; (h) assess the potential impact of industrial systems on AMR in the environment Applied, (i) develop innovative technological, policy, social, economic and regulatory approaches to mitigate AMR in the environment [1].

Regional Perspective

Currently, 5 countries (i.e. Indonesia, Malaysia, Pakistan, Bangladesh, and Sri Lanka) in Asia Regions were piloting and participating in this ESBL Ec Tricycle Project [3,6,7]. Tricycle Project was carried out and facilitated by enhanced collaboration across the Tripartite and UNEP. On the other hand, The Fleming Fund also will provide ongoing support to Tricycle coordination and training, and providing regional technical assistance and quality assurance for 9 countries in Asia regions (i.e. Bangladesh, Bhutan, Indonesia, Nepal, Sri Lanka, Myanmar, Laos, Pakistan, Timor-Leste) [8].

Related to the methodology, the impact of different farming practices and settings on the emergence, transmission and persistence of AMR needs to be assessed [1]. Therefore, The Tripartite will carry out work [Figure 1] at the country level in collaboration with UNEP and national laboratories and academia in the human, animal, plant, food, and environmental sectors, to carry out assessments of antimicrobial residues and AMR at the critical control points in manufacture, distribution, use, and disposal of antibiotics to the environment from the human, animal, food, and plant sectors [3]. The assessment took samples from human blood for human health, samples from chicken poultry products for animal health, and samples from the gutter and waste for environment. The E. coli germs were isolated and their resistances were observed. This has become a joint activity among the sectors involved [10].

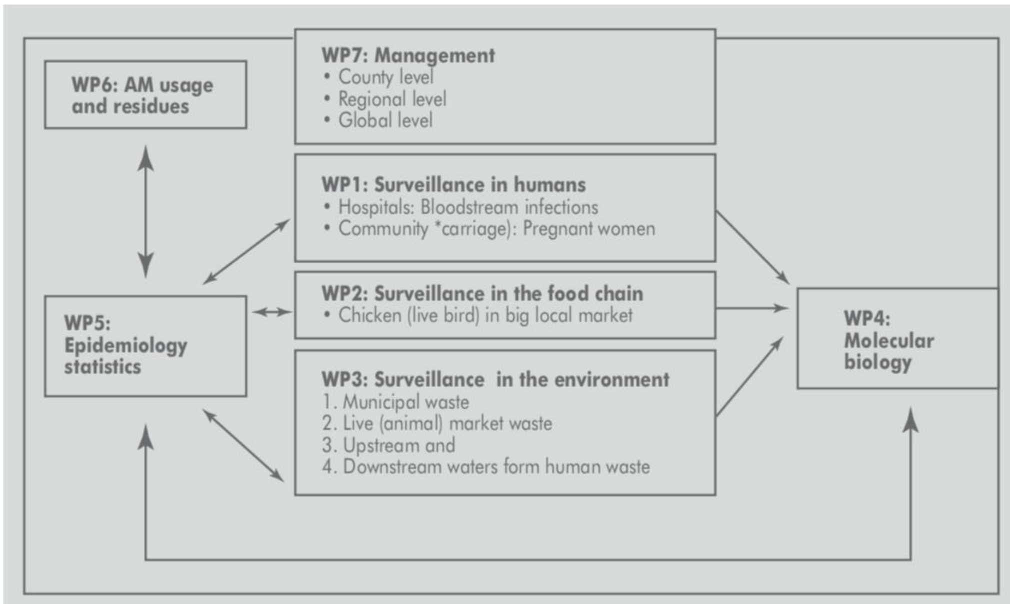

Figure 1. Framework of Tricycle Program [2,9]

The goal is to develop epidemiological and microbiological methodologies that can be carried out in an identical manner in any country, even those with limited resources. It should be possible for results to be analyzed globally or by region and country; subsets of ESBL Ec isolates shall be archived for in-depth molecular analysis to characterize genetic properties and changes over time [5]. This following items be current requirements to support Tricycle project, such as:

1. Resources (i.e. financing capacity, workforce, information system and technologies, laboratory equipment and reagents, etc.)
2. Integrated, harmonized, standardized protocol followed by workshop and training
3. Technical assistance (data collection, data management, analysis, reporting)
4. Including all relevant sectors in integrated surveillance coordination [9,11]

Generally, many LMICs facing lack of robust legal and regulatory frameworks; lack of robust systems for collecting and analyzing data due to lack of resources and capacity; lack of trained laboratory and clinical personnel; weak laboratory and communication infrastructure; and poor coordination among the human, animal and other sectors [4,11]. On the other hand, the chosen granularity (year, country) the project will not generate data on a specific subpopulation in a given country or variations within the year. It will not permit us to infer relationships or causality between the rates in the three sectors explored [2]. However, the implementation of activities was hampered because of the nCoV-2019 pandemic.

Further Expectations

The WHO Integrated Global Survey protocol has been developed [6], however evaluation of the guidelines is needed for protocol improvements to prepare for scaling up going forward. Integrated, harmonized, standardized protocols need to be developed by taking into account the domain of effectiveness and efficiency of the Tricycle Project, especially for LMIC settings. It also could be an opportunity to survey concomitantly the presence of other target bacteria or resistance mechanisms (carbapenem-resistant enterobacteria are often cited) either by phenotypic or genomic methods [2]. In subsequent efforts, outputs from this project could readily be coupled with surveillance data on the use of antibiotics in animals and humans, as well as tracking major sources of their release into environment and assessing the impact of prevention and control measures.

Integrated surveillance is further proposed to be strengthened through use of the IT systems for centralization of AMR surveillance data in partnership with WHONET and JANIS (Japan Nosocomial Infection Surveillance). Awareness was also identified as a priority, and a template for creating targeted communications strategies for AMR in SEAR is being developed [6]. On the other hand, surveillance of antibiotic resistance in aquatic environments and farmed soil environments used for livestock and crop production, are priorities. Some experts suggested a nested approach to surveillance including a focus on priority pathogens or bacterial indicators such as E. coli, e.g. Tricycle ESBL producing E. coli combined with a culture-independent resistance gene- focused metagenomic and/ or a high-throughput qPCR method to investigate the entire resistome [12].

Perspective in Country Level

Cambodia

In order to implement the Multi-sectoral Action Plan (MSAP) on Antimicrobial Resistance in Cambodia 2019-2023, the One Health approach in the Tricycle project is used to strengthen the involvement of related key stakeholders. Several face-to-face meeting activities including training and workshops have been planned. All data gathered in WPs will be entered into WHONET and some of the current requirements are related to laboratory equipment and reagents, technical assistance (including data collection and analysis), and financial support.

Lao P.D.R

The three sectors (i.e. human health, food chain (animals), and the environment) will select and design as institutions and focal points to coordinate the implementation of the ESBL-Ec Tricycle methodology. Available data already suggest there is a high prevalence of resistant organisms in Lao PDR, such as ESBL-producing Gram-negative organisms, mostly E. coli and Klebsiella pneumoniae, in blood isolates. Therefore, activities such as training workshops, assessment visits, provision of laboratory reagents, and advocacy are planned. Some sites that will be used are hospitals, community, market, and capital or biggest city.

Myanmar

Currently, Myanmar is in the implementation stage for the AMR surveillance system that applies the integrated One Health approach. Key stakeholder identification for the implementation of the Tricycle program has also been planned. The team will have three portions to detect surveillance in human, food chain, and environment; and data will be collected by using free WHONET software. Meeting activities, workshops, and trainings have been planned and some have been carried out via online. Some of the current requirements are related to separate budgets, human resources (data collection, data analysis), and technical support (reagents, primers, and techniques for molecular biology).

Cambodia

A. Background

The ESBL Ec Tricycle AMR surveillance project supports the concept that a simplified, integrated, trans-sectoral surveillance system of bacterial resistance to antibiotics could be implemented on a global basis. It would bring critical, timely and invaluable information to assess and track globally and over time the efficacy of the various control programs of AMR and would cover as many countries as possible, including the poorest ones.

The proposed surveillance will focus on a single key indicator, the frequency rates of ESBL-E. coli, measured yearly in strictly identical and controlled conditions in the three major sectors (tricycle) that are the human, the food-chain and the environment. The choice of this unique target is because ESBL-Ec are responsible for severe morbidity and mortality in humans, with significant associated health costs and disease burdens. Although ESBL-Ec do not represent all aspects of AMR, they can be considered a highly relevant and representative indicator of the magnitude and leading edge of the global AMR problem (1).

There were several studies related to ESBL E Coli in Cambodia whereby participation of all the 3 main stakeholders were not warranted, for instance:

- In 2019, a study on Meat and Fish as Sources of Extended-Spectrum β-Lactamase–Producing Escherichia coli, Cambodia concludes that the "integrated epidemiologic and genomic methods to characterize community, clinical, and environmental data support concerns that the dissemination of antimicrobial drug–resistant bacteria from food animals to humans may be more likely in low- and middle-income countries. This finding is concerning because meat consumption is projected to drastically increase in these countries, and animal production that relies on routine antimicrobial drug use is being promoted to meet this demand. Particularly for low- and middle-income countries such as Cambodia, implementation of multi-sectoral strategies to combat antimicrobial resistance from a One Health perspective must be supported, and food safety should be prioritized" (2).

- In 2018, a study on "Beta-lactam resistance among Enterobacteriaceae in Cambodia: The four-year itch" whereby a total of 10 415 samples were tested during the study period. Among the 1233 Enterobacteriaceae isolated, the most commonly isolated organisms were Escherichia coli (789; 63.9%) and Klebsiella pneumoniae (243; 19.8%). The species of bacteria identified differed significantly between the patients in the community and those in the hospital: E. coli was found mainly in community patients (chi-square for trend = 21.2, $p < 0.0001$) and K. pneumoniae in hospital patients (chi-square for trend = 8.2, $p < 0.0001$) (3).

- In 2019, a study on Carriage of carbapenemase- and extended-spectrum cephalosporinase-producing Escherichia coli and Klebsiella pneumoniae in humans and livestock in rural Cambodia; gender and age differences and detection of blaOXA-48 in humans" concluded that "faecal carriage of E. coli and K. pneumoniae harbouring extended-spectrum cephalosporinase genes are common in the Cambodian community, especially in women and young children.

Exposure to animal manure and slaughter products are risk factors for intestinal colonization of ESCE/K in humans" (4).

- A study in 2016 on "Occurrence and molecular characteristics of antimicrobial resistance of Escherichia coli from broilers, pigs and meat products in Thailand and Cambodia provinces" was carried out to investigate prevalence and characteristics of AMR in commensal E. coli isolates from broilers, pigs and their meat in the area along the Thailand–Cambodia border. AMR phenotype, class 1 integrons, ESBL and localization of resistance determinants were analyzed (5).

- A review of antimicrobial resistance in Cambodia published in 2019 found out that between 2000 and 2018, twenty-four papers were included for final analysis, with 20 describing isolates from human populations. Escherichia coli was the most commonly described organism, with median resistance rates from human isolates of 92.8% (n = 6 articles), 46.4% (n = 4), 55.4% (n = 8), and 46.4% (n = 5) to ampicillin, 3rd generation cephalosporins, fluoroquinolones, and gentamicin respectively (6).

B. <u>Rationale</u>

In 2015, WHO adopted the AMR common surveillance known as Global Antimicrobial Surveillance System (GLASS) and since April 2016, Cambodia has been enrolled with GLASS whose index bacteria include: Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Staphylococcus aureus, Streptococcus pneumoniae, Salmonella spp, Shigella spp and Neisseria gonorrhoeae (7).

To formalized a comprehensive response to AMR in Cambodia, a Multi-sectoral Action Plan on Antimicrobial Resistance in Cambodia (MSAP) 2019-2023 was officially adopted by the Ministry of Health (MOH), Ministry of Agriculture, Forestry and Fisheries (MAFF) and Ministry of Environment (MOE) in early 2020 (8). Therefore, the MSAP 2019-2023 identified seven strategic areas where actions should be focused over the next five years:

1. Building human capacity for antimicrobial resistance
2. Containing AMR through good practices
3. Evidence generation through surveillance and laboratories
4. Governance and coordination to reduce antimicrobial resistance
5. Increasing public awareness
6. Rational use of antimicrobial medicines
7. Research and innovation for antimicrobial resistance

C. <u>Objectives</u>

The main objectives of the project are to:

- Reduce the global burden of AMR and
- Adopt One Health approach to combat AMR in Cambodia.

D. Methodology

The project is organized by splitting it into three parallel core work packages (WPs) dealing with resistance in humans, animals and the environment respectively. All data gathered in these WPs will be entered into WHONET which a readily available WHO-sponsored software for surveillance of antibacterial resistance with existing training courses and online manuals that will continue to be supported and made available in the various WHO regions. Additional WPs will be inserted in the project as described below including management, epidemiology and statistics, molecular biology and usage.

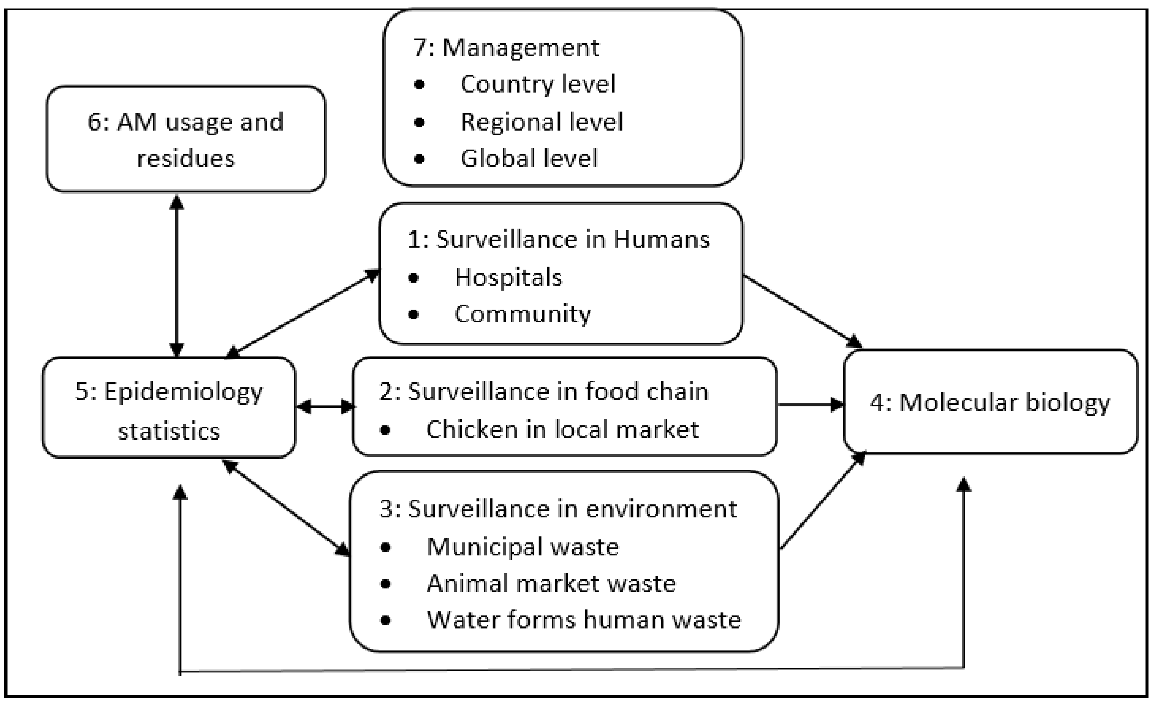

Figure 1: Framework of Tricycle program

E. Plan activities

Due to the Covid-19 pandemic situation, face-to-face meetings including training and workshop could not be organized due to possible disease transmission and some travel restriction but some latest ICT development could leverage the different needs of the project. However this following items as current requirement to support Tricycle project.

- Inception meeting, workshops and training
- Laboratory equipment and reagents
- Technical assistance in different aspects of the project implementation including data collection and analysis
- Financial support

F. Stakeholder

The ESBL Ec Tricycle AMR surveillance project involve three major sectors (tricycle) that are the human, the food-chain and the environment sectors. With the 3 key stakeholders already engaged, the most relevant strategic objectives to further and specifically collaborate to implement the Tricycle programme are Strategic Objective 2: To strengthen evidence generation through functional human, agriculture and environment laboratories and effective surveillance mechanisms and Strategic Objective 7: Build research and innovation to support policy, good practices, implementation, monitoring and evaluation of AMR activities. This is to emphasize that the prospect for implementing the Tricycle programme, even with separate focus, will be built on a strong national background and will also complement a comprehensive national effort to combat AMR.

G. Expected Outcome

It is expected that related key stakeholders will participate in this project under One Health approach and the results will be satisfactory and useful and the Tricycle project will be recognized and advocated for an increasing number of countries to be enrolled in the following years.

Lao P.D.R

A. Background

Antimicrobial resistance (AMR) has emerged as a key global health challenge for the years ahead with major economic consequences, particularly in low- and middle-income countries. The UN advocates a global, holistic, "One Health" approach to the problem involving the joint effort of WHO, OIE, FAO and UNEP in the Tripartite plus agreement[1], which addresses the problem of AMR through the Global Action Plan on AMR. This is reflected in the National Strategic Plan on AMR in Lao PDR 2019-2023, in which multi-sectoral interventions using a One Health approach are proposed. An efficient, robust and multi-sectoral surveillance system is a central tool to steer this action and assess its effectiveness. In Lao PDR, a national AMR surveillance system, consisting of a national reference laboratory and 4 surveillance sites, has been established and data is shared to the Global AMR Surveillance System (GLASS). The animal sector has already implemented AMR surveillance in pigs, with a view to expand to chickens, in numerous provinces around the country. While information is shared between these two sectors, the surveillance systems remain functionally distinct, due to differences in surveillance objectives, and target bacterial species. The environment sector is also currently not involved in AMR surveillance in Lao PDR. Such surveillance systems are commonplace in countries with limited resources.

For this reason, the Advisory Group on Integrated Surveillance on Antimicrobial Resistance (AGISAR) and the WHO have developed the Global Surveillance on extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli Protocol, the "Tricycle Protocol". This protocol allows for the implementation of a simplified, integrated trans-sectoral surveillance system for bacterial resistance to antibiotics. This integrated surveillance protocol uses a One Health approach that is built upon principles of WHO surveillance tools, such as the AGISAR Guidance on Integrated Surveillance on AMR in foodborne bacteria[2] and the GLASS[3] . The surveillance focuses on a single key indicator that these two programs recommend: the frequency rates of ESBL-producing E. coli (ESBL-Ec), thereby utilizing and building upon existing country systems. The proposed name is "Tricycle", after its three-wheeled namesake, to demonstrate the idea that it will simultaneously address three aspects of bacterial resistance (human health, food chain (animals), and the environment) using a One Health approach, in a simple and feasible manner designed to provide robust and valid statistically-based surveillance outcomes using minimal resources.

There is also an opportunity for additional exploratory analyses to be conducted at the national, regional or global levels following the implementation of ESBL-Ec Tricycle surveillance. This depends on the availability of additional specific resources.

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[1] https://www.who.int/antimicrobial-resistance/interagency-coordination-group/Tripartite_Plus_update_info_session_011018.pdf

[2] https://apps.who.int/iris/bitstream/handle/10665/255747/9789241512411-eng.pdf;jsessionid=00352D196259367C5C4895FF0B94BBD7?sequence=1

[3] https://www.who.int/glass/en/

In the core part of the protocol, ESBL-Ec is determined yearly under identical and controlled conditions in humans, the food chain and environment. The protocol comprises epidemiological and microbiological procedures specifically designed to be conducted in a harmonized manner in all countries, even those with limited resources. Based on this surveillance approach, countries can build on this approach and develop a more comprehensive and enhanced integrated surveillance system on AMR and include other pathogens such as Salmonella and Campylobacter and other food animals, food, provinces or cities. Such a national integrated surveillance system can allow countries to gain a better understanding of the relationship between AMR rates and antimicrobial consumption (AMC) and use (AMU) in different sectors, and can be used to develop and monitor control and prevention strategies on AMR.

B. Rationale

Available data already suggest there is a high prevalence of resistant organisms in Lao PDR, such as ESBL-producing Gram-negative organisms, largely E. coli and Klebsiella pneumoniae, in blood isolates. AMR surveillance in the animal sector has identified E. coli as an important pathogen. The environment sector is currently performing coliform testing of wells and boreholes.

E. coli as a commensal bacterium has been selected as the microorganism indicator for a number of reasons. It is a cross-cutting bacteria found in the human, animal and environment sectors and is a species of Enterobacterales, an important and commonly isolated Gram-negative family, and can represent antimicrobial resistance patterns for this Gram-negative group. E. coli is also a bacterial species that can be easily cultured and isolated in any laboratory with minimal resources (does not require complex methodologies like Salmonella and Campylobacter) and so it can be implemented in sectors with no bacteriology capacity or low laboratory resources.

It is acknowledged that ESBL E. coli does not represent the overall global problem of AMR and there are many other aspects aside from this single microorganism and resistance trait. However, the existing literature shows (i) great variations in the rates of ESBL colonization in humans in and between countries and prevalence trends over time ; (ii) variable prevalence among farm animals, and evidence that some human morbidity linked to ESBL-Ec is due to antibiotic usage in the food chain and to the presence of ESBL-Ec in the environment ; (iii) interventions leading to a decreased exposure to antibiotics in animals or humans have been followed by a decrease in ESBL E. coli occurrence rates; and (iv) infections caused by ESBL-producing Gram-negative organisms require treatment using critically-important antimicrobial drugs.

Therefore, notwithstanding the fact that the comparison of these studies is sometimes difficult as different sampling schemes and methods have been used in the past, ESBL-Ec are a relevant and representative proxy for the magnitude and trends of the global AMR problem. These bacteria also represent a turning point in the evolution of AMR. They are typically resistant to most available antibiotics, with the result that affected patients often must be treated with drugs of last resort such as carbapenems or colistin. Access to these drugs may be limited in some settings, including in Lao PDR. However, the overall use of these antimicrobials has greatly increased over the last few years, promoting, in turn, the selection of carbapenem-resistant Enterobacteriaceae against which none of the currently available antibiotics in Lao PDR are readily effective. This is already resulting in treatment failure and increased mortality. However, solid epidemiological data on the associated health burden are still lacking.

C. Objectives

1. A common, simplified, and integrated multi-sectoral surveillance system designed to detect and estimate the prevalence/quantity of a simple and representative microorganism indicator, E. coli, producing a specific resistance mechanism via extended-spectrum beta-lactamases (ESBL), in three key sectors, namely, humans (hospital and community), the food chain, and the environment. Repeated annually, this will provide a common set of metrics to follow over time.
2. Improvements and expansion of national integrated surveillance systems for AMR with the addition of more bacterial species, food animals, and geographic locations.
3. Feasibility to the monitoring of prevalence trends at the national, regional and global levels.

D. Methodology

Figure 1 shows the structure of the integrated surveillance system and the interaction of the different Working Packages. The ESBL-Ec Tricycle protocol for sampling and isolating ESBL-Ec has been developed for three fundamental packages. A molecular testing (sequencing) package was included to provide support in the analysis of the genetic characteristics of the ESBL-Ec isolates from the different sectors and to analyze and compare among sectors and find relatedness together with information about the use and consumption of antimicrobials in the human and animal sectors. With the information of the different packages, countries will have the benefit of identifying and estimating the main drivers and be able to design and develop strategies to contain AMR.

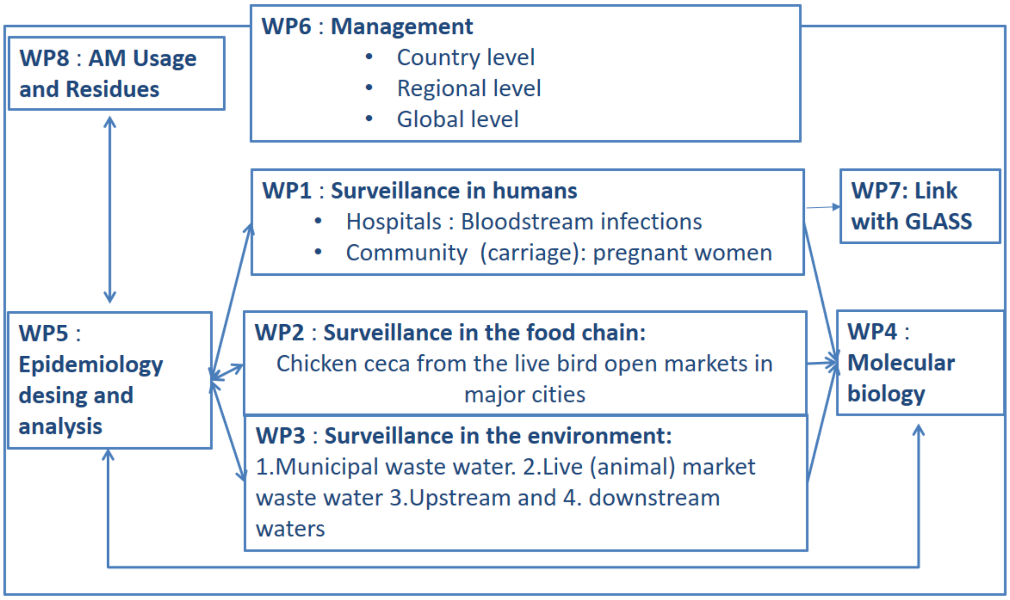

Figure 1. ESBL Ec Tricycle structure.

Figure 2 shows each sector with the sites selected to be sampled, sample subjects by sector and the number of samples to be collected per year.

Sector	Sites	Sample subject	Sample	No. of samples	Links
Human	Hospital	Inpatient	Bacteremias	5000 blood cultures/year	GLASS specimen sample
	Community	Pregnant women	stool/rectal swab	100	Minimal number
Animal	Market	Chicken	Cecal	240/year 20/month	Most common food animal in countries
Environment	Capital or biggest city	Communal sewage	Waste water	8-12 rounds per year 4 samples per round 2 cities (suggested)	Suggested 1 round per month. River samples: AMR related with environment Waste water: AMR related with community
		Market sewage	Waste water		
		River Downstream	Water		
		River Upstream	Water		

Figure 2. Sites and sampling framework

E. Plan activities

1. **Training workshop**

With the support of WHO and Fleming Fund, a training workshop will be delivered to the focal points designated by the national authorities to learn about the ESBL-Ec Tricycle methodologies by sector and the principles of integrated surveillance on AMR in order to facilitate implementation.

2. **Assessment visit to support the selections of sites and implementation of the laboratory methodologies**

An assessment visit will be conducted with a group of experts in order to facilitate site selection and implementation of methodologies in the laboratories.

3. **Provision of laboratory reagents and materials to implement the surveillance**

Reagents and materials to implement the laboratory methodologies will be provided for the initial year. Countries will aim to establish as this surveillance as a routine activity in the national AMR surveillance system in the following years and scale up to add other cities, bacterial pathogens, food animals and environments to develop a national integrated surveillance system on AMR. If this is not yet possible, countries may seek support from partners to continue implementation.

4. **Data collection and analysis support**

Continual support will be established with the national AMR committee, national coordinator and focal points from each sector for this integrated surveillance in order to analyze and develop a national report.

5. **Continue support and advocacy to continue building the national integrated surveillance system**

The GLASS ESBL-Ec Tricycle module is being developed in order to triangulate GLASS data with ESBL-Ec Tricycle data to understand the trends regional and globally and to facilitate the identification of new approaches and initiatives to increase capacity of countries.

F. Stakeholder

The three sectors will each select and designate an institution and focal point to coordinate the implementation of the ESBL-Ec Tricycle methodology.

A national focal point will be designated as the national coordinator of the surveillance and will lead and manage the implementation, and coordinate with the different focal points to support and follow up the implementation, data collection and analysis and prepare the national report.

Suggested institutions to be part of ESBL-Ec Tricycle surveillance and activities to be conducted by each sector in Lao PDR include:

1. Human health

- Collect samples at hospital and perform testing at the National Center for Laboratory and Epidemiology (NCLE) through the existing AMR surveillance system
- Collate and share a report with the animal and environment laboratories on a monthly basis

2. Animal health

- Collect samples from chickens and perform testing at the National Animal Health Laboratory (NAHL) through the existing AMR surveillance system in the animal sector
- Report the result to NCLE on a monthly basis

3. Environmental health

- Collect water samples (waste water and river) and perform testing at environmental laboratory
- Report the result to NCLE on a monthly basis

G. <u>Expected Outcome</u>

- Tricycle Program should address three aspects of bacterial resistance (human health, food chain (animals), and the environment) using a One Health approach.
- To provide robust and valid statistically-based surveillance outcomes even those with limited resources setting.
- To develop and monitor control and prevention strategies on AMR, AMC and AMU.

Myanmar

A. Background

Tricycle Program means the development of a globally harmonized protocol for a simplified and integrated AMR surveillance system with a single key indicator: the frequency of ESBL-Ec in humans, the food chain, and the environment (1). In fact, Myanmar is now in the implementation stage for antimicrobial resistance (AMR) surveillance system and currently we are directing into the integrated approach for AMR survey and one health approach. However, this AMR program is highly challenging particularly for low and middle income countries, where testing capacities are limited and budgets are not enough, to focus on a great number of pathogens and susceptibilities of each. That is why, WHO Advisory Group on Integrated Surveillance of AMR (AGISAR) proposed to emphasize on a single indicator Escherichia coli since 2015 (2).

The choice of this unique target was because extended-spectrum beta-lactamase producing E.coli (ESBL-Ec) was responsible for severe morbidity and mortality in humans, with significant associated health costs and disease burdens. In addition, the prevalence of ESBL-Ec was rising globally. According to a meta-analysis from 2000 to 2018, the proportion of ESBL-Ec based on MIC in human, animal and environmental/food isolates was 42.4%, 63.2% and 28.6% respectively. The prevalence of those based on the DDM in human, animal and environmental/food isolates was 13%, 26.3% and 25% respectively (3). Animal isolates had a higher prevalence than human isolates and possible reasons might be due to uncontrolled use of antibiotics in domestic animals as well as dietary supplements. Congestion of ESBL-Ec contaminated food and water by human and contact with environment can cause the higher resistance level in human (3).

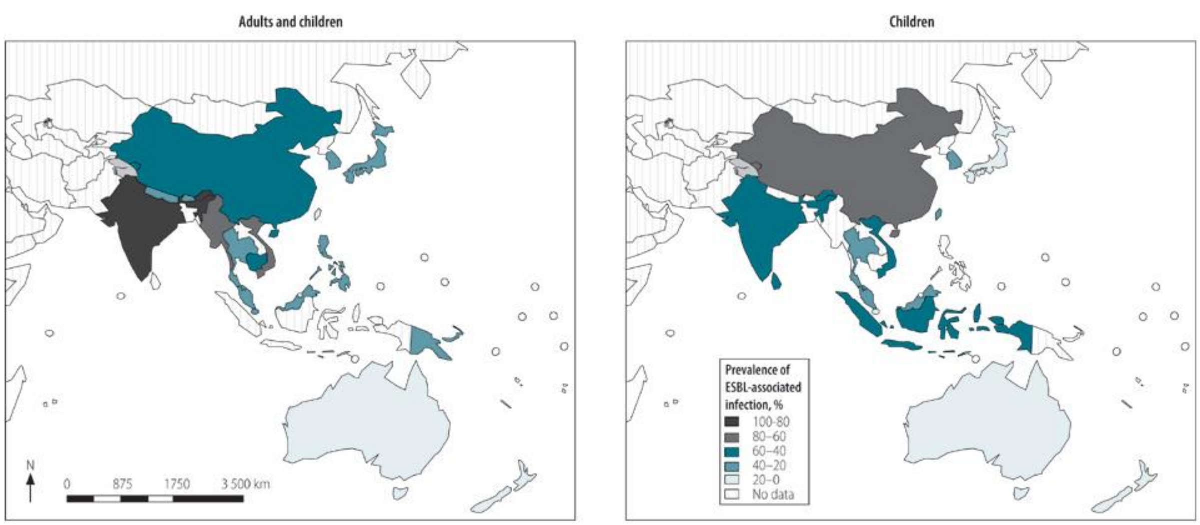

Figure 1: Map of prevalence of extended-spectrum β-lactamase-associated infection in South-East Asia and Western Pacific countries (https://www.who.int/bulletin/volumes/97/7/18-225698/en/)

In Animal aspect, one study in 2015 screened of antibiotic residues in three common fresh water fish including tilapia (Tilapia nilotica), catfish (Clarias batrachus) and rohu (Labeo rohita) collected from two local retailed outlets and one local market within Nay Pyi Taw area, Myanmar. The results indicated that a significant percent of tested aquaculture products were found to be antibiotic screening test positive, and there is a general lack of knowledge about the purpose and proper usage of antibiotics by aquaculture producers (9). Another study in 2009 screened antibiotic residues in chicken muscle, liver and kidney by using microbial inhibition test, Swab Test on Animal Food. They identified the antibiotic residue positive samples in all local markets (10). Those results of antibiotic residues in animals are one of the drivers of AMR globally. However, there was no proper research for the prevalence of ESBL-Ec in animals in Myanmar.

B. Rationale

Myanmar is a developing countries and limited budgets, inadequate human and technical resources makes it difficult to emphasize a load of pathogens. That's why, we will target on high morbidity and mortality ESBL-Ec according to WHO AGISAR proposal. The reason to choose ESBL-Ec was due to the rising trend of ESBL-Ec worldwide including Myanmar. The prevalence rate of ESBL-Ec in Myanmar was 38% (16/42 isolates) in 2014 and 36.9% (157/426 isolates) in 2017 (4) (5). It was comparable with other countries such as Thailand, Cambodia and Pakistan with the prevalence of 30%, 48.2% and 40% respectively (6) (7) (8). On the other hand, Tricycle program is a vital role to identify the global burden of AMR. Without harmonizing of human, animals and environment, it is not feasible to combat AMR.

C. Objectives

- To reduce the global burden of AMR
- To unite all efforts to combat AMR not only in human but also in animal food chain and environment

D. Methodology

In general, Myanmar will have three portions to detect surveillance in human, food chain and environment running in parallel and data will be collected by using free WHONET software. After that, we will have additional packages including molecular biology, AM usage, epidemiology statistics and management as following. These efforts will be strongly supported by The Fleming Fund and WHO (3).

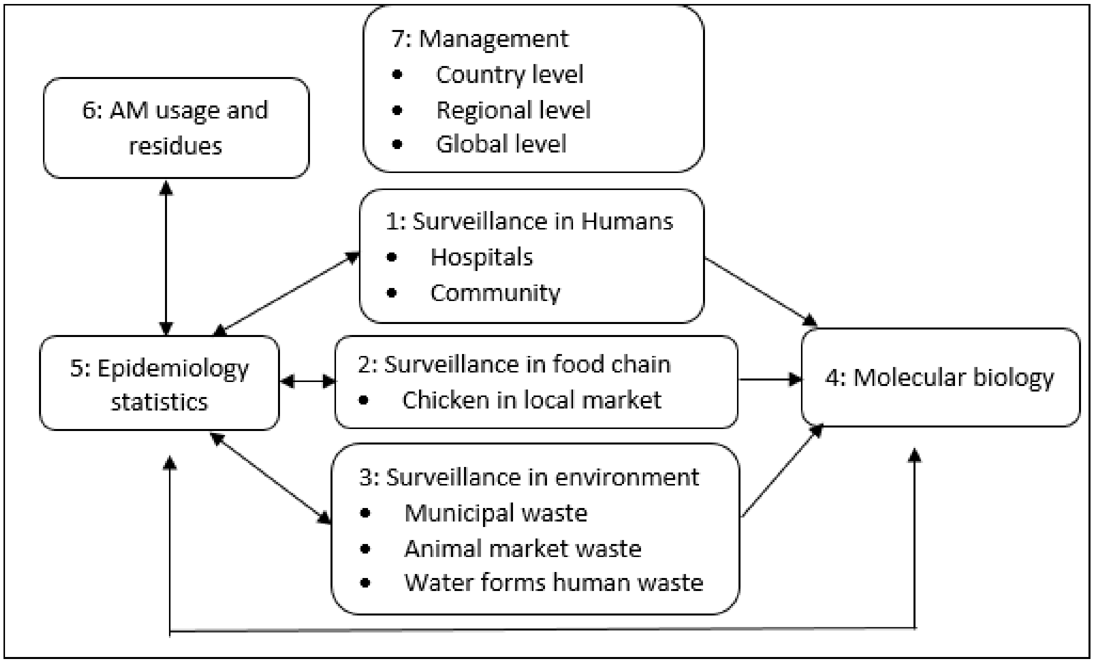

Figure 2: Framework of Tricycle Program

E. Plan activities

Myanmar team are planning to identify key stakeholders and personnel to participate in and to implement Tricycle program soon after the pandemic outbreak of novel Corona virus-2019. This is following items as plan activities that Myanmar team will conduct with related multi-stakeholder:

1. To initiate multiple stakeholders meeting for planning
2. To propose the strategic plans with operational details
3. To prepare for surveillance in human, animals and environment
4. To strengthen the molecular laboratory technique
5. To analyze data statistically and report the results
6. To monitor and evaluate plan for successful implementation of the activities

F. Stakeholder

Because of nCoV-2019 pandemic, all of the meetings, workshops, trainings and workplaces are closed in this period. We are still working from home via telecommunication and most are delayed. However some current requirement that need to be considered to support multi-stakeholders collaboration are:

1. Separate budget for Tricycle program
2. Workshops and trainings for that program
3. Human resources for data collection, data analyst, coordinator and lab technicians
4. Technical support such as reagents, primers and technique for molecular biology

G. Expected Outcome

Antimicrobial resistance (AMR), a global public health threat, is very sophisticated not targeting a specific disease like other infectious diseases such as HIV, malaria, TB, etc. Once antibiotic resistance occur, it is related to a whole global usage of antibiotic misuse in human, animal and agriculture. In fact, it affects a myriad of various bacterial species, each with its own pathogenicity, epidemiology and clinical consequences. Therefore, it is rather broad, complex and challenging. Myanmar is one of the countries running NAP AMR in line with GAP to combat AMR and now we are targeting into integrated surveillance of AMR taking a step-wise approach. Despite ESBL-Ec do not represent the all aspects of AMR, it could be a highly representative indicator of the magnitude and leading edge of the global AMR problem.

Myanmar is expecting that the successful implementation of this AMR project will bring together all the critical players from human, animal and environment relating with national one health strategy to bring down the level of AMR and reduce its spread.

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