The Animal/Human Interface With A Focus On Low And Middle Income Countries: Antimicrobial Resistance in Southeast Asia

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1. Summary

Antimicrobial resistance (AMR) is one of the greatest challenges of modern public health. AMR is spreading internationally with devastating consequences, causing substantial morbidity, mortality and economic losses. AMR threatens to render once treatable infections incurable and, as current antimicrobials lose efficacy, has the potential to reverse years of medical progress. The phenomenon of AMR is a global problem intrinsically linked to human health and behaviour, animal health, food-production, agriculture and the environment. AMR, a natural evolutionary response to antimicrobial exposure, has been precipitated in recent years by substantial increase in rational and irrational antimicrobial usage and poor infection control measures.

Southeast Asia is a uniquely dynamic region of the world, currently experiencing unprecedented economic development and population growth. Antimicrobial use (AMU) and AMR are on the rise in Southeast Asia, aided by regional drivers such as a prosperous food-production industry heavily reliant on antimicrobials, loosely regulated access to antimicrobials, low awareness (from the public, health professionals and farmers), widespread irrational prescribing and self-medication, and abundance of low quality or counterfeit drugs. Combined with high prevalence of infectious disease and weak diagnostic capacity, particularly in primary healthcare settings, Southeast Asia can be considered as global hub for AMR emergence. This contributes to the global spread

of AMR as bacteria are readily transported to other parts of the world by international travellers, or by international trade of animals and goods. The AMR burden in Southeast Asia is high, surveillance capacity is present, but fragmented and heterogeneous across the region.

Important knowledge gaps remain concerning AMU and AMR in Southeast Asia. As much of the AMR data have been generated in healthcare settings, much remains to learn about the extent of AMR in humans and animals in the community. AMR-associated morbidity and economic cost (particularly the wider societal costs) have been difficult to quantify, as solid data are rare. Similarly, data on AMU in Southeast Asia are scarce, despite the consensus that AMU is large (and increasing) in Southeast Asia for humans and food-production animals. The effect of AMU in animals on AMR in humans has been difficult to measure directly. Finally, quantifying the impact of interventions (education, regulation, stewardship) on AMU and AMR remains challenging.

We propose that a collaborative AMR surveillance network throughout Southeast Asia could contribute to address the knowledge gaps described above by collecting, analysing and disseminating information about AMU and AMR in a standardised way. Existing infrastructure may form the backbone of the network, which could subsequently be expanded via the development of additional surveillance sites. In addition to surveillance according to the recommendations of the WHO, the network should be designed to support advanced molecular technologies that may help answer questions

about AMR gene flow between species and emergence of resistant strains at the human-animal-environment interface. Longitudinal sampling should span humans and animals both in health care and community settings to define the current state of AMR and monitor long term trends. Data on AMU should be collected where possible, and mathematical modelling and GIS technology could be integrated to create a comprehensive picture of AMU in Southeast Asia. Investigating knowledge, attitude and practices through social research will be part of our surveillance effort, because understanding human behaviours underlying excessive and irrational AMU is crucial to reduce AMU and, eventually, control AMR.

2. Introduction - Antimicrobial resistance: a growing global public health concern

In 1928 Alexander Fleming initiated a medical revolution by discovering penicillin, which was used from the 1940s to cure previously life-threatening infections. In 1945, during his Nobel Lecture, Fleming foretold the risks of antimicrobial resistance (AMR): "The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily under dose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant." (1). Unfortunately, these words of warning did not spread as universally as the use of the antimicrobials that he had discovered.

The development of AMR is a natural evolutionary response to antimicrobial use (AMU) (2): microorganisms under selective pressure develop mechanisms to avoid killing, either by spontaneous mutations, or by acquisition of genetic elements conferring resistance via naked DNA uptake or transferred from other bacteria, a phenomenon known as horizontal gene transfer (3). As a consequence, microorganisms loose susceptibility against drugs to which they responded before (4). In recent years, the emergence and spread of AMR organisms is thought to have been precipitated by (i) a dramatic increase in use (both appropriate and inappropriate) of antimicrobial agents in human, animals and agriculture and (ii) failure to prevent the spread of resistant organisms due to sub-standard infection control measures (5,6).

Today, AMR in bacteria, viruses and parasites is a growing concern worldwide. The World Health Organization (WHO) considers AMR "one of the greatest challenges in public health" (7) and has warned about the possibility of "a post-antimicrobial era", in which common infections and minor injuries could again become common killers (4). AMR is already diminishing our ability to treat and control infections, causing higher morbidity, mortality, longer hospital stays and higher treatment costs. As many drugs lose efficacy, treatment relies increasingly on last-resort drugs, and the need for alternative/new antimicrobial agents only becomes more urgent. If there is no response, it has been estimated in a recent report from the United Kingdom that AMR could claim 10 million lives a year and cumulatively cost the world 100 trillion USD by 2050 (8).

AMR is a multifactorial and complex problem intrinsically linked to human health and behaviour, but also intertwined with animal health, food production, agriculture and the environment (9). In our interconnected world where people, animals and goods are increasingly mobile, AMR in one region cannot be isolated from AMR worldwide. AMR is a global problem that requires global solution. However, this report is limited by request to the current status of AMR in Southeast Asia. Southeast Asia is commonly regarded to be a hotspot for AMR emergence, and addressing the issues across this region should be viewed as a crucial part of the solution (10).

This report aims to (i) explore Southeast Asia-specific drivers of AMR, (ii) map the current AMR situation in Southeast Asia, (iii) identify knowledge gaps and pending questions, and (iv) present a vision for a surveillance network in Southeast Asia. This

report focuses on low- and middle income countries (LMICs) in Southeast Asia, namely Myanmar, Thailand, Cambodia, Lao PDR, Vietnam, Malaysia, The Philippines and Indonesia (Figure 1). For the pusposes of this review, HIV, TB and malaria are not included.

Figure 1. Southeast Asia situation map, including the percentage of *E. coli* isolates that were resistant to third generation cephalosporins in Southeast Asia in 2012-13; data compiled from (4,11)
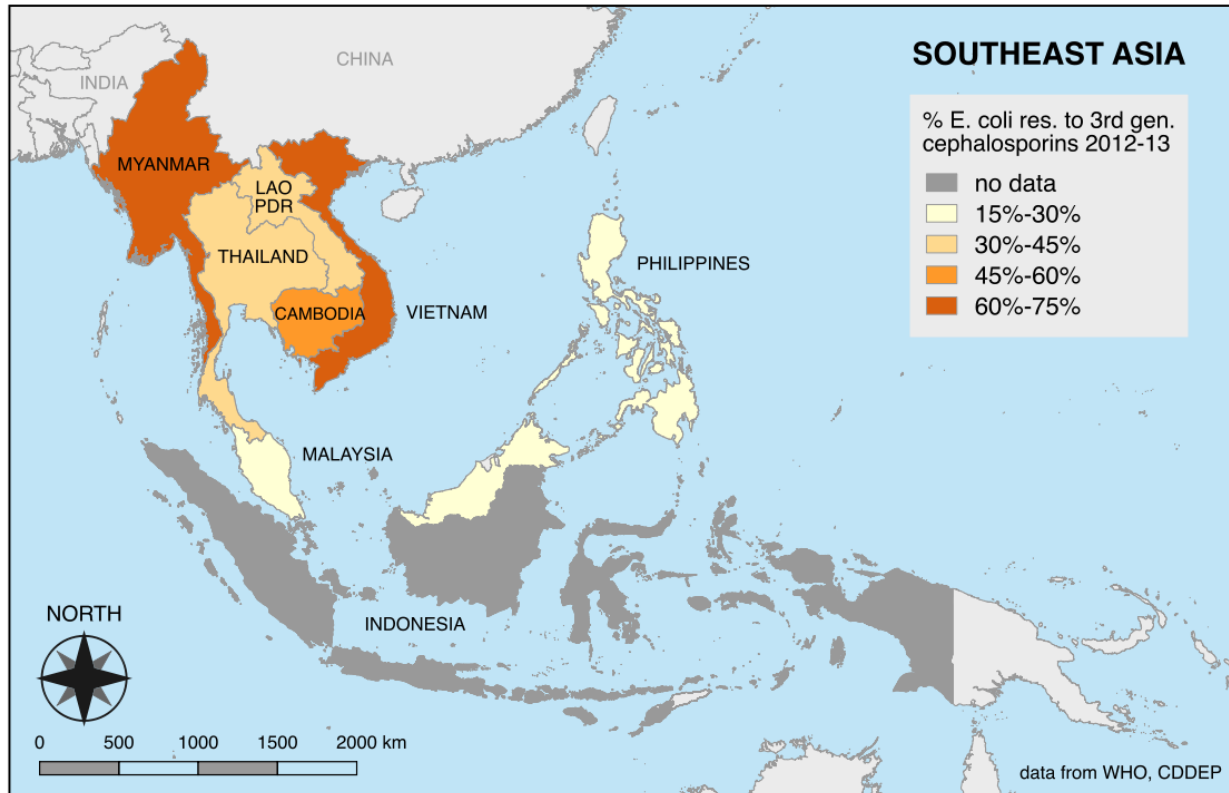
3. The drivers of AMR in Southeast Asia

AMR driving factors are plentiful (Figure 2) and how these may impact on the emergence of AMR in Southeast Asia is outlined below.

3.1. Economic development and population growth stimulate the demand for antimicrobials

Southeast Asia is a highly diverse and dynamic region, which has long been recognized as a focal point for existing and emerging infectious diseases (10,12). In recent decades, Southeast Asia has experienced tremendous, yet heterogeneous, socioeconomic development and population growth (10) and Table 1. The rapid development of transport infrastructures has dramatically improved mobility of people, animals and goods (12).

Prosperity and population growth across the region have triggered an increase in demand for animal protein. The per capita consumption of animal protein has increased by 45% between 1990 and 2000 in the Mekong region (Cambodia, Lao PDR, Thailand and Vietnam) (10). In addition to the expansion of the domestic market, meat and fish exports have increased considerably. Throughout the whole region, between 1995 and 2013/14, livestock production doubled, and fish production tripled (Table 1). Vietnam is currently the third largest producer of aquaculture products, its production being surpassed only by China and India (13,14). Currently, large-scale, intensive livestock and fish production systems operate alongside more traditional fishing and farming

practices, as well as mixed aquaculture-livestock systems, all of which rely heavily on the use of antimicrobials (10,14–18). The increase in demand for animal protein is likely to contribute to AMR as estimates suggest that the consumption of antimicrobials for food production surpasses use in humans (18). The switch to more intensive farming methods has increased the demand for antimicrobials, and market reforms have improved their availability. AMU restrictions usually exist, but they are seldom enforced, and/or often ignored by drug sellers and farmers. The consequence is that antimicrobials for animal use across the region is poorly regulated, which amplifies the probability of excessive or inappropriate use (10,13,19–23).

Figure 2. Schematic of the development and spread of AMR, drivers and the tools for mitigation
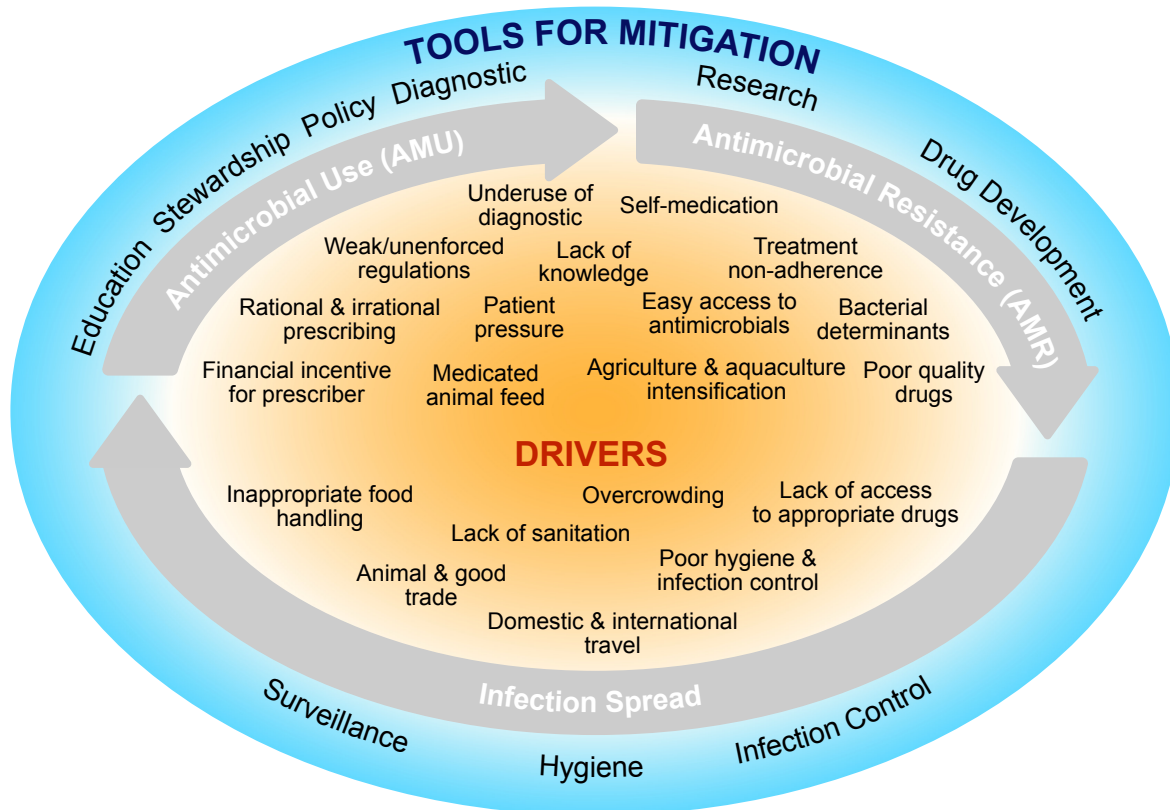
Table 1. Socioeconomic development of Southeast Asia 1995-2014

Indicator[a]	Cambodia			Indonesia			Lao PDR			Malaysia		
	1995	2014[b]	% change	1995	2014[b]	% change	1995	2014[b]	% change	1995	2014[b]	% change
Population (millions)	10.7	15.3	43	197.0	254.5	29	4.9	6.7	38	20.7	29.9	44
Life expectancy	55	68	24	65	69	6	56	66	18	72	75	4
Infant mortality rate (per 1000 live births)	88	26	-70	51	24	-53	97	52	-46	12	6	-50
GDP per capita (USD)	322	1095	240	1026	3492	240	363	1794	394	4280	11307	164
Livestock production index 1995-2013[c]	69.8	89.6	28	79.5	139.9	76	72.9	125.8	73	88.2	138.8	57
Total fisheries production (million tons)	0.11	0.75	562	4.39	20.88	376	0.04	0.15	274	1.25	1.99	59
CO_2 emission 1995-2011 (ton per capita)	0.1	0.3	200	1.1	2.3	109	0.1	0.2	100	5.8	7.9	36

(a) source: World Bank, http://www.worldbank.org
(b) if not stated otherwise
(c) Livestock production index includes meat and milk from all sources, dairy products such as cheese, and eggs, honey, raw silk, wool, and hides and skins, reference year 2004-2006=100

Table 1 (continued) Socioeconomic development of Southeast Asia 1995-2014

Indicator[a]	Myanmar			Philippines			Thailand			Vietnam		
	1995	2014[b]	% change	1995	2014[b]	% change	1995	2014[b]	% change	1995	2014[b]	% change
Population (millions)	44.7	53.4	20	69.8	99.1	42	59.3	67.7	14	72.0	90.7	26
Life expectancy	60	66	10	66	68	3	70	74	6	72	76	6
Infant mortality rate (per 1000 live births)	69	41	-41	34	23	-32	24	11	-54	31	18	-42
GDP per capita (USD)	ND	1204	ND	1061	2873	171	2856	5977	109	288	2052	613
Livestock production index 1995-2013[c]	33.4	191.9	475	64.2	126.5	97	99.2	130.2	31	49.5	147.4	198
Total fisheries production (million tons)	0.82	5.05	513	2.81	4.69	67	3.59	2.70	-25	1.47	6.33	329
CO_2 emission 1995-2011 (ton per capita)	0.2	0.2	0	0.9	0.9	0	2.8	4.5	61	0.4	2.0	400

(a) source: World Bank, http://www.worldbank.org
(b) if not stated otherwise
(c) Livestock production index includes meat and milk from all sources, dairy products such as cheese, and eggs, honey, raw silk, wool, and hides and skins, reference year 2004-2006=100

3.2. Antimicrobials in human medicine

Antimicrobials are amongst the most frequently prescribed drugs in human medicine worldwide (2). Studies in Vietnam show, for example, that more than 50% of drugs used in human medicine are antimicrobials (19), and that antimicrobials were the most commonly sold drugs in urban and rural pharmacies (23). The potential efficacy of antimicrobials and their low-cost explain their usage. Early symptoms induced by infections with many bacteria, viruses and parasites are comparable. Therefore, it is typically difficult to diagnose patients presenting with a non-specific illness (such as a fever, diarrhoea or a respiratory tract infection) and to determine which patients need antimicrobials. This is a problem particularly in primary healthcare facilities where the diagnostic capacity can be minimal. Rapid diagnostic tests for malarial parasites has greatly improved the situation for malaria, and has reduced unnecessary treatment with antimalarial drugs in endemic settings (24). Unfortunately, tests to further discriminate between common viral and bacterial infections are either not available or rarely used in LMICs (25). As bacterial infections can progress quickly, antimicrobials are often prescribed as a safeguard without confirmation of a bacterial aetiology. As a consequence, antimicrobials are often unnecessarily used for viral infections. These difficulties partly explain why antimicrobials are commonly overused in LMICs (6). A study from Vietnam found that one third of hospitalized patients received an antimicrobial in the absence of correct medical indication (26), perhaps partly because many hospitals in Vietnam lack adequate capacity and sufficiently trained staff to isolate the infecting bacteria and determine their antimicrobial susceptibility profile (19,20).

Self-medication is common in Southeast Asia, possibly because self-treatment is often less expensive and more convenient than visiting a doctor for a consultation (19,27,28). As a result, antimicrobials are commonly purchased without a prescription, and studies show that 90% of antimicrobials are sold without a prescription in pharmacies in Vietnam (despite a prescription-only regulation in Drug Law since 2005) and that in Indonesia 90%-100% of pharmacies fulfilled antimicrobial requests without a prescription (21,23,29,30). Pharmacists often satisfy the demand for antimicrobials for fear of losing customers, as antimicrobials represent a large portion of their income, and because prescription-only regulations are infrequently enforced (23). A similar study in Thailand demonstrated that shops often sold antimicrobials to customers complaining of corryzal symptoms, acute diarrhoea or dysuria (31).

3.3. Antimicrobial awareness, knowledge and prescribing practices

The risk of inappropriate AMU through self-medication, unregulated access and irrational prescribing is magnified by the fact that knowledge about antimicrobials and their use is low in Southeast Asia amongst the public, drug sellers and doctors (23,29,32,33).

A lack of knowledge regarding medication among the general public stimulates irrational demand for antimicrobials and/or treatment non-adherence, two important drivers of AMR. For example, a study in Malaysia demonstrated that over half of the patients discontinued their antimicrobial treatment once symptoms disappeared (34). Treatment non-compliance was associated with limited knowledge about antimicrobial function and

low AMR awareness (34) but sometimes was also the result of limited financial means. As a pharmacist in Vietnam explains: "Patients want antimicrobials, but can only afford half a cure, and as a consequence under-dose themselves" (23).

In a recent survey amongst doctors in Lao PDR (35), virtually all doctors (96.6%) were aware of the seriousness of the problem of AMR, yet almost one third (29.8%) agreed that prescribing unnecessary antimicrobials was harmless. Three quarters (76%) of the doctors agreed that antimicrobials were overused in hospitals and the community, but less than half (47.1%) thought that it was happening in their own hospital. This mind-set was mirrored by a study in Malaysia, where 83% of medical students surveyed acknowledged that AMR was a national problem, but only 63% thought the problem existed in the teaching hospital to which they were affiliated (36). In the cited Laotian study, more than half of the doctors had no information regarding local AMR patterns for the etiologic agents of typhoid fever and hospital-acquired pneumonia (35).

Furthermore, the study in Lao PDR (35) confirms the difficulty of prescribing an appropriate antimicrobial (which was recognized by 72.5% of the doctors). Almost all (96.6%) doctors surveyed in Lao PDR would have welcomed more training on antimicrobial prescribing, as would 88% of medical students surveyed in Malaysia (36). In Lao PDR, the primary source of information for antimicrobial prescribing were the national guidelines, but information from pharmaceutical companies came second, potentially creating a conflict of interest as companies may privilege antimicrobial sales over scientific evidence when providing recommendations. In a further study from

Malaysia, 21.6% of doctors acknowledged that even when they judged antimicrobials to be unnecessary for treatment, they occasionally prescribed them to comply with patients requests (37). Half of the doctors surveyed in Lao PDR agreed that patients expectations influenced antimicrobial prescribing (35). Similarly, interactions with health professionals in Thailand revealed that irrational antimicrobial prescribing is commonly associated with the prescriber's low understanding of antimicrobials and their usage, and the perceived pressure from patients that expect or request antimicrobial treatment (38).

A recent study demonstrated that despite the existence of national antimicrobial guidelines (updated in 2014), antimicrobial overprescribing is frequent in primary care facilities in Malaysia (39). Upper respiratory tract infection (URTI) was the most common presentation for antimicrobial prescription, accounting for almost half (49.2%) of the prescriptions. This illustrates the overuse of antimicrobials for a condition that is often self-limiting and generally of viral aetiology. Antimicrobials were prescribed to 46.2% of the patients diagnosed with URTI in Malaysia but, as comparison, only to 17% and 5% of patients diagnosed with URTI in the Netherlands and in Hong Kong, respectively (39). It was further observed that antimicrobial prescribing was higher in private than public primary care clinics. Notably, in LMICs (including two Southeast Asian countries), AMR was found to be associated with out-of-pocket health expenses in the public sector (40). The authors speculate that higher costs in the public sector divert patients towards the private sector, where overprescribing of antimicrobials is more frequent due to stronger financial incentives (supplier-induced demand). Indeed in Vietnam, many patients tend

to self-medicate or use the private sector as it is cheaper than using the public sector (27). Out-of-pocket health spending is projected to stay high in Vietnam, as the out-of-pocket to total health expenditure ratio is expected to be 43.5% in Vietnam by 2040, more than twice the predicted 2040 world average of 20.6% (41).

3.4. Antimicrobials in agriculture and aquaculture

Antimicrobial overuse, misuse and lack of awareness are not restricted to human medicine, but also exist in veterinary use linked to food production. Livestock and fish producers in Southeast Asia are under immense pressure to satisfy both a growing domestic demand and the export industry. Farms and aquaculture systems producing for the domestic market benefit from loose regulations, while the use of antimicrobials is more strictly controlled in export products, particularly to Europe and the United States (13,19).

Antimicrobial agents are routinely used in livestock production in Southeast Asia (i) to treat infections (therapeutic), (ii) to prevent infection (prophylactic), (iii) to treat asymptomatic animals belonging to a group where other animals have disease symptoms (metaphylactic), and (iv) at sub-therapeutic concentrations to promote growth (growth promoters, which is increasingly controversial). In contrast with human medicine, where subjects are treated individually, in animal production antimicrobials are normally administered to groups of animals (herds/flocks/ponds) (22,42). Studies in Vietnam have shown that in poultry production systems antimicrobials are predominantly used to prevent rather than to treat infections (15,16). One study in Vietnam suggests that AMU

in household chicken farms in the Mekong delta region is approximately six times greater than reported in some European countries (15). In addition commercial feeds for pigs and poultry are often medicated (17).

According to the FAO, virtually all classes of antimicrobials important for human medicine are also used in animals (16). In Vietnam, antimicrobials considered of critical importance for human medicine by the WHO are used in poultry and pig farms (penicillins, third generation cephalosporins, quinolones, aminoglycosides, polymyxins and macrolides) (15,43,44). In Vietnam most antimicrobials destined for animal production are available over the counter, and the veterinary pharmacist is a major source of advice for farmers (15,16).

In Southeast Asia, the use of antimicrobials is also particularly common in intensive aquaculture, as well as in integrated agriculture-aquaculture systems where humans, vegetable/rice fields, livestock and aquaculture ponds are in close proximity (10,45). For example, studies reveal that antimicrobials are routinely used in most (>70%) aquaculture systems in Vietnam, including farms raising fish and shrimp (13,46). These included many antimicrobials on the WHO list of critically important antimicrobials for human use, including beta-lactams and the quinolones (13). All tilapia (fish) farmers interviewed for a study in Thailand reported using antimicrobials (47). Antimicrobials in aquaculture are usually administered in feed, and therefore given indiscriminately to healthy and infected shrimp or fish (13,14). In addition, many antimicrobial products used in aquaculture in Vietnam did not contain the concentration of antimicrobials

advertised on the label, or provided erroneous dilution information (48). Farmers are usually not well informed about antimicrobials, and are often not aware of the appropriate regulations (13). As aquaculture farms often lack appropriate waste treatment, antimicrobials are eventually released in the surrounding environment where high levels of antimicrobials residues have been measured (45,46).

The use of antimicrobials and the generation of AMR on farms may lead to the transmission of AMR organisms and genetic elements coding for resistance to humans (49). Transmission mechanisms include direct contact with animals, consumption of foods of animal origin and dissemination through animal waste (50). The problem is amplified by the integrated agriculture-aquaculture farming systems commonly found in Southeast Asia, which often have poor bio-containment (51). Some aquaculture systems are sustained by adding human and livestock waste, creating the optimal context for antimicrobial residues, AMR genes or AMR bacteria present in livestock or humans to transfer to aquaculture, and subsequently to environmental water that is ultimately consumed by humans and animals. As an illustration, 87% of the *Escherichia coli* isolated from the Matang mangrove estuaries in Malaysia were resistant to at least 1 of 15 antimicrobials tested, and 34% were resistant to 3 or more antimicrobial classes (52). The authors hypothesized an association between the high level of multi-drug resistance (MDR) and the proximity to fishing villages lacking adequate sewage and sanitation.

3.5. Drug access and quality

General access to antimicrobials has improved across the region, thus the consumption of antimicrobials has had both a positive effect (preventing illness and death for millions) and a negative effect (inappropriate AMU is a major driver of AMR). It is worth noting that lack of timely access to good quality antimicrobials remains a reality for many (53). In a recent study from Lao PDR, one in five doctors declared that the antimicrobial prescribed was more influenced by what was available at the hospital than by the presumed etiologic agent (35). In the same study, 38.3% of the participants considered that some of the antimicrobials available at their hospital were of poor quality.

It is difficult to ascertain the overall quality of antimicrobials available in Southeast Asia. According to WHO, up to 10% of all drugs worldwide (including antimicrobials) are counterfeit. Antimicrobials (mainly antimalarials and antibacterials) are the most frequent counterfeited drugs (54). Counterfeit drugs include drugs which are incorrectly dosed, contain the wrong active ingredient, contain no active ingredient, are of sub-standard quality or are wrongly packaged (55). Worldwide, sales of counterfeit drugs is a lucrative business that generates an estimated $75 billion a year (56). A study found that Southeast Asia produced more than three quarters (78%) and consumed almost half (44%) of counterfeit antimicrobials globally (55), while a recent review confirmed that almost half of all counterfeit antimicrobials were found in Asia (54). A further study found that 31% of drugs tested had an active pharmaceutical ingredient at least 15% lower (or higher) than indicated on the label (57), and a study in Cambodia showed that 14.5% of drugs tested (including antimicrobials) were unacceptable with respect to the

quantity of active ingredient documented in the packaging (58). This is particularly

alarming in the context of AMR, as antimicrobial drugs in which the active

pharmaceutical ingredient is of low quality or low dosage exposes pathogens to sub-

therapeutic doses, which again favours the development of resistance.

4. The current AMR situation in Southeast Asia

4.1. The AMR burden in Southeast Asia

In order to help determine the magnitude of the AMR problem globally, the WHO recently collected information available worldwide regarding AMR for seven common bacterial pathogens (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, Non-typhoidal *Salmonella*, *Shigella* species, and *Neisseria gonorrhoea*). These data were published in the "Antimicrobial Resistance – Global Report on Surveillance" in 2014 (4). This report highlighted the lack of systematic data collection concerning AMR in Southeast Asia, and describes the problem as "burgeoning and often neglected" (4). As an illustration, the percentage of *Escherichia coli* resistant to third generation cephalosporins in Southeast Asia is summarised in Figure 1. Some recent trends are listed below.

4.1.1. MDR Gram-negative bacteria

A recent publication reviewed the epidemiology and molecular characterization of MDR Gram-negative bacteria in Southeast Asia (59). MDR Gram-negative bacteria were found to be a major contributor to the AMR burden in Southeast Asia. The prevalence of carbapenem-resistant organisms is rising in Southeast Asia, which is particularly alarming, as carbapenems are considered to be one of the few last resort options for treating infections caused by MDR Gram-negative organisms. *Acinetobacter baumannii* was the most frequently isolated carbapenem-resistant organism associated with hospital-acquired infections in Southeast Asia.

AMR is not restricted to pathogens and commensal organisms can also act as a sink and a source for AMR genes. For example, the prevalence of carriage of Extended-Spectrum Beta-Lactamase (ESBL)-producing *Escherichia coli* in the stool of community residents was 71.9%, 51.0% and 69.3% in Lao PDR, Vietnam and Thailand, respectively (60). Another community survey in Thailand demonstrated that 26.4% of food and open water sources were contaminated with ESBL-producing *Escherichia coli* (31). A study in Lao PDR revealed that 23% of children attending pre-school facilities were colonized with EBSL-producing *Enterobacteriaceae* (85% *E. coli* and 20% *K. pneumoniae*) (61). Similarly, 44% of *E. coli* isolated from children with UTIs were resistant to third generation cephalosporins via undetermined mechanisms in Cambodia (62). A study conducted on the Thailand-Myanmar border in non-pregnant adults with symptoms of urinary tract infections (UTIs) in 2013-2014 reported that 20.9% of *E. coli* cultured produced ESBLs, a percentage five times higher than in a similar setting in pregnant women in 2004-2006, confirming the rapid increase in AMR in Southeast Asia (63). A recent study in Vietnam showed a high prevalence of resistance of *E. coli* isolated from pig and chicken farms: most notably against ampicillin (97.8% and 94.4% for chickens and pigs, respectively), ciprofloxacin (73.3% and 21.1%), gentamicin (42.2% and 35.6%) and colistin (22.2% and 24.4%) (16). Furthermore, the colistin-resistance gene *mcr-1*(64) has been found in some Southeast Asia in *E. coli* strains isolated between 2012 and 2015 (65) from (i) humans in Cambodia (66), (ii) human and pigs in Lao PDR (67), (iii) human, pigs, chicken and water in Malaysia (68,69), (iv) humans in Thailand (67), and (v) from chicken and pigs in Vietnam (16,70).

Salmonella subspecies *enterica* serovar Typhi causes typhoid fever, which is common in some parts of Southeast Asia (71). MDR *S.* Typhi (defined as resistance against ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole) is commonly isolated in Southeast Asia (72). Recently, a particular lineage of this multidrug-resistant subclade, which is common across the region, has been associated with fluoroquinolone treatment failure in Nepal (73). A recent study in Thailand demonstrated that non-typhoidal *Salmonella* were present in 82%, 62% and 20% of pork, chicken meat and lettuce samples, and a high proportion of those isolates were resistant to multiple antimicrobial agents (including ampicillin, chloramphenicol, nalidixic acid, sulfamethoxazole-trimethoprim, and tetracycline) (74). In the Philippines, randomly selected *S. enterica* isolates from slaughtered swine in Manila were all resistant to at least one antimicrobial agent, and 15% were MDR (75).

Shigella spp. are the most common cause of bacterial dysentery worldwide. *Shigella sonnei*, which has historically been the genus dominant in Europe, is now beginning to dominate in many LMICs, including in Southeast Asia, where it is progressively replacing *Shigella flexneri* as the prevailing species (76,77). With respect to the spread of AMR, this epidemiological shift from *Shigella flexneri* to *Shigella sonnei* is particularly concerning in a place like Southeast Asia where many drivers of AMR are present. Indeed, it has been shown *Shigella sonnei* is more likely to develop resistance against broad-spectrum antimicrobials than *Shigella flexneri*, perhaps due to its ability to accept and maintain horizontally transferred DNA (77). Furthermore, it appears that Asia is the

focal hub for the emergence and spread of fluoroquinolone resistant *S. sonnei. S. sonnei* with this phenotype have been isolated globally but are associated with travel to South and Southeast Asia, thus exemplifying the role of international travel in AMR organisms trafficking (Figure 3) (78). Recently, the *mcr-1* colistin-resistance gene has been reported in an *S. sonnei* isolate from Vietnam (79). This was the first description of the *mcr-1* gene in *Shigella*.

The high prevalence of AMR Gram-negative bacteria in Southeast Asia is not just a concern locally, but also for the rest of the world. Recently in South Korea, an epidemic of *Shigella sonnei* resistant to both third generation cephalosporins and fluoroquinolones was traced back to a traveller returning from Vietnam (80), exemplifying the ease with which disease and AMR can spread in an increasingly connected world (Figure 3). Similarly, a recent study investigating the risk-factors for travel-related acquisition of antimicrobial resistant *Enterobacteriaceae* showed that Asia was a particularly high-risk area for the acquisition of ESBL-producing or ciprofloxacin-resistant *Enterobacteriaceae* (81,82). Another study investigating the effect of international travel on the gut resistome of healthy individuals demonstrated that travel to Southeast Asia or the Indian subcontinent was associated with high acquisition rate of AMR genes in the microbiome of travellers returning to the Netherlands (83).

Figure 3. The phylogenetic structure of ciprofloxacin resistant Shigella sonnei in an international context

Unrooted maximum likelihood phylogeny of Central Asia III, comprised of 97 *S. sonnei* sequences. Branch colours indicate region of isolation (where no travel history is confirmed) or region of recent travel (where travel history confirmed) according to the keys. For isolates with confirmed recent travel, a coloured circle at the tip indicates the region where the isolate was collected (multiple coloured circles are indicative of multiple isolates). Labelled arrows indicate branches where the mutations *gyrA*-S83L, *gyrA*-D87N, *gyrA*-D87G and *parC*-S80I have arisen. Blue background shading denotes isolates exhibiting ciprofloxacin resistance conferred by triple mutations (*gyrA*-S83L, *parC*-S80I and *gyrA*-D87G (or *gyrA*-D87N)). Subpopulations A and B, are highlighted in the darker blue shaded areas, denoting clonal expansions in Southeast Asia and Europe/America, respectively. Numbers above major branches indicate bootstrap support values, and horizontal scale bar denotes th e number of nucleotide substitutions per site (From reference 78).
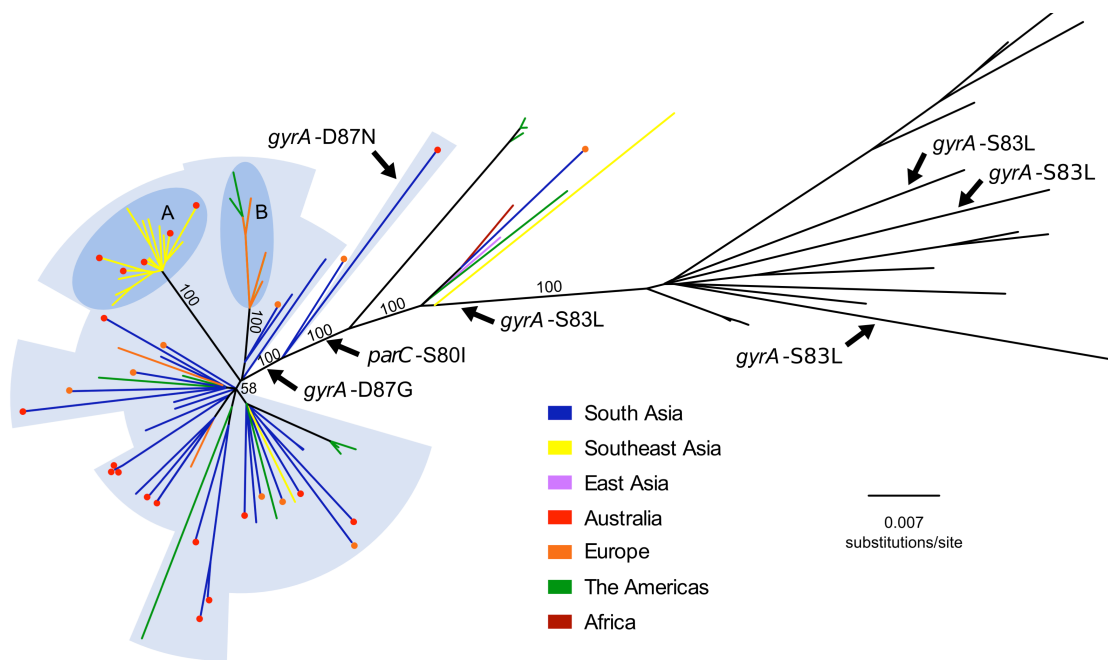
4.1.2. MDR Gram-positive bacteria

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been proposed to be common in health facilities in Asia, but recent data on MRSA prevalence in Southeast Asia is limited (84). In the community, recent studies reported a 7.9% prevalence of MRSA carriage in the rural and urban population in Vietnam (85), and a prevalence of 6% in elderly adults (over 60) in Jakarta, Indonesia (86). Other studies found an 8.1% prevalence of MRSA carriage in patients on hospital admission in Thailand (87) and Indonesia (88). Recently, ceftaroline-non-susceptible MRSA isolates have been reported in Thailand and the Philippines (89).

MDR *enterococci* have been isolated from livestock and animal-related products in Malaysia, Thailand, Vietnam and Indonesia (22,90–92). Given the pivotal role of these countries in livestock and fish production, export of food products may fuel the spread of AMR via the consumption of incorrectly handled (or undercooked) meat or fish products. As an illustration, 75% of pangasius (Cat fish) samples imported into Switzerland from Vietnam were contaminated with *E. faecalis* (93) Furthermore, MDR *enterococci* have been detected in coastal waters (including recreational coastal waters) in Malaysia, and in water bodies such as agricultural wells, rivers, and canals in Thailand (94–96), thus providing the possibility of water-borne transmission of resistant *enterococci*. Recently, vancomycin-resistance was reported in *E. faecalis* and *E. faecium* clinical isolates from patients in Malaysia (97) and Thailand (98).

While the consensus is that the AMR burden is large in Southeast Asia in terms of morbidity, mortality and economic cost, it is difficult to quantify the exact AMR burden due to the lack of standardised and comprehensive data. A recent estimate based on secondary data provided by 1,023 hospitals suggests that in Thailand, infections caused by AMR bacteria caused an additional 3.24 million days of hospitalization and 38,481 deaths in 2010 (99). The same study estimated that the antimicrobials necessary to treat AMR infections cost 202 million USD in 2010, and the total costs associated with AMR-related morbidity and premature deaths was 1.3 billion USD.

4.2. The current AMR surveillance capacity in Southeast Asia

While it is widely accepted that excessive and unregulated AMU and AMR emergence are preoccupying issues in Southeast Asia, vast knowledge gaps remain concerning the extent of the problem, both for AMU and AMR. This may be due to the fact that surveillance capacity is largely heterogeneous across the region. Some AMR surveillance systems are functional, other may have good infrastructure but lack trained personnel, equipment and reagents, and some areas within Southeast Asia have little capacity altogether, leaving large zones without data. Below, we describe some networks that are or have contributed to surveillance efforts for AMR in Southeast Asia.

4.2.1. The Asian Network for Surveillance of Resistant Pathogens (ANSORP)

The Asian Network for Surveillance of Resistant Pathogens (ANSORP) was created in 1996 to perform surveillance of AMR in Asia (100). ANSORP is an independent, non-governmental, not-for-profit international network for collaborative research on antimicrobial agents and infectious disease in the Asia Pacific region. The goal of ANSORP is to develop international strategies, action plans and to provide a platform for effective control and prevention of AMR in Asia. ANSORP is active in 113 hospitals in 65 cities throughout 14 countries (Saudi Arabia, Sri Lanka, India, China, South Korea, Japan, Hong Kong, Taiwan, Thailand, Vietnam, Philippines, Malaysia, Singapore, Indonesia).

4.2.2. The Vietnam Resistance project (VINARES)

The Vietnam Resistance (VINARES) project was initiated after the publication of a report (21) that identified sub-optimal infection control, inadequate laboratory diagnostic capacity, and inappropriate AMU as main drivers of AMR in Vietnam (20). VINARES is a capacity-building initiative aimed at strengthening antimicrobial stewardship in Vietnam, particularly in the areas of (i) infection control and healthcare-associated infections, (ii) antimicrobial consumption and (iii) microbiological analysis and reporting capacity. VINARES is a collaborative effort between Vietnamese healthcare professionals, the Wellcome Trust Major Overseas Programme (WT-MOP) and Linköping University (Sweden). Sixteen hospitals throughout Vietnam participate in the VINARES collaboration.

4.3. The Vietnam Wellcome Trust Major Overseas Programme (WT-MOP)

The primary objective of the Vietnam WT-MOP is to perform clinical research that has a major impact on local and global health. The network created by the WT-MOPs in Asia (Vietnam and Thailand MOPs) has a unique presence across the region and leads focussed responses to the major and rapidly evolving challenges of healthcare in Asia. Local capacity building through training and enhancing laboratory infrastructure has been a major component of the research conducted through this organisation in last 30 years. The WT-MOPs have central units in Bangkok, Thailand (MORU) and in Ho Chi Minh City, Viet Nam (OUCRU). One of the core themes across the region is to make defining a contribution to the understanding of infectious diseases transmission and susceptibility; to develop new tools to prevent, control and treat AMR organisms; improve clinical outcomes for the major endemic and emerging infectious and non-infectious diseases; and enhance public health policy in the region. The WT-MOPs in Asia are a major contributor to AMR research and surveillance in the region and aims to perform key clinical trials to improve treatment regimens and intervention studies to reduce AMU and AMR in human communities and farming systems.

4.3.1. The Global Antimicrobial Resistance Partnership (GARP)

The Global Antimicrobial Resistance Partnership (GARP) was launched in 2008 to amplify the voice of LMICs at the AMR discussion table. The goal of GARP is to formulate and promote locally relevant policies related to AMU and AMR in low- and middle-income countries (101). This is achieved by catalysing discussions between local experts in order to analyse the AMU and AMR situation, identify knowledge gaps, and propose locally relevant solutions that can be implemented in the public and/or private sector. GARP is funded by the Bill and Melinda Gates Foundation (101), and

has active programmes in 8 countries: Kenya, India, Vietnam, South Africa, Mozambique, Tanzania, Nepal and Uganda. After a 3-5 year period of initial funding by GARP, countries are expected to sustain the activities they chose to pursue.

4.3.2. PulseNet Asia Pacific

PulseNet Asia Pacific was established in 2002 as a network of laboratories active in performing molecular subtyping of bacteria. The goal of PulseNet is to enable timely exchange of DNA fingerprinting data on pathogens causing foodborne outbreaks in the Asia Pacific region. PulseNet Asia Pacific focuses on laboratory capacity building, training, quality assurance, protocol evaluation, standardization, and communication enhancement. Current members of the network are Australia, Bangladesh, China, Hong Kong, India, Japan, Korea, Malaysia, New Zealand, Philippines, Taiwan, Thailand and Vietnam. PulseNet Asia Pacific is part of PulseNet International, a worldwide network of laboratories that use standardised genotyping methods and real-time information sharing to enhance surveillance and provide early warning of food and waterborne disease outbreaks, as well as emerging pathogens.

4.3.3. The National Antimicrobial Resistance Surveillance Thailand (NARST)

The National Antimicrobial Resistance Surveillance Thailand (NARST) is a collaborative network that was initiated in 1998 through funding by the WHO. Its goal is to strengthen the surveillance program for AMR and standardise laboratory practices in Thailand. NARST collects data from 33 hospitals throughout Thailand (102). The Thailand AMR Containment and Prevention (AMRCP) Program was created in 2011 to contain and

limit the emergence and spread of AMR bacterial strains in Thailand. AMRCP's operational actions are guided by the "One Health" principle, and some of the actions scheduled for the 2012-2016 period include: (i) estimating the AMR burden in Thailand, (ii) developing laboratory and information technology for surveillance of AMR, antimicrobial use and hospital-acquired infections, (iii) improving the understanding of how AMR develops and spreads, (iv) generating local evidence for promoting responsible use of antimicrobials (iv) regulating the use and distribution of antimicrobials in humans and food animals, and (v) conducting research and development of diagnostics, therapy and prevention of antimicrobial-resistant bacterial infections (99).

4.4. The WHO, FAO and OIE tripartite collaboration

The World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE) have united in a tripartite collaboration that promotes a holistic, multisectorial and comprehensive "One Health" approach to the AMR threat. Their ultimate goal is to (i) ensure that antimicrobial agents stay effective and useful to cure disease in humans and animals, (ii) promote prudent and responsible use of antimicrobial agents, and (iii) ensure global access to good quality medicine. As part of this effort, the OIE is developing a database to collect harmonized data on AMU in animals worldwide, as there is currently a major knowledge gap as to the quantities and types of antimicrobial agents used in agriculture, particularly in LMICs.

This (non-exhaustive) description of the existing networks and initiatives demonstrates that the fight against AMR is gaining momentum in Southeast Asia. However, the landscape is fragmented, and better coordination, collaboration and data sharing between the networks may be beneficial to assess AMR burden, evaluate current surveillance capacity, disseminate information and avoid redundancy of people, resource and laboratory capacity.

4.5. Availability of AMU data in Southeast Asia

Many high-income countries stabilized or reduced their antimicrobial consumption between 2000 and 2010, but consumption has increased in many LMICs, particularly the BRIC countries (6,18,103). AMU is the main driver of AMR, and therefore the collection of data on AMU in humans, agriculture and aquaculture is crucial to understand AMR development in Southeast Asia. Unfortunately, identifying reliable estimates for AMU in humans and animals is extremely challenging.

It has been reported that per capita antimicrobial consumption increased in Thailand and Malaysia between 2000 and 2010 and in Vietnam between 2005 and 2010, decreased in Indonesia and the Philippines, while no data are available for Myanmar, Cambodia and Lao PDR (6). AMU in primary healthcare is an important driver of AMR (104), but data on AMU in primary care setting in LMICs is scarce. A previous literature review has shown that the proportion of patients receiving antimicrobials in primary healthcare has increased worldwide in the past decades (39,105), but again nationwide estimates are lacking for many Southeast Asian countries (106,107). Recently, a study

on a representative sample of primary healthcare clinics in Malaysia aimed to fill this knowledge gap, and showed that one in five patient encounters in a primary care clinic resulted in an antimicrobial prescription (39). The rate of prescription was much higher in the private (30.8%) than in the public (6.8%) clinics. Such studies are precious to fill the knowledge gap concerning AMU in Southeast Asia and understand better the drivers of AMR.

Globally, it has been estimated that veterinary AMU for food production will increase by 67% by 2030, and that one third of this increase will be caused by a shift towards intensive livestock production in middle-income countries. Some of the greatest increase in AMU (>200%) between 2010 and 2030 are expected in Myanmar and Indonesia (18). Despite this prediction, data on AMU in animal populations is scarce, particularly in LMICs, where the bulk of the AMU increase is expected. A survey from the OIE in 2012 showed that only 27% of its members had official systems to collect quantitative data on AMU in livestock production (108). Similarly, in a recent attempt to quantify AMU in animals worldwide, estimates regarding AMU in animals could be obtained from 32 high income countries only (18). To fill this important knowledge gap, OIE is developing a database containing country-level AMU data; this database was not available online at the time of writing.

4.6. Antimicrobial stewardship and policy in Southeast Asia

There is no universally accepted definition of antimicrobial stewardship (AMS), but the term refers to the strategies, policies, guidelines and tools used to promote and

increase the appropriate use of antimicrobials (109,110). The goal of AMS is to ensure effective treatment for patients with bacterial infection and to reduce unnecessary antimicrobial use in order to limit the development of AMR and preserve efficacy of antimicrobials.

In 2011, the 11 members of the WHO South East Asia Region (Bangladesh, Bhutan, Democratic People's Republic of Korea, India, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, Thailand, Timor-Leste) signed the "Jaipur Declaration", in which they recognized the seriousness of the AMR problem and committed to act in order to safeguard the efficacy of antimicrobial drugs. The members agreed to "promote behavioural change in prescribers and communities through continuous training, educational campaigns with process and outcome measures for rational use of antimicrobial agents and emphasizing antimicrobial resistance in medical, dental, veterinary and pharmacy curricula". This became known as a seminal stewardship initiative in the region. A recent report showed that a year after signing the Jaipur Declaration, 42% of the countries involved had national AMS standards in Asia; the worldwide proportion was 52% (110). 63% of the hospitals surveyed in Asia reported having AMS standard, which is close to the worldwide average of 62%. No data were available specifically for Southeast Asia.

In Thailand, the Antimicrobial Smart Use (ASU) initiative was introduced in 2007 to promote the rational use of antimicrobials (38). Guided by the assumption that optimizing AMU requires a behavioural change, the ASU initiative attempted to rectify

common misconceptions that patients have about antimicrobials, by clarifying that: (i)

antimicrobials are not anti-inflammatory drugs, (ii) antimicrobials are potentially

dangerous drugs, and (iii) upper respiratory tract infections, diarrheal diseases and

superficial cuts can be cured without antimicrobials. In addition, the ASU initiative tried

to change prescription practices among health professionals, through education and

training. In a further study in Thailand on how to improve AMS programs in hospitals,

the addition of trained infectious diseases clinical pharmacists to the normal

consultation decreased the amount of inappropriate prescribing of antimicrobials (111).

Recently, Vietnam expanded their National Action Plan to combat drug resistance with

an Aide-Memoire in which 4 ministries committed to jointly implement the action plan

across multiple sectors, improve AMR awareness and strengthen rational drug use and

infection control – all of which fall under AMS (see case study, source:

www.wpro.who.int).

Case study: AMU and AMR in Vietnam

In 1986, the Vietnamese government initiated economic reforms known as the Đổi Mới, which initiated a transition towards a more market-driven economy. In the following 30 years, income and life expectancy increased, access to healthcare and drugs improved, and childhood mortality decreased (21) (Table 1). With great foresight, the Vietnamese Ministry of Health declared in its National Drug Policy (1996) "antimicrobials are very important drugs for treatment, and therefore it is necessary to regulate the prescription, antimicrobial use, and antimicrobial resistance of most common bacterial pathogens as well as improve laboratory diagnosis." In 2005, the Drug Law introduced a prescription-only regulation for antimicrobials (21). Unfortunately, it is have not been solidly enforced and access to antimicrobials remains relatively unregulated today (19).

In parallel, population growth, increasing buying power and economic development in Vietnam have stimulated the demand for antimicrobials in human health, but also in animals, due to the thriving livestock industry, constantly expanding to satisfy a growing domestic meat market as well as export (Table 1). In 2010, Vietnam was the third largest producer of aquaculture products behind China and India (112).

Use of antimicrobials is widespread in hospitals. A recent study found that in adult intensive care units (ICU) in Vietnam, almost 85% of the patients were receiving antimicrobials (113). The most frequently used antimicrobials in ICUs were broad-spectrum beta-lactam antimicrobials (3rd, 4th generation cephalosporins and carbapenems) and fluoroquinolones (113). The propagation of resistant strains is

facilitated by the fact that hospitals are old and overcrowded, understaffed, and only the larger facilities have diagnostic capacity suitable for bacterial culture and AMR profiling (19,113).

The use of antimicrobials (often in self-medication due to financial reasons) in the community is frequent in Vietnam: a study in rural Vietnam in 1999 showed that 91% of children with suspected acute respiratory tract infection (ARI) received broad-spectrum penicillins. These drugs were self-prescribed for more than three quarters of these individuals (28). From 1999 to 2007 penicillin-resistant *Streptococcus pneumoniae* increased from 8% to 75% in this same community that was investigated for antimicrobial usage (114). Self-medication in Vietnam is facilitated by the fact that pharmacies easily agree to sell antimicrobial without prescription (23).

Use of antimicrobials in also widespread in livestock production and aquaculture, including antimicrobial used to treat human disease including, beta-lactams, aminoglycosides, macrolides, tetracyclines, (fluoro)quinolones, phenicols, pleuromutilins, lincosamides, sulfonamides, diaminopyrimidine (trimethoprim) and colistin (16,19). The situation is similar in aquaculture, and a survey found that 72.3% of aquaculture farms raising fish and shrimp used feed containing antimicrobials (13). This is further complicated by the fact that agriculture-aquaculture systems where aquaculture ponds, animals and fields are in close proximity, often without bio-containment are common. This increases the risk of environmental release of antimicrobials and AMR bacteria, promoting AMR gene exchange or amplification. In agriculture and aquaculture,

antimicrobials are usually used for economic reasons (improve production by avoiding disease) and with poor knowledge about antimicrobials and regulations (13,19).

Some recent facts about AMU and AMR in Vietnam:

- 57% of the total quantity of antimicrobials used in commercial feeds for poultry and pigs in Vietnam consisted of antimicrobials regarded by WHO of importance for human medicine, including amoxicillin, colistin, tetracyclines, neomycin, lincomycin, and bacitracin (17)
- In 85.1% of Vietnamese aquaculture systems, aquaculture animals are raised with other farm animals (chicken or pigs), or within or alongside rice fields (13)
- 37.5% of *E. coli* isolated from pigs on farms or about to be/recently slaughtered near Hanoi contained the recently described colistin-resistance gene *mrc-1* (70)
- 26.9% of fish and shrimp samples bought in local markets in Vietnam contained antimicrobial residues (13)
- In retail chicken and pork meat, 66% of *Salmonella* was MDR and 78.4% were resistant to at least one antimicrobial (90)
- Ho Chi Minh City had the highest rate of resistance in *Campylobacter* isolated from chicken (compared to Dakar in Senegal, Yaounde in Cameroon, Nouméa in New Caledonia, and Antananarivo in Madagascar) (115)

- In Central Vietnam, *Salmonella spp* were detected in 46.3% and 71.7% of poultry and swine farms surveyed, respectively; 72% of isolates were resistant to 1 of 14 antimicrobial tested, with high rates of resistance to ampicillin, chloramphenicol, ciprofloxacin, sulphamethoxazole and tetracycline (116)

- In adult ICUs in Vietnam, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* had carbapenem resistance rates of 89.2%, 55.7%, and 14.9% respectively (113).

- Approximately one third of patients received antimicrobials inappropriately in Vietnamese hospitals (26).

The political and medical leaders in Vietnam recognized the seriousness and the likely impact of the AMR problem and in 2013 Vietnam became the first country in the Western Pacific WHO region to approve a National Action Plan to combat drug resistance (source: www.wpro.who.int). In 2015, this was taken a step further by signing of an Aide-Memoire in which the Ministry of Health, Ministry of Agriculture and Rural Development, Ministry of Trade and Industry and Ministry of Natural Resources and Environment committed to jointly coordinate the implementation of the national action plan across different sectors, improve health workers and public awareness with respect to AMR, enhance and improve national surveillance capacity for AMU and AMR, ensure adequate supply of quality essential drugs and strengthen safe and rational drug use and infection control across sectors.

5. Knowledge gaps

5.1. AMR in the community

AMR surveillance is being currently performed across the region, but via an *ad hoc* approach and mainly in healthcare setting. This method is limited as the hospital-associated AMR burden only represents a fraction of the total problem in Southeast Asia. Unfortunately, data originating from systematic longitudinal sampling of healthy individuals and animals is rare, leaving large knowledge gaps regarding the AMR burden in the community and in livestock/fish production in Southeast Asia. We surmise that integrated surveillance incorporating healthcare settings, the community and livestock/aquaculture farms is required to generate a more comprehensive understanding of the AMR issue across the region.

5.2. AMR-specific morbidity, economic cost and societal costs

While the general consensus is that AMR-associated morbidity, mortality and economic costs are enormous, there is currently no reasonable estimate of these for Southeast Asia (the same could be argued for the vast majority of the world). The difficulty in directly estimating the morbidity and economic cost of AMR is notorious, which is associated with the scarcity of data required for such analyses (117,118). Beyond morbidity, mortality and economic cost, it is also critical to estimate the wider societal cost of AMR, including the loss of efficacy of current antimicrobials (and its effect on modern medicine), as well as detrimental effects of AMR on human capital, labour force, gross domestic products and economic growth.

Generating evidence regarding the human, economic and societal cost of AMR is essential to define the magnitude of the problem, as well as raising regional awareness, promoting attitude changes and assisting governments in developing policies required to tackle AMR. Understanding the effect of AMR on clinical outcome is the first, and the most necessary, step towards evaluating the economic cost of AMR.

5.3. Data on AMU

Despite the prediction that a shift towards more intensive livestock production in middle-income countries will cause a large increase in global AMU, data on AMU is generally scarce, particularly in LMICs in Southeast Asia (18). Surveillance systems that monitor AMU in food-production animals exist in relatively few number of countries, all of which are high income and outside of Southeast Asia (119). As AMU is the key driver of AMR, a paucity of data for AMU is a significant knowledge gap that requires filling urgently.

Precise estimates for AMU in humans is difficult to obtain as a large proportion of the antimicrobials sold are done so without prescription, or without any formal official record of their sale. In addition, substandard, spurious, falsely labelled, falsified and counterfeit (SSFFC) antimicrobials are thought to be commonly found on the market in Southeast Asia (54,55), this is a further obstacle when accurately estimating AMU. Quantifying AMU in animals is, arguably, more challenging due to the heterogeneity of procurement channels, the number of producers, vague or incorrect labelling on feed packaging, and the fact that antimicrobial-containing feed is often used by farmers without supervision

or being prescribed by a trained veterinarian. These issues may be tackled at source (i.e. through sales channels, importation and manufacture), however the amounts entering the various supply chains still need to be assessed accurately.

Despite the difficulty, estimating AMU is important for (i) defining the extent of the AMR problem, (ii) detecting pockets of overuse/misuse, (iii) understand the local drivers, (iv) monitoring trends and (v) quantifying the effect of potential interventions aimed at reducing AMU.

5.4. Influence of AMU in animals on AMR in humans

The consensus is that AMU in livestock production and aquaculture is an important driver of AMR in humans, data regarding this association are available from Europe, but are limited in Southeast Asia (120,121). In particular, little or no data are available for mixed livestock/aquaculture/agriculture systems and semi-intensive production systems prevalent in developing countries and their impact on AMR in humans, despite the fact that AMU is widespread and antimicrobials are used without veterinary supervision. Further, defining the main AMR gene flow routes between animals and humans (and vice versa) and understanding the various sinks and sources of AMR genes and organisms are particularly important for understanding the risks of gene transfer and emergence/spread of AMR organisms that operate at the animal-human-environment interface.

5.5. Quantification of the impact of interventions

While it is recognized that education, stewardship, policy, better diagnostics, better infection control can all optimize AMU and potentially decrease AMR, it is difficult to quantify their individual impacts on AMU and AMR. There is, therefore, an important knowledge gap as to which intervention (or component of an intervention) is the most efficient at reducing AMU and/or AMR. Filling this gap is important for permitting the design of targeted interventions aimed at reducing AMU and AMR, particularly in resource-limited settings. While essential, this is not a straightforward task, and will require an efficient data collection system to collect homogeneous AMU and AMR information over time and space across the region.

6. A vision for an AMR surveillance network in Southeast Asia

6.1. The goal of a network – surveillance geared towards research

In the light of the widespread (and vastly unregulated) AMU in humans, animals and aquaculture in Southeast Asia and the extent of the AMR problem, we recommend the development of a collaborative surveillance network. The network should be sustainable and its overriding role should be to better understand AMU and AMR in order to fill current knowledge gaps, guide interventions, inform policy, reduce AMU and ultimately reduce AMR-associated disease in Southeast Asia and beyond. Specifically, this surveillance network will be instrumental for (i) establishing accurate baseline estimates of the scale of AMU and AMR in Southeast Asia, (ii) detecting outbreaks of AMR associated infections early (and to react rapidly), (iii) monitoring long-term trends in both AMU and AMR, (iv) quantifying AMR-associated morbidity, mortality and economic cost, (v) measuring the effect of interventions aimed at reducing AMU and AMR and (vi) supporting future research efforts.

The primary focus of the network should be to improve and/or develop surveillance capacity for AMR in Southeast Asia. However, we additionally suggest that such a surveillance network should generate data that will be compatible with (and stimulate), future research on AMR. Better synergies should be developed between surveillance and research, as both initiatives will likely (at least partially) be supported by similar funders, collaborations, field sites and infrastructures.

6.2. *Suitable locations, current capacity, existing networks and collaboration*

Available data suggests that a considerable surveillance infrastructure, capacity and know-how already exist across Southeast Asia. However, we conclude that this infrastructure is heterogeneous throughout the region and highly fragmented. When possible, we suggest that existing AMR surveillance infrastructure should be used as a priority as this avoids the waste of resources and redundancy of equipment and data. AMR surveillance infrastructure that currently exists but is not operational (underused, absence of trained personnel, lack of reagents) should be developed. Where no infrastructure exists but is required, it should be developed *de novo*. The Asia WT-MOP provide an example of how this might work, as they are comprised of an extensive network of institutes and laboratories (Thailand, Vietnam, Laos, Cambodia, Indonesia, Nepal and access into other locations), which could be used form a basis for an AMR surveillance network. Once established, through standardised approaches, the network could be expanded beyond this infrastructure to include other organisations and facilities. Using an established research structure across the region circumnavigates some of the political issues in working with government agencies and departments.

For a sustained approach to AMR, medical (and veterinary) microbiology laboratories need be improved, developed or created as needed throughout the region in order to ensure that all locations have a uniform standard for the culture and identification of organisms and to determine antimicrobial susceptibility profiles. Collaboration and coordination between existing surveillance sites and networks need be initiated to

develop synergies and avoid redundancy of infrastructure and personnel. Sample collection, analysis (particularly the determination of AMR profiles) and result dissemination need to (i) be standardised across all sites within the network and (ii) follow the recommendations of recognised guidelines, such as the WHO's Global Antimicrobial Resistance Surveillance System (GLASS), in order to ensure longitudinal comparability with other surveillance networks worldwide (122). Reporting of results should occur via a common platform that permits real-time information sharing across Southeast Asia and worldwide. These data should be available in a manner that is clear, useful and interpretable for the general public, healthcare professionals and policy-makers.

In order to ensure a long-term commitment and sustainability of the surveillance program, capacity building should be performed and integrated with locally relevant projects and local ownership of the field sites/infrastructure should be guaranteed. In order to facilitate the development of surveillance activities and infrastructure in places where limited capacity exists currently, teams from laboratories with greater experience, knowledge and know-how should train, coach and mentor sites with a less well-developed surveillance/research infrastructure. This approach should result in uniform procedures (sampling, analysis and reporting) across the network, and improve the overall quality control.

6.3. Technology for AMR surveillance – classical methods and new technologies

The primary aim of a Southeast Asian AMR surveillance network should be to gather and distribute standardised data regarding AMU, AMR and the AMR-associated disease burden. Therefore, we propose that a surveillance network follows standardised procedures suggested in WHO's GLASS guidelines to collect specimens, determine resistance profiles and share data (WHONET) (122). However, the network also needs to be able to adapt for the implementation of advanced molecular biology techniques (e.g. whole genome sequencing (WGS), gene arrays and PCR). These techniques should be introduced to improve quality of surveillance and answer specific questions, such as elucidating the directionality and gene flow between species and to understand the major reservoirs of AMR genes and organisms. Whilst such techniques are currently the in realm of well-funded research organizations, PCR detection of AMR genes and WGS are begin to emerge into mainstream diagnostic and public health laboratories in high and middle-income countries. In conclusion, AMR is having a major impact on shaping bacterial populations across the region and is a key driver facilitating the international trafficking of bacterial pathogens. Whilst currently expensive and technically demanding, WGS of sentinel organisms should become an essential component of a Southeast Asian surveillance programme. WGS will be a fundamental method for understanding how AMR bacteria emerge and spread nationally, regionally and intercontinentally.

A partner capable of handling the large amount of data will be required to centralize, process and disseminate results. Trust and transparency between local data-collecting sites and the central data handling entity will need be maintained across the network to ensure a reliable flow of data.

6.4. Sampling strategies

We suggest that both humans and animals need to be sampled using the same protocol in sentinel sites, which should include healthcare facilities, the community and farms (places cultivating animals for human consumption (see below)). In healthcare settings, it is essential that data on AMR is collected in combination with patient data (e.g. diagnostic, treatment and most importantly, outcome) to generate a better understanding of the impact of AMR on patient health, healthcare systems, medical and societal costs, loss of productivity and the how that relates to outcome and mortality. Probably the most effectual syndrome to measure would be bloodstream infections. AMR plays a pivotal role in treatment and disease outcome in these bloodstream infections, and blood culture is relatively straightforward to standardise and compare data. Furthermore, blood culture can be established and performed in sentinel healthcare facilitates, or blood samples can even shipped and processed in a central reference laboratory if required.

It is worth noting that AMR-associated disease burden in healthcare setting represents only a small fraction of the total AMR burden, therefore community (human) and farm (animal) sampling should be performed in the same locations. Repeated cross-sectional

sampling in "healthy" (asymptomatic) humans and animals needs to be performed in conjunction with disease sampling to longitudinally assess the prevalence of AMR in the general human population and in animals and to monitor trends and changes over time. Sampling in the community could be performed randomly or on specific groups that can be followed over time (e.g. school-age children and farm workers, etc.). It would be prudent and cost-effective to sample animals in central sentinel locations where. We suggest that animals could be sampled prior to being slaughtered at slaughter locations, to assess the rate of AMR gene transfer into the food chain. Further, sampling butchered meat samples in markets could be performed in tandem, as consumption is the most likely mechanism of AMR genes entering the food chain.

6.5. Collection of data on AMU

Accumulating data on AMU in humans and animals is challenging but essential for assessing AMR. Information concerning AMU is can be obtained from various sources including published literature, reports, health authorities, veterinarian authorities, the pharmaceutical industry and animal feed manufacturers, suppliers and distributers. In addition, the World Organization for Animal Health (OIE) is currently developing a global database regarding AMU in farm animals at country-level, which could be incorporated into any surveillance system as it becomes publicly available. Individual-level data on AMU could also be obtained from farmers, hospitals, community based doctors or community members via surveys, interviews or sales records. Measuring antimicrobial access and quality should be included in the surveillance activities in order to understand selective pressures driving specific AMR phenotypes.

Furthermore, GIS technology could be used to populate and extrapolate maps of AMU throughout Southeast Asia based on available and newly generated data. Lastly, mathematical modelling will become a useful tool to infer a baseline understanding of the interaction of AMU and AMR across the region.

6.6. Investigating knowledge, attitude and practices regarding AMU and AMR through social research

AMU and AMR have a large behavioural component and understanding the human behaviours underlying excessive and irrational AMU should be an important component of any AMR surveillance effort. Antimicrobials are often seen as "wonder drugs" of which their true potential, limitations and long-term effects are not always understood. Qualitative research (e.g. surveys, interviews and focus-groups) are required to generate data and better knowledge, attitudes and practices with respect to (i) AMU in humans, (ii) AMU in agriculture and aquaculture, (iii) antimicrobial prescribing habits, (iv) self-medication and treatment compliance in Southeast Asia.

A particular focus should be placed on understanding the drivers of irrational prescribing by healthcare professionals, irrational demand by members of the public and irrational use by farmers. Generating robust evidence concerning current practices will improve awareness and will hopefully promote attitude change. In healthcare settings, there is a critical need to (i) broadly advertise the importance of diagnostic testing, (ii) understand the local, national and regional barriers to better diagnostic testing and (iii) assess what

combination of cost, reagent accessibility, time and training are restricting better

diagnostic approaches.

7. Conclusions

AMU and AMR are increasing across Southeast Asia, yet there is no sustained approach across the region to assess longitudinal AMU and AMR patterns. A collaborative AMR surveillance network which is collecting, analysing and disseminating standardised data throughout the region and beyond will be instrumental for filling current knowledge gaps concerning AMU and AMR. Further, this network is needed to establish baseline measurements for the prevalence of AMR and longitudinally measure trends. Ultimately, better surveillance capacity will contribute to guiding clinical interventions, inform policy, and ultimately reduce AMU and AMR-associated disease.

A sustainable integrated network should be built upon existing infrastructure, and expand to new locations to become region wide. Capacity building, training, collaboration and data sharing will be essential to improve surveillance capacity across Southeast Asia. Surveillance efforts should be embedded into locally relevant projects to ensure the long-term commitment of staff and local authorities. Such a network should focus on surveillance and the development of local capacity, which should be compatible with future research projects. Modern molecular technologies will become integral, and as they become more affordable should be made part of routine diagnostics and a sustained surveillance system.

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9.   Appendix

Key local partners in Southeast Asia:

Cambodia   Cambodia-Oxford Medical Research Unit (COMRU), Siem Reap

Indonesia   Eijkman-Oxford University Clinical Research Unit (EOUCRU), Jakarta

Lao PDR   Lao-Oxford-Mahosot Hospital Wellcome Trust Research Unit, Vientiane

Malaysia   The University of Malaya, Kuala Lumpur

Myanmar   Myanmar-Oxford Clinical Research Unit (MOCRU), Yangon

Philippines   San Lorenzo Hospital, Manila

Thailand   Mahidol Oxford Tropical Medicine Research Unit, Bangkok

Vietnam   Oxford University Clinical Research Unit, the Hospital for Tropical

Diseases, Ho Chi Minh City

National Hospital for Tropical Diseases, Hanoi

With additional support from:

Australia   The University of Melbourne

Singapore   The Genome Institute of Singapore, Nanyang Technological University

South Korea The International Vaccine Institute (IVI)

UK   The Sanger Institute, Cambridge University

The London School of Hygiene and Tropical Medicine

University of Oxford