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ANTIBIOTIC RESISTANCE: IMPLICATIONS OF HOSPITAL PRACTICES FOR PUBLIC HEALTH

A study from Hanoi, Vietnam



La Thi Quynh Lien

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Antibiotic resistance: implications of hospital practices for public health

A study from Hanoi, Vietnam

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In the memory of my father

“Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.”

– Marie Curie

PREFACE

In 2001, I passed the entrance examination of Hanoi University of Pharmacy (HUP). My father, who constantly encouraged me to study, was thrilled and held a party in celebration of my success. During the first semester at HUP, I won a scholarship to study pharmacy at Saint Petersburg State Chemical Pharmaceutical Academy, Russia. After being graduated from the academy, I returned to Vietnam in 2008 and started working as a lecturer at HUP.

Being a junior lecturer at the only Vietnamese university specialised in training pharmacists, where the cut-off score of the entrance examination has always been among the top universities across the country, I did not feel "good enough". I strongly desired to be trained at a prestigious, high-ranking university in the world.

It can be said that my PhD journey started during evening English classes, to times when my scholarship applications for PhD programmes abroad were rejected, until I received a scholarship from the Vietnamese Government to study PhD in Sweden. At the time, I was fortunate to be involved in a research project aimed to strengthen the management of antibiotics and antibiotic resistance in Vietnam led by Professor Cecilia Stålsby Lundborg, who later became my principle supervisor.

Antibiotic resistance is a global challenge, particularly for low- and middle-income countries, and requires global action. I hope this thesis will contribute, in part, to the provision of evidence and respective solutions in the battle against antibiotic resistance globally.

Now that I am at the end of my PhD journey, I do not think that being “good enough” really matters. I have learned and grown so much throughout this journey, not only in my professional but also my personal life. For me, a big achievement on this journey is “understand more... and fear less”.

ABSTRACT

Background: Antibiotic resistance is a global challenge, requiring urgent attention. Inefficient hospital infection control and hospital dissemination of antibiotic residues and antibiotic resistant bacteria contribute to the spread of antibiotic resistance.

Overall aim: To explore hospital practices in relation to the spread of antibiotic resistance in a rural and an urban hospital in Hanoi, Vietnam.

Methods: The thesis consists of one qualitative (II) and three quantitative studies (I,III&IV). In **Paper I**, a cross-sectional study, questionnaires consisting of items on knowledge and practices of infection control were collected from 339 hospital staff. For analysis, total knowledge or practice score ranged from 0-15. In **Paper II**, individual interviews and focus group discussions were conducted with a total of 50 doctors, nurses and cleaning workers. A semi-structured guide on infection control was used. Content analysis was applied. In **Papers III & IV**, wastewater samples were collected every month over one year in the two hospitals; quantities of antibiotics used were also collected in the rural hospital. High-performance liquid chromatography-tandem mass spectrometry was used to determine antibiotic concentrations; standard disk diffusion and E-test were applied for antibiotic susceptibility testing in 265 *Escherichia coli* (*E. coli*) isolates from the water samples; polymerase chain reactions were used to detect antibiotic resistance genes.

Results: The majority of hospital staff showed good knowledge of infection control and had good or adequate practice scores. Median knowledge scores were 11.8 (6.8-13.9) and 12 (1.4-14.5) ($p=0.17$); median practice scores were 11.4 (4.7-15.0) and 12.4 (1.0-15.0) in the rural and the urban hospitals respectively ($p=0.003$). Cleaning workers had lower knowledge as well as practice scores than doctors and nurses (I). The staff acknowledged poor infection control practices during interviews and groups discussions. They pointed out various difficulties but were not aware of the situation of healthcare-associated infections in their hospitals (II). In hospital wastewater samples, studied antibiotics were present both before and after wastewater treatment: 70.5µg/L before treatment and 34.0µg/L after treatment per month in the rural hospital; 93.5µg/L before treatment and 32.4µg/L after treatment per month in the urban hospital. A significant correlation with the quantities used was found for ciprofloxacin ($r = 0.78$; $p = 0.01$) and metronidazole ($r = 0.99$; $p < 0.001$) (III). Resistance to at least one of the studied antibiotics was detected in 83% of *E. coli* isolates; multidrug resistance was found in 32%. The highest resistance prevalence was found for co-trimoxazole (70%). Forty-three percent of isolates were ESBL-producing, with the *bla*_{TEM} gene being more common than *bla*_{CTX-M}. Co-harboring of the *bla*_{CTX-M}, *bla*_{TEM} and *qepA* genes was found in 46% of isolates resistant to ciprofloxacin (IV).

Conclusions: Hospital staff was generally knowledgeable about infection control, but was not aware of the situation in their own hospital. Although practice scores were good or adequate for the majority of the staff, in fact, the practices seem to remain poor. Antibiotic residues were present in hospital wastewater both before and after wastewater treatment at concentrations that can promote the development of antibiotic resistance. Antibiotic-resistant *E. coli* along with genes coding for cephalosporin resistance *bla*_{CTX-M} and *bla*_{TEM}, and a gene coding for ciprofloxacin resistance *qepA* were highly prevalent among the isolates.

Key words: antibiotic resistance, hospital infection control, hospital staff, hospital wastewater, antibiotic concentrations, antibiotic resistance genes, Vietnam

LIST OF SCIENTIFIC PAPERS

- I. **Lien LTQ**, Chuc NTK, Hoa NQ, Lan PT, Thoa NTM, Riggi E, Tamhankar AJ, Stålsby Lundborg C. Knowledge and self-reported practices of infection control among various occupational groups in a rural and an urban hospital in Vietnam. *(Submitted)*
- II. **Lien LTQ**, Johansson E, Lan PT, Chuc NTK, Thoa NTM, Hoa NQ, Phuc HD, Tamhankar AJ, Stålsby Lundborg C. Staff awareness of hospital infection control – a qualitative study from a rural and an urban hospital in Vietnam. *(Submitted)*
- III. **Lien LTQ**, Hoa NQ, Chuc NTK, Thoa NTM, Phuc HD, Diwan V, Dat NT, Tamhankar AJ, Stålsby Lundborg C. Antibiotics in wastewater of a rural and an urban hospital before and after wastewater treatment, and the relationship with antibiotic use – a one year study from Vietnam. *Int J Environ Res Public Health*. 2016 Jun 14;13(6)
- IV. **Lien LTQ**, Lan PT, Chuc NTK, Hoa NQ, Nhung PH, Thoa NTM, Diwan V, Tamhankar AJ, Stålsby Lundborg C. Antibiotic resistance and antibiotic resistance genes in *Escherichia coli* isolates from hospital wastewater in Vietnam. *Int J Environ Res Public Health*. 2017 Jun 29;14(7)

These papers will be referred to in the text by their Roman numerals (I-IV). Published papers are reproduced with permission from the publishers.

CONTENTS

1 BACKGROUND	1
1.1 Antibiotic resistance	1
1.1.1 The global situation of antibiotic resistance	1
1.1.2 Antibiotic resistance and factors contributing to its spread	2
1.2 Hospital infection control	4
1.3 Dissemination of antibiotic residues and antibiotic resistant bacteria from hospitals	5
1.3.1 Antibiotic residues in hospital wastewater	5
1.3.2 Antibiotic-resistant bacteria in hospital wastewater	6
1.4 Vietnam	7
1.4.1 Country demographics	7
1.4.2 The Vietnamese healthcare system	8
1.4.3 Antibiotic resistance in Vietnam	9
1.4.4 Hospital infection control in Vietnam	10
1.4.5 Dissemination of antibiotic residues and antibiotic resistant bacteria from Vietnamese hospitals	10
1.5 Study rationale	11
2 AIM AND OBJECTIVES	12
3 METHODS	13
3.1 Overview	13
3.2 Study settings	15
3.3 Sampling and data collection	16
3.3.1 Questionnaire (Paper I)	16
3.3.2 Individual interviews and focus group discussions (Paper II)	17
3.3.3 Repeated sampling of water and collection of antibiotic use data (Papers III and IV)	18
3.4 Analysis of water samples	19
3.4.1 Quantitative determination of antibiotics (Paper III)	19
3.4.2 Antibacterial susceptibility testing and detection of antibiotic resistance genes (Paper IV)	19
3.5 Data analysis	20
3.5.1 Questionnaire data (Paper I)	20
3.5.2 Qualitative data (Paper II)	21
3.5.3 Data on antibiotic concentrations in water samples and hospital antibiotic use (Paper III)	23
3.5.4 Data on antibiotic resistance and antibiotic resistance genes among <i>Escherichia coli</i> isolates (Paper IV)	23
3.6 Ethical permission	23
4 MAIN FINDINGS	24
4.1 Knowledge, practices and perceptions of infection control among hospital staff (Papers I and II)	24
4.1.1 Staff knowledge (Papers I and II)	24

4.1.2 Staff reported practices (Papers I and II)	25
4.1.3 Comparison of knowledge and practices across occupation groups (Papers I and II)	27
4.1.4 Staff awareness of the hospital situation (Paper I)	27
4.2 Antibiotic concentrations and antibiotic-resistant bacteria in hospital wastewater (Papers III and IV)	29
4.2.1 Antibiotic concentrations in hospital wastewater and the relationship to hospital antibiotic use (Paper III)	29
4.2.2 Antibiotic resistance and antibiotic-resistance genes in <i>Escherichia coli</i> isolates from hospital wastewater (Paper IV)	31
5 DISCUSSION	34
5.1 Hospital infection control (Papers I and II)	34
5.1.1 The need for continued training and tailor-made education for hospital staff and cleaning workers	34
5.1.2 Possible discrepancies between self-reported and 'actual' practices	35
5.1.3 Lack of resources for infection control: poorer conditions in the rural versus the urban setting	36
5.1.4 Infection control practices: the know-do gap	37
5.1.5 Improving infection control practices: making data on healthcare- associated infections available for hospital staff	38
5.2 Dissemination of antibiotic residues and antibiotic resistant bacteria from hospitals (Papers III and IV)	39
5.2.1 Relationship between antibiotic concentrations in hospital wastewater and hospital antibiotic use	39
5.2.2 Reduction of antibiotic concentrations in hospital wastewater after wastewater treatment	40
5.2.3 Antibiotic residues in hospital wastewater may promote the selection of resistant bacteria	40
5.2.4 High prevalence of antibiotic-resistant <i>Escherichia coli</i> in hospital wastewater before and after wastewater treatment	41
5.2.5 Detection of extended-spectrum beta-lactamases-producing <i>Escherichia</i> <i>coli</i> isolates	43
5.2.6 Detection of antibiotic resistance genes: genetic evidence of antibiotic resistance	43
5.2.7 Public health implications	44
5.3 Factors influencing hospital infection control and hospital dissemination of antibiotic residues and antibiotic resistant bacteria	46
5.4 Methodological considerations	48
6 CONCLUSIONS	52
7 POLICY IMPLICATIONS AND FUTURE RESEARCH	53
8 ACKNOWLEDGEMENTS	54
9 REFERENCES	57
10 APPENDICES	68

LIST OF ABBREVIATIONS

ARG	Antibiotic resistance gene
CLSI	Clinical and Laboratory Standard Institute
E. coli	Escherichia coli
ECOFF	Epidemiological cut-off
ESBL	Extended-spectrum beta-lactamases
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FGD	Focus group discussion
GDP	Gross domestic product
HAI	Healthcare-associated infection
HIV	Human immunodeficiency virus
LMIC	Low- and middle-income country
MDR	Multidrug resistance
MIC	Minimum inhibitory concentration
MoH	Ministry of Health
MRSA	Methicillin-resistant Staphylococcus aureus
MSC	Minimal selective concentration
OR	Odds ratio
PCR	Polymerase chain reactions
PNEC	Predicted no-effect concentration
SMART	Study for Monitoring Antimicrobial Resistance Trends
UV	Ultraviolet
WAT	Wastewater after treatment
WBT	Wastewater before treatment
WHO	World Health Organization

1 BACKGROUND

1.1 Antibiotic resistance

1.1.1 The global situation of antibiotic resistance

A warning about antibiotic resistance was raised by the Scottish medical doctor and microbiologist Alexander Fleming already in 1945 in his Nobel Prize speech for discovering the first antibiotic penicillin. Since then, concerns about antibiotic resistance and its consequences have increased and it has now escalated into a global crisis [1-3]. Both the United Nations and the World Health Organization (WHO) have stressed that a “post-antibiotic era” is a very real picture and that urgent attention is required globally [2, 4, 5].

The recent, and so far the first, global report on antimicrobial resistance by WHO, published in April 2014, indicates alarmingly high rates of resistance in the bacteria which cause common infections in healthcare facilities as well as in the community [2]. The three bacteria of greatest concern are *Escherichia coli* (*E. coli*) resistant to third-generation cephalosporins and fluoroquinolones, *Klebsiella pneumoniae* resistant to third-generation cephalosporins and carbapenems, and methicillin-resistant *Staphylococcus aureus* (MRSA). *E. coli* exists in normal intestinal flora of humans and animals. Nevertheless, it is the most common cause of healthcare and community-acquired urinary tract infections, blood stream infections and the leading cause of foodborne infections worldwide [2]. High prevalence of antibiotic-resistant *E. coli* has been reported [2]. Eighty-six percent (86%) of blood *E. coli* isolates in an Indian tertiary care hospital were resistant to third-generation cephalosporins [6].

The presence of extended-spectrum beta-lactamases (ESBL) in Enterobacteriaceae have exacerbated the global situation of antibiotic resistance. ESBLs hydrolyse and inactivate beta-lactam antibiotics, including third-generation cephalosporins [7]. ESBL-producing bacteria have also shown co-resistance to quinolones, aminoglycosides, and sulphonamides, contributing to the emergence of multidrug resistance (MDR) [8]. For example, in a Kenyan hospital, 90% of ESBL-producing *E. coli* isolates have been demonstrated to be resistant also to fluoroquinolones [9].

Antibiotic resistance causes prolonged illnesses and excessive deaths. More than 200,000 newborns are estimated to die each year due to infections for which effective antibiotics are unavailable [4]. Infection by resistant bacteria will in many cases also lead to increased financial burden on individuals as well as the healthcare system. Last line antibiotics are often more expensive and less available [2]. Chandy et al. (2014) indicated that patients with

antibiotic-resistant infections had to pay 700 USD more than those with infections susceptible to first-line antibiotics in India. This equates to more than a year of wages for an Indian rural male casual worker [10].

Antibiotic resistance is more devastating in low- and middle-income countries (LMICs) where resources are limited and the incidence of infectious diseases is relatively high. At the same time, on these countries it is difficult for infection prevention and control strategies to become effective [11].

1.1.2 Antibiotic resistance and factors contributing to its spread

Antibiotic resistance has been defined as the ability of bacteria to change in ways that resist the effects of drugs – “that is, the germs are not killed, and their growth is not stopped” [12]. The evolution of resistance therefore occurs naturally in bacteria when exposed to antibiotics. However, antibiotic misuse accelerates the process and multiple factors can contribute to its spread. Figure 1 shows potential pathways by which antibiotic resistance may spread. The extent of these pathways may differ depending on different bacteria, different types of resistance, and also various locations and environments [13].

The WHO’s global action plan on antimicrobial resistance emphasises the importance of using a ‘One Health’ approach [14]. The ‘One Health’ concept considers human health, the health of animals and of the environment as one entity – they are interconnected [15]. In relation to antibiotic resistance, the ‘One Health’ approach is crucial because antibiotic resistance can spread between human, animals and the environment as it is shown in the Figure 1.

This thesis will focus on the hospital-related aspect of these pathways, specifically on hospital infection control and hospital dissemination of antibiotic residues and antibiotic-resistant bacteria and resistance genes into the environment.

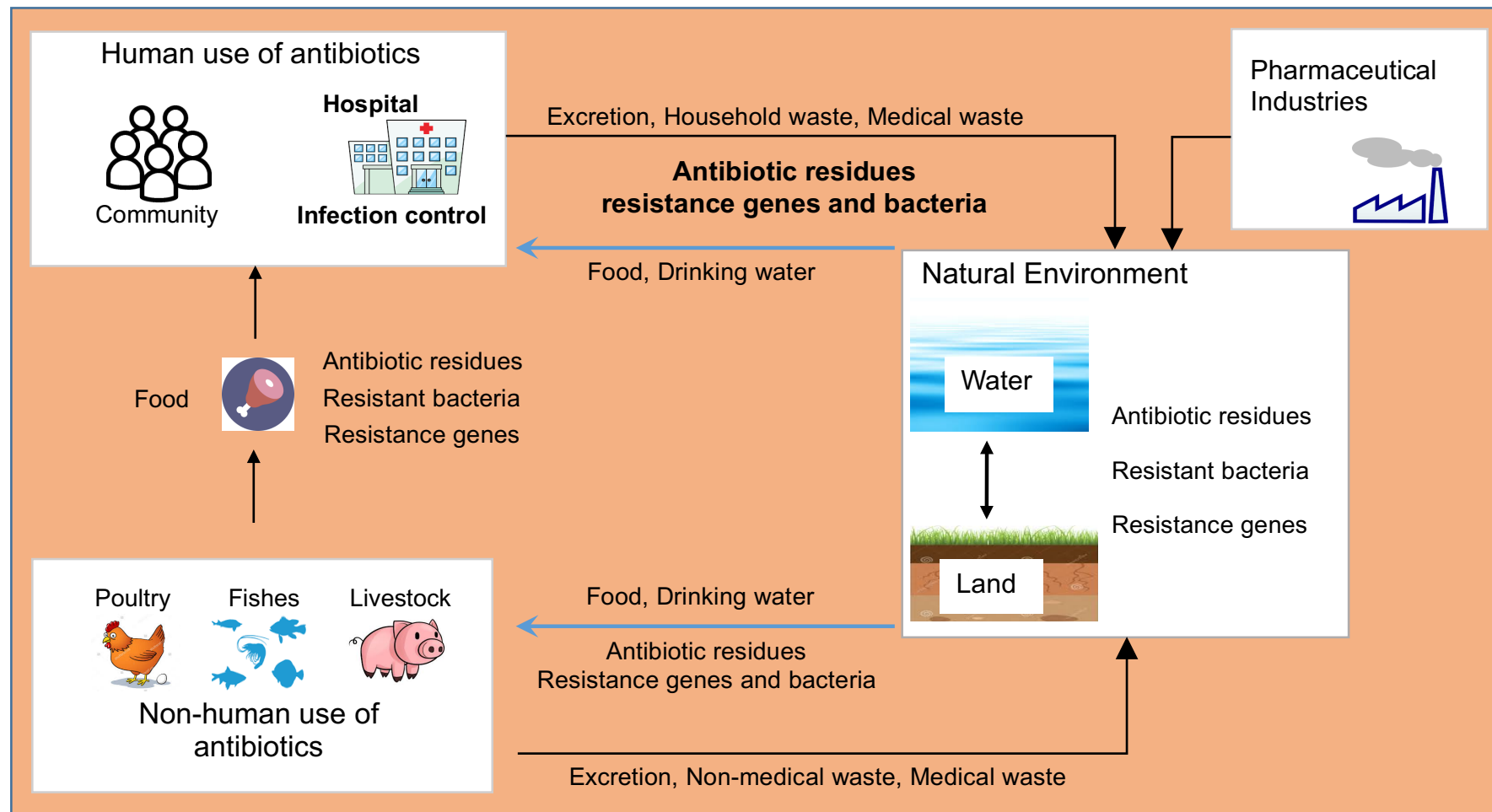


Figure 1. Potential pathways for the spread of antibiotic resistance (Adapted from Stålsby Lundborg and Tamhankar BMJ Vol 358: p. j2440, 2017)

1.2 Hospital infection control

Poor infection control favours the spread of microorganisms in healthcare facilities, causing healthcare-associated infections (HAIs) [16]. HAIs result in additional antibiotic prescriptions, further contributing to the development of antibiotic resistance in bacteria. At the same time, the spread of bacteria including resistant pathogens in hospitals and other healthcare facilities contributes significantly to the increasing global burden of antibiotic resistance. In Europe, the death toll from HAIs caused by multidrug-resistant bacteria is estimated to exceed 25,000 per year and the problem might be more aggravated in other parts of the world [14].

In individual healthcare facilities, behaviour of healthcare workers plays a 'key' role in implementing infection control programmes [17]. A systematic review on the topic concluded that "compliance to infection control precautions is internationally suboptimal" [18]. The core problem is healthcare workers' inadequate application of infection control guidelines [18, 19]. Particularly in LMICs, the issue of insufficient healthcare worker performance needs urgent attention [20]. An important determinant for the high burden of HAIs in those settings is paucity of knowledge and lack of compliance with basic infection control measures such as hand hygiene [21, 22]. Therefore, assessing existing knowledge and practices among healthcare workers is important in developing successful infection control programmes.

A know-do gap in infection control practices has been pointed out in various studies. Paudyal et al. (2008) indicated that although all doctors surveyed in two government and three private Nepalese hospitals understood the importance of handwashing, only about half complied with the recommended practice [23]. In another study conducted with 1,444 nurses in China (2010), the nurses' knowledge about standard precautions was deemed to be average whereas compliance with standard precautions was found to be low [24]. According to Tenna et al. (2013), healthcare workers from two university hospitals in Ethiopia were found to have a good understanding of the importance of hand hygiene and tuberculosis infection control principles. However, this knowledge did not translate into effective implementation of infection control practices [25].

Studies on healthcare workers' knowledge and practices of infection control are largely quantitative. Qualitative approaches are useful for describing contextual factors and latent influences, helping to understand barriers and facilitators for healthcare worker behaviour in more depth. Few qualitative studies have been published showing a comprehensive view on

hospital staff's perceptions of various issues in infection control, not only hand hygiene or medical waste management [26, 27]. In a qualitative study on barriers and motivators affecting tuberculosis infection control practices of healthcare workers in Russia, Woith et al. (2012) indicated that knowledge deficit and negative attitude are the main barriers for appropriate infection control practices [26]. Ider et al. (2012) also reported suboptimal knowledge and attitudes of Mongolian healthcare workers as challenges that hinder the effective implementation of infection control programmes along with other barriers, such as poor funding and inadequate management [27].

Current literature on knowledge, perceptions and practices of infection control has mainly been conducted with nurses, doctors or medical students. Only one Indian study has to my knowledge been conducted also with cleaning workers besides doctors, nurses, administrators and medical students (2012) [28]. The role of cleaning workers in hospital infection control is important as both they and their work can be a vector of disease transmission in hospitals.

1.3 Dissemination of antibiotic residues and antibiotic resistant bacteria from hospitals

1.3.1 Antibiotic residues in hospital wastewater

Antibiotics are important medicines used in hospitals for treating infectious diseases including HAIs. Large amounts of antibiotics can be released into hospital wastewater due to excretion of used antibiotics and incorrect disposal of unused compounds, which can later be discharged into the environment. The occurrence of antibiotics in the aquatic environment can promote the selection of antibiotic-resistant bacteria [29].

The concern about antibiotic pollution of the environment in general, and that of aquatic ecosystems in particular, is increasing globally [30, 31]. Hospital effluents and even hospital wastewater treatment plants represent an important source for releasing antibiotics as well as antibiotic-resistant bacteria into the environment [30, 32]. Diwan et al. (2013) concluded that “antibiotics are released in hospital wastewater continuously, daily and all year round” [33]. They detected various antibiotics in hospital effluents, ranging from 1.4 µg/L to 236.6 µg/L [34].

Among antibiotic groups, various studies have detected fluoroquinolones at high concentrations in hospital effluents [29, 34-38]. According to Rodriguez-Mozaz et al. (2015), ciprofloxacin and ofloxacin were present in the effluent of a Spanish hospital at

concentrations ranging from 13.78 µg/L to 14.38 µg/L, respectively. They have also been found in other hospital effluents, also at high concentrations [39-41]. In Hanoi, Vietnam, Duong et al. (2008) found that the concentrations of ciprofloxacin and norfloxacin in hospital wastewater ranged from 1.1 µg/L to 44 µg/L and from 0.9 µg/L to 17 µg/L, respectively [42].

1.3.2 Antibiotic-resistant bacteria in hospital wastewater

As mentioned earlier, antibiotic residues in the environment can lead to selective pressure on bacteria, favouring the development of antibiotic resistance in bacteria. Significant correlations between the concentration of antibiotic residues and the prevalence of antibiotic-resistant bacteria have been observed in hospital effluent [43]. Akter et al. (2012) indicated that all *E. coli* isolates from the tested hospital wastewater of Bangladesh showed MDR patterns which correlated with the antibiotics used by the practitioners to treat patients [44]. Similar presence of multidrug-resistant *E. coli* in the sewage connected to hospitals in southern Austria has been documented by Reinthaler et al (2003) [45]. Another study indicated that effluents from both the studied hospitals in south Ethiopia contained antibiotic-resistant bacteria (*Salmonella*, *Shigella*, *Staphylococcus aureus* and *E. coli*), and that these were released to receiving water bodies [46].

Along with antibiotic-resistant bacteria, the presence of antibiotic resistance genes (ARGs) in the aquatic environment is a problem due to the possibility of gene transfer from bacteria harbouring resistance genes to susceptible bacteria [29]. Hundreds of ARGs encoding resistance to a broad range of antibiotics have been found in microorganisms located not only in hospital wastewater, but also in wastewater treatment plants, surface water, groundwater, and even drinking water [47]. Findings by Rodriguez-Mozaz et al. (2015) revealed that ARGs such as *bla_{TEM}* (resistance to beta-lactams), *ermB* (resistance to macrolides), *qnrS* (reduced susceptibility to fluoroquinolones), *tetW* (resistance to tetracyclines) and *sulI* (resistance to sulfonamides) were detected at high concentrations in hospital effluent samples in Spain. They also found the incomplete removal of antibiotics and ARGs in wastewater treatment plants, which severely affected a receiving river [38].

Globally, national (and/or regional) regulations have rarely defined how to treat hospital effluent for antibiotic residues and antibiotic-resistant bacteria before its disposal – either discharge in public sewage for treatment at a municipal wastewater treatment plant or discharge into a surface waterbody [48]. The situation is likely worse in LMICs, where treatment is not commonly adopted and direct discharge of raw hospital wastewater into surface rivers is common practice [48]. Such water can be released into waterbodies,

wherefrom it might be used for irrigation or later end up in water used for household purposes, resulting in resistant infections in humans and animals. Hospital dissemination of antibiotic residues and antibiotic-resistant bacteria along with ARGs can exacerbate the situation of antibiotic resistance, posing a danger to public health.

1.4 Vietnam

1.4.1 Country demographics

The Socialist Republic of Vietnam is located on the Indochina Peninsula in Southeast Asia, bordered by China to the north, Laos to the northwest, Cambodia to the southwest and the Pacific Ocean to the east. It covers approximately 331,000 km². The country is largely humid and tropical, although its climate tends to vary across regions. In the mountains and plateaus in the north, temperatures can vary from 5°C to 37°C. Temperatures vary less in the southern plains, normally ranging between 21°C and 28°C.

With a population of more than 92 million inhabitants, Vietnam is the 14th most populous country in the world. Its population density is approximately 310 persons/km². Roughly 35% of the population lives in urban areas with a growing rate of urbanisation. The total fertility rate per woman is 2.09 [49]. There are more than 54 ethnic groups with Kinh being the majority (nearly 86%). The official language is Vietnamese. The median age is 31 years, relatively young compared to 39-41 years in Europe.

Starting in 1986, economic and political reforms have rapidly promoted the country's economic growth and transformed Vietnam from one of the poorest nations in the world to a lower middle-income country. In 2016, the gross domestic product (GDP) per capita in 2016 was 2,185 USD [50]. Along with the economic growth, Vietnam has made significant progress in reducing poverty and providing basic services. Key developments and health indicators of the country are presented in Table 1.

Table 1. Basic indicators for Vietnam [49-52]

Indicators	Year	Values
Population (million)	2016	92.695
GDP per capita (USD)	2016	2,185
Poverty rate (<1.25USD/day) (%)	2015	7.0
Fertility rate per woman	2016	2.09
Sex ratio (males per100 females)	2016	97.3
Adult literacy rate (%)	2009	93.5
Life expectancy at birth (years)	2015	76
Infant mortality rate (per 1,000 live births)	2016	17.3
Under-five mortality rate (per 1,000 live births)	2016	21.6
Maternal mortality rate (per 100,000 live births)	2015	54
Low birth weight rate (<2,500 gr, %)	2011	5.1
Prevalence of underweight, weight for age (% of children ≤ 5)	2010	12
Number of physicians per 10,000 inhabitants	2015	8.0
Number of pharmacists per 10,000 inhabitants	2015	2.2

1.4.2 The Vietnamese healthcare system

Since 1986, when the government began to implement market reforms, Vietnam's healthcare system has been transformed into a mixed public-private system, making healthcare more accessible. Despite rapid expansion of the private sector, the public sector remains the main source of healthcare services as well as health research and training.

The public healthcare system consists of four administrative levels: central, provincial, district and commune (Figure 2). Central hospitals are under direct control of the Ministry of Health (MoH) and are located in large cities (mostly in Hanoi – the capital). These hospitals provide medical services as top referral hospitals. Provincial/district hospitals are under the control of provincial/district health departments and are responsible for the medical care of the people from respective provinces and districts. Commune health centers are managed by the district health centers and are responsible for the primary healthcare of the local people.

Administrative level		Public sector	Private sector
Central	Ministry of health	<ul style="list-style-type: none"> • National/central hospitals • Preventive institutes • Research/professional institutes • Medical schools • Pharmacy schools • Pharmaceutical companies 	Private hospitals Private clinics Private pharmacies Private drugstores Private medical schools
Provincial	Provincial health departments	<ul style="list-style-type: none"> • Provincial hospitals • Preventive health centers • Drug control centres • Secondary medical schools • Pharmaceutical companies 	
District	District health centers	<ul style="list-style-type: none"> • District hospitals/policlinics • District health centres 	
Commune	Commune health stations	Commune health stations	

Figure 2. The Vietnamese healthcare system
 (Adapted from Thuan et al. BMC Health Serv Res 8, 162 (2008))

1.4.3 Antibiotic resistance in Vietnam

Vietnam has high prevalence of antibiotic resistance [53, 54]. According to the Study for Monitoring Antimicrobial Resistance Trends (SMART) 2010-2013, Vietnam has one of the highest rates of Gram-negative ESBLs-producing bacteria and the highest rates of cephalosporin resistance in the Asia-Pacific region [53]. According to Dyar et al. (2012) 60% of *E. coli* isolates from children were resistant to three or more commonly used antibiotics in rural Vietnam already in 2007 [55].

Importantly, increases in antibiotic resistance rates have been reported in various studies. From 1999 to 2007, penicillin-resistance rates in *Streptococcus pneumoniae* increased from 8% to 75% in Ba Vi district [56]. An increase in tetracycline and chloramphenicol resistance (from 81 to 99% and from 2.5% to 13% respectively) was observed in *Streptococcus suis*, the leading cause of bacterial meningitis in adults in Vietnam, over an 11-year period [57]. High carbapenem-resistance rates are found in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [58]. A national action plan on prevention and control of drug resistance including antibiotics has been in place. However, of note is the fact that what has been studied and reported is most likely only the tip of the iceberg [*personal communications with the head of*

a microbiology department in a national hospital].

1.4.4 Hospital infection control in Vietnam

As aforementioned, poor hospital infection control contributes to the spread of antibiotic resistance. Many Vietnamese hospitals are old and overcrowded, making effective infection control challenging. Occupancy can exceed 100%, particularly during communicable disease outbreaks. The MoH estimates the average bed occupancy rate across all levels of public hospitals to be approximately 130% [59]. Patient overload in public hospitals is one of the most considerable challenges across the Vietnamese healthcare system, compounded by the fact that patients tend to seek health care in public hospitals rather than their commune health stations. With high workload, it is difficult to ensure adequate practices including infection control.

The first regulation which raised hospital infection control was the MoH's regulation on hospital organisation, conditions and activities, issued in 1997 [60]. During the last decade, efforts have been made to improve the system for infection control, however, much is still unknown regarding the compliance with regulations. An indicator for the quality of infection control in hospitals is the rate of HAIs. High rates of HAIs in Vietnamese hospitals have been reported [61, 62]. An MoH survey on 9,345 patients at 10 public hospitals indicated an HAI rate of 6%, with hospital-acquired pneumonia accounting for 55% [63]. Another survey on 3,671 patients at 15 intensive care units across the country showed an HAI rate of 27.3%, including infections caused by antibiotic-resistant bacteria. The rates of antibiotic use in Vietnamese hospitals were high, ranging up to 99.5% [64]. Although Vietnam is part of the WHO clean hands campaign, low compliance to hand hygiene (13.4%) has been reported [65]. To the best of my knowledge, there has been no study published from Vietnam regarding knowledge, perceptions and practices of infection control among hospital staff.

1.4.5 Dissemination of antibiotic residues and antibiotic resistant bacteria from Vietnamese hospitals

Nearly 50% of Vietnamese hospitals lack wastewater treatment plants [66, 67]. Regulations on healthcare waste management exist, specifying that every hospital must have a wastewater treatment plant. Nevertheless, no standards have yet been established on how hospital wastewater should be treated in terms of antibiotic residues and antibiotic-resistant bacteria.

Evidence on the hospital dissemination of antibiotic residues and antibiotic-resistant bacteria into the environment in Vietnam is very scarce. To the best of my knowledge, there has been

only one study published, which attempted to investigate residual concentrations of fluoroquinolones and the fate of resistant *E. coli* in hospital wastewater. This study used grab sampling, indicating the presence of ciprofloxacin and norfloxacin residues, and susceptible *E. coli* isolates in the treated wastewater samples [42]. It has however been suggested that continuous sampling should be used to determine antibiotic concentrations in hospital wastewater as grab sampling only provides the quantities of antibiotics present in wastewater at the time of sampling [33].

1.5 Study rationale

The increasing spread of antibiotic-resistant bacteria is a major public health problem. In combating with antibiotic resistance, an important development is the endorsement of a global action plan at the 69th World Health Assembly in 2015. One of the ‘core pillars’ of this plan is global surveillance [68]. In Vietnam, like in many other LMICs, where standard surveillance systems have not yet been established, studies are needed to understand the extent of the problem, providing evidence and baseline data in order to design contextualised interventions.

Various factors contribute to the spread of antibiotic resistance including inadequate hospital infection control and the discharge of antibiotic residues and antibiotic-resistant bacteria from hospitals into the environment. Studies on these topics in Vietnam are lacking. This study was therefore undertaken to understand the problem, contributing to the global action in combating with antibiotic resistance.

2 AIM AND OBJECTIVES

The overall aim was to explore hospital practices in relation to the spread of antibiotic resistance in a rural and an urban hospital in Hanoi, Vietnam.

The specific objectives were to:

- (i) assess hospital staff's knowledge and reported practices of infection control [**Paper I**],
- (ii) explore hospital staff's perceptions on infection control practices including healthcare-associated infections, hand hygiene and healthcare waste management [**Paper II**],
- (iii) determine the level of antibiotic residues in hospital wastewater in relation to hospital antibiotic use [**Paper III**], and
- (iv) determine selected antibiotic resistance patterns and antibiotic resistance genes of *Escherichia coli* isolated from hospital wastewater [**Paper IV**].

3 METHODS

3.1 Overview

To fulfil the aim and objectives, this thesis is based on four papers (Figure 3). The studies were conducted in two general hospitals in Hanoi: one rural and one urban hospital. A mixed method approach was used including both quantitative and qualitative methods (Table 2).

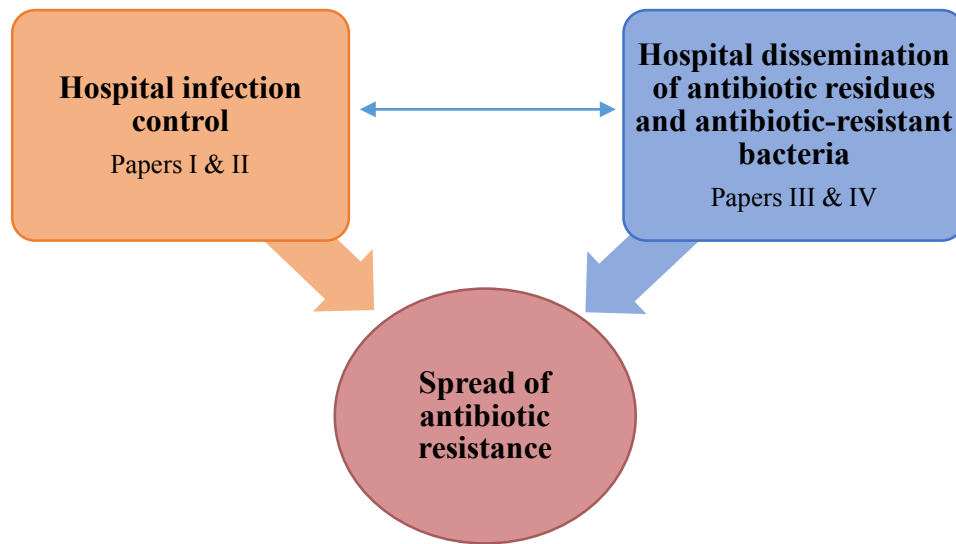


Figure 3. Study framework

Paper I – a quantitative cross-sectional study using a questionnaire to evaluate and compare knowledge and reported practices of hospital infection control among various occupation groups.

Paper II – a qualitative study using individual interviews and focus group discussions (FGDs) to understand the perceptions of infection control among hospital staff.

Paper III – a quantitative repeated cross-sectional study over a one-year period, collecting wastewater samples in the two hospitals and antibiotic quantities delivered to all inpatient wards every month in the rural hospital to investigate levels of antibiotic residues and its relationship with the hospital antibiotic use.

Paper IV – a quantitative repeated cross-sectional study using the same water samples as in Paper III to examine the prevalence of phenotypic resistance to selected antibiotics and antibiotic resistance genes among *E. coli* isolates from the water samples.

Table 2. Overview of papers' topics and methods

Paper	Topic	Study design	Sampling and data collection	Data analysis
I	Staff knowledge and self-reported practices of hospital infection control	Quantitative cross-sectional	Questionnaire among 339 hospital staff of varying occupations	- Knowledge score - Practice score - Mood's median test - Ordinal logistic regression
II	Staff perceptions on hospital infection control	Qualitative	6 individual interviews and 6 focus group discussions with in total 50 hospital staff of varying occupations	Manifest and latent content analysis
III	Antibiotic residues in hospital wastewater and the relationship with hospital antibiotic use	Quantitative one-year repeated cross-sectional	- Continuous sampling of water samples every month - Solid-phase extraction and high-performance liquid chromatography-tandem mass spectrometry for determination of antibiotic concentrations - Quantities of antibiotics delivered to all inpatient wards every month	- Descriptive statistics - Wilcoxon signed rank test - Spearman's correlation
IV	Antibiotic resistance and antibiotic resistance genes in <i>Escherichia coli</i> isolates from hospital wastewater	Quantitative one-year repeated cross-sectional	- Same water samples as for study III - Standard disc diffusion and E-test for antibiotic susceptibility testing - Combined disc diffusion for detection of production of ESBL - Polymerase chain reactions for detection of antibiotic resistant genes	- Prevalence of resistance to each studied antibiotic, to at least one of the studied antibiotics, and multidrug resistance - Prevalence of selected ARGs - Fisher's exact test

NOTE. ESBL: extended-spectrum beta-lactamases; ARG: antibiotic resistance gene

3.2 Study settings

The study was conducted in one rural and one urban hospital in Hanoi (Figure 4). Hanoi, located in northern region of Vietnam, is the capital and the country's second largest city by population. It has 12 urban districts, one town and 17 rural districts. Approximately 49% of the roughly 7.3 million registered inhabitants live in urban areas and 51% in rural areas [49].

Hanoi is also one of the country's largest centres in terms of the healthcare system. In 2013, when the studies were conducted, there were 16 central hospitals, 27 urban provincial hospitals, 14 rural district hospitals, 30 district health preventive centres and 577 commune health stations [69].

The rural hospital is located in a rural district, 60 km north-west of the Hanoi centre. At the time of the study, it had 220 beds with 46 doctors, 110 nurses, 12 midwives and 12 cleaning workers. The catchment area was the rural district where the hospital is located.

The urban hospital is provincial by administration, situated in the center of Hanoi. It was 520-bedded and employed 181 doctors, 392 nurses, 32 midwives and 35 cleaning workers at the time of the study. Patients were mainly from within the city.

Both hospitals were general hospitals having typical inpatient wards such as infectious diseases, internal medicine, obstetrics, pediatrics, surgery and intensive care unit, and pharmacy departments. The urban hospital had a microbiology department equipped with a microbiological laboratory. In the rural hospital, there was a laboratory department, however, it lacked a microbiological laboratory.

In both settings, hospital wastewater was collected into a wastewater treatment plant which functioned using filtration followed by chemical and biological treatment. Treated wastewater went into a local draining system which was then discharged to nearby rivers.



Figure 4. Study settings

3.3 Sampling and data collection

3.3.1 Questionnaire (Paper I)

A questionnaire was developed based on the WHO, the Vietnamese MoH guidelines on infection prevention and control, and the authors' previous experience [70, 71]. It consisted of two sections, one on knowledge and another on infection control practices. It was piloted with 20 respondents followed by a few modifications prior to the data collection for the study.

The questionnaire included, in addition to the participants' demographic characteristics (age, sex, workplace, qualification), 15 items each for knowledge and practice. A question on reasons for non-compliance with hand hygiene, an important measure for infection prevention and control, was also included. The questions had closed response alternatives where respondents could select one or more alternatives as instructed (Appendix 1).

Questionnaires were distributed to hospital staff ensuring to recruit staff with different occupations (physicians, nurses, midwives, cleaners) and from various departments. The questionnaires were then collected and assessed for completeness. Finally, 144 questionnaires

from the rural hospital and 195 from the urban hospital were included in the analysis. Participants' demographic characteristics are presented in Table 3.

Table 3. Study participant's demographic characteristics

Characteristics	Total (N=339) n (%)	Rural hospital (n=144) n (%)	Urban hospital (n=195) n (%)
Age (years) (35.8±11.1)	(n=292)	(n=129)	(n=163)
20-30	144 (49.3)	78 (60.5)	66 (40.5)
31-40	52 (17.8)	18 (13.9)	34 (20.9)
41-50	46 (15.8)	13 (10.1)	33 (20.2)
51-60	50 (17.1)	20 (15.5)	30 (18.4)
Sex	(n=326)	(n=137)	(n=189)
Male	44 (13.5)	25 (18.2)	19 (10.1)
Female	282 (86.5)	112 (81.8)	170 (89.9)
Occupation	(n=335)	(n=142)	(n=193)
Physicians	92 (27.4)	44 (31.0)	48 (24.9)
Nurses/midwives	224 (66.9)	94 (66.2)	130 (67.4)
Cleaning workers	19 (5.7)	4 (2.8)	15 (7.8)

3.3.2 Individual interviews and focus group discussions (Paper II)

To better understand the findings from the questionnaire, individual interviews and focus group discussions (FGDs) were also organised with hospital staff. A semi-structured guide was developed based on literature and the authors' previous experience [28, 72]. It consisted of discussion points on HAIs, hand hygiene and waste management in order to gain understanding in greater detail, variety and clarity (Appendix 2).

Purposive sampling was used to select the participants with varying backgrounds in terms of age, sex, experience and qualifications, aiming to capture a range of views. Six interviews (three in each hospital) were conducted with hospital managers as they have an overview of hospital activities. Six FGDs (three in each hospital) were conducted with hospital staff by occupation: one with doctors, one with nurses and one with cleaning workers (Table 4). Participation was voluntary. Staff was given information about the aim of the study and written consent was obtained.

Interviews and FGDs were carried out in Vietnamese by two researchers, in locations chosen by the participants. A research assistant recorded the sessions and took notes during the

discussions. Neither the researchers nor the research assistants were employed by any of the two hospitals and none had any direct relationship to the participants. The interviews/discussions were carried out until no new information was emerged. The interviews lasted between 45 and 60 minutes whilst the FGDs lasted between 60 and 120 minutes.

Table 4. Characteristics of participants in individual interviews and focus group discussions

Interview code	Hospital	Category of staff	Number of participants	Years of experience
II 1	Urban	Hospital manager	1	28
II 2	Urban	Hospital manager	1	27
II 3	Urban	Hospital manager	1	31
II 4	Rural	Hospital manager	1	26
II 5	Rural	Hospital manager	1	14
II 6	Rural	Hospital manager	1	32
FGD 1	Urban	Doctors	6	1-25
FGD 2	Urban	Nurses	7	1-27
FGD 3	Urban	Cleaning workers	9	1-12
FGD 4	Rural	Doctors	8	1-20
FGD 5	Rural	Nurses	8	1-32
FGD 6	Rural	Cleaning workers	6	1-2
Total			50	

NOTE. II: individual interview; FGD: focus group discussion

3.3.3 Repeated sampling of water and collection of antibiotic use data (Papers III and IV)

In 2013, wastewater samples from each hospital were taken both before and after wastewater treatment. Water was sampled on a monthly basis, during a weekday of the last week of that month.

Each sampling event in both the hospitals followed the same procedure: wastewater was collected continuously for 24 hours before and after treatment, using a pump with a flow speed of 40 drops/min. The pumps were connected to closed containers surrounded by ice and protected from light [33]. For each type of water, wastewater before treatment (WBT) and wastewater after treatment (WAT), three litres of water were collected over 24 hours. Water samples of 500 mL each were stored in amber glass bottles, wrapped in silver foil and clearly labelled. The water bottles were then placed in an ice box with a lid, protected from light and transferred within 12 hours for analysis.

Water samples were tested for concentrations of selected antibiotics at a chemical laboratory; Hanoi Drug and Cosmetic Testing Centre (**Paper III**). To examine the prevalence of antibiotic resistance as well as ABR genes among *E. coli* isolates (**Paper IV**), the water samples were tested at the microbiological laboratory of Bach Mai Hospital, located in central Hanoi. In the testing laboratories, samples were stored at -20°C.

Water sampling could not be done in the urban hospital from June to August 2013 as the hospital and its wastewater treatment plant were under reconstruction during this period. In total, 42 water samples were collected; 18 from the urban hospital and 24 from the rural hospital.

To investigate the possible association between antibiotic concentrations detected in the hospital wastewater and hospital antibiotic use (**Paper III**), data on quantities of antibiotics delivered to all inpatient wards were collected from the Department of Pharmacy in the rural hospital. Unfortunately, this was not feasible in the urban hospital since it was undergoing refurbishment.

3.4 Analysis of water samples

3.4.1 Quantitative determination of antibiotics (Paper III)

To test the level of antibiotic residues in the water samples, seven antibiotics were selected based on the antibiotic prescription patterns in the inpatient wards of the hospitals, the degree of metabolism of antibiotics in the human body and the environmental impact of an antibiotic [30, 73]. The studied antibiotics were: (i) metronidazole, (ii) sulfamethoxazole, (iii) trimethoprim, (iv) ceftazidime, (v) ciprofloxacin, (vi) ofloxacin, and (vii) spiramycin.

The method described by Diwan et al. was used [73]. In brief, water samples were filtered through a 0.45-μm filter membrane and acidified with formic acid to pH 3.0, and subjected to solid phase extraction for isolating analytes. The analytes were then subjected to a high-performance liquid chromatography-tandem mass spectrometry (Shimadzu LCMS-8030) for the quantitative determination of the selected antibiotics.

3.4.2 Antibacterial susceptibility testing and detection of antibiotic resistance genes (Paper IV)

From the water samples, coliforms were detected and isolated on selective and differential media [74]. Coliform isolates were then sub-cultured on *Brilliance*TM UTI agar to collect

presumptive *E. coli* isolates. Following biochemical confirmation by standard tests, confirmed *E. coli* isolates were tested for antibiotic susceptibility. In total, 265 *E. coli* isolates from the water samples were tested; 158 isolates from the rural hospital (WBT = 84; WAT = 74) and 107 isolates from the urban hospital (WBT = 60; WAT = 47).

The antibiotic panel selected for testing was a part of the antibiotic list routinely tested in clinical laboratories: (i) amoxicillin/clavulanic acid, (ii) ceftazidime, (iii) ceftriaxone, (iv) ciprofloxacin, (v) co-trimoxazol (trimethoprim/sulfamethoxazole), (vi) fosfomycin, (vii) gentamicin, and (viii) imipenem.

The standard Kirby Bauer disc diffusion method was used for antibiotic susceptibility testing. Results were interpreted as resistant, intermediate or susceptible using the Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI M100 – 2013) [75]. Zone diameters from the disc diffusion method were also compared with epidemiological cut-off (ECOFF) values [76]. Minimum inhibitory concentrations (MICs) were checked for ciprofloxacin and ceftazidime using E-test method.

For isolates resistant to third-generation cephalosporins, ESBL production was tested using the combined disc diffusion method. Genotypic confirmation was done by polymerase chain reactions (PCR). Genes coding for betalactam resistance, *bla*_{CTX-M} and *bla*_{TEM}, were tested in ESBL-producing strains and *qepA* gene was tested for ciprofloxacin resistant strains [77, 78].

3.5 Data analysis

3.5.1 Questionnaire data (Paper I)

For each question, a score from 0 to 1 was given. For questions where only one alternative could be chosen, 1 point was given for a correct response and 0 for an incorrect answer. For questions where more than one alternative was possible, 1 point was given if all alternatives were correct and 1/n points (n=number of alternatives) for each alternative with a correct response. Knowledge or practice scores were calculated and then summed up to gain the total score for each individual.

The total knowledge or practice scores for each respondent ranged from 0 to 15. Scores were divided by quartile. The first cut-off corresponded to the 2nd quartile (7.5) and the second cut-off to the 3rd quartile (11.25). Thus, a total score of <7.5 was considered poor knowledge/practice, 7.5 to <11.25 was considered adequate knowledge/practice and ≥11.25 was considered good knowledge/practice.

Mood's median test was applied to compare median knowledge/practice scores. A post-hoc analysis of ordinal logistic regression models was performed to test the difference in the scores among occupation groups within/between the two hospitals, adjusting for age and sex. Bonferroni correction was used to adjust p -values for multiple comparisons. The statistical analyses were performed in R 3.3.1 using packages "RVAideMemoire", "ordinal" and "lsmeans" [18].

3.5.2 Qualitative data (Paper II)

Audio recordings were transcribed verbatim to produce transcripts of narrative text in Vietnamese. The transcriptions were checked by one of the researchers. Thematic content analysis was applied for the analysis [79].

The text was read thoroughly to obtain a sense of the whole and divided into 'meaning units'. 'Meaning units' are parts of the text that carry specific meanings related to the study objective. Each meaning unit was condensed and labelled with a code. Coding and quotation selection were carried out in Vietnamese, then coding results and selected quotations were translated to English. This process was checked by one of the authors, who was fluent in both Vietnamese and English. Relevant codes were grouped into a category. Categories were discussed among the authors until sub-themes and a main theme were identified. Examples of the coding process are presented in Table 5.

Table 5. Example of the coding process

Condensed meaning units	Codes	Category
<ul style="list-style-type: none"> - HAIs are the infections occurring in patients in the hospital after admission - During stay in the hospital, patients can get infections 	Occurring in the hospital	HAIs
<ul style="list-style-type: none"> - There are infections incubating for 5 days, 7 days and even longer, so the infections may appear after discharge 	Appearing after discharge	
<ul style="list-style-type: none"> - Surgical site infections; hospital-acquired endometrial infections; stitches infection after giving birth - Nosocomial digestive disorders; catheter placement infection; nosocomial tuberculosis 	Examples of HAIs	
<ul style="list-style-type: none"> - Noncompliance with hand hygiene - Doctors don't wash their hands after each patient - Not absolute compliance with aseptic discipline 	Through hospital staff	
<ul style="list-style-type: none"> - Through needle puncture or bandage - Sharing bowls or cups 	Through medical equipment and domestic utensils	
<ul style="list-style-type: none"> - HIV and hepatitis B can be transmitted through needle puncture - Hospital waste can be a source of diseases - Hospital waste if not properly managed can lead to HAIs 	Through hospital waste	
<ul style="list-style-type: none"> - Infected air; tuberculosis cases from one patient to another - Respiratory infections can be transmitted within the hospital 	Through the air	

NOTE. HAI: healthcare-associated infection; HIV: human immunodeficiency virus

3.5.3 Data on antibiotic concentrations in water samples and hospital antibiotic use (Paper III)

Antibiotic concentrations are presented in $\mu\text{g/L}$. The quantities of antibiotics used are presented in grams per month (g/month). Wilcoxon signed-rank test was performed to compare antibiotic concentrations in wastewater before and after treatment for each hospital. To examine the correlation between quantities of antibiotics used and antibiotic concentrations in the wastewater, Spearman's correlation was applied. All statistical analyses were performed using Stata 12 (StataCorp LP, College Station, TX, USA).

3.5.4 Data on antibiotic resistance and antibiotic resistance genes among *Escherichia coli* isolates (Paper IV)

Percentage of resistant isolates to each studied antibiotic, to at least one of the studied antibiotics, and MDR among *E. coli* isolates were calculated. Bacterial isolates were considered multidrug-resistant when they were non-susceptible to at least one agent in three or more antibiotic categories [21]. Fisher's exact test was performed to test the difference between the resistance prevalence before and after wastewater treatment in each hospital using Stata 12 (StataCorp LP, College Station, TX, USA).

3.6 Ethical permission

The research project has been approved by Hanoi Medical University Review Board in Bio-Medical Research (N0. 116/HMU IRB, 2012 December 21st). Permission to conduct the studies was acquired from both the hospitals involved in the study. Participants in the qualitative study and respondents to the questionnaire were informed about the studies and that confidentiality was maintained throughout by the researchers. Written consent was obtained from participants of individual interviews and FGDs. Participants were informed that they could withdraw from the study at any time without any implications. By answering the questionnaire, it was assumed that the respondents consented to participate.

4 MAIN FINDINGS

The main findings derived from papers I-IV are summarized and presented below. The section is divided into two main sub-sections: knowledge, practices and perceptions of infection control among hospital staff (papers I and II) and antibiotic concentrations and antibiotic-resistant bacteria and ARGs in hospital wastewater (papers III and IV).

Quotes from FGDs and individual interviews are presented in italics to illustrate the findings. Within the quotes, explanations are given in square brackets.

4.1 Knowledge, practices and perceptions of infection control among hospital staff (Papers I and II)

4.1.1 Staff knowledge (Papers I and II)

In both the hospitals, the majority of respondents showed good knowledge of infection control (rural hospital: 65%, urban hospital: 73%). The median knowledge scores were 11.8 (6.8-13.9) and 12 (1.4-14.5) in the rural and urban hospitals respectively ($p=0.17$).

Interviewees and FGD participants were generally knowledgeable about the definition of HAIs and its types. They pointed out different examples of HAIs: surgical site infections, hospital-acquired endometrial infections, post-partum suture infections, hospital-acquired digestive disorders, catheter placement infections, hospital-acquired respiratory tract infections, hospital-acquired tuberculosis, and hospital-acquired HIV through needle stick injuries.

“An HAI, firstly, is an infection that the patients have acquired in the hospital or in another healthcare facility, secondly symptoms of infection, that were absent at the time of admission, have appeared during 48 hours after admission.” (nurses – rural hospital)

The staff were aware of the importance of hand hygiene in preventing HAIs. They understood that all health measures in hospitals were hand-related, therefore if hand hygiene procedures were complied with, the incidence of HAIs could be reduced significantly.

“Hand hygiene is very important because it contributes to preventing HAIs. If everybody followed hand hygiene procedures, the rate of HAIs could be reduced a lot.” (nurses – rural hospital)

At the same time, the staff acknowledged the role of healthcare waste management, particularly waste classification, in infection control. They were very well aware on how to classify the waste in respective types of bags and containers.

“There are four types of waste: healthcare waste – in yellow bags, domestic waste – green bags, hard things in black bags and white bags for recycled waste...Sharp things are put into yellow boxes, and then the boxes are put into black bags.” (cleaning workers – rural hospital)

4.1.2 Staff reported practices (Papers I and II)

Similar to the knowledge score, most staff in both hospitals had good or adequate reported practice, hereafter referred to as practice scores. The median practice scores were 11.4 (4.7-15.0) and 12.4 (1.0-15.0) in the rural and urban hospital respectively. Reported practices in the urban hospital were likely to be better than the rural one ($p=0.003$).

Nevertheless, poor practices of infection control were emphasised during interviews and group discussions in both the hospitals.

“We do training every year but the [infection control] practices are still poor.” (hospital manager – urban hospital).

“Standard procedures exist but are not followed. Trainings are organised but the [infection control] practices remain poor.” (hospital manager – rural hospital)

The staff acknowledged various difficulties in infection control practice in their hospitals: poor budget for infection control, lack of facilities and insufficient equipment and supplies, lack of staff specialised in infection control, patient overload, and poor awareness of hospital staff as well as patients and their relatives.

“We have to use one oxygen generator with the same oxygen tube over two to three days, even 10 days for several patients, causing high risks for getting HAIs.” (doctors – rural hospital)

Various reasons for non-compliance with hand hygiene were reported (Figure 5). In both hospitals, the two leading reasons were emergencies (rural hospital: 75.7%, urban hospital: 75.9%) and high workload (rural hospital: 58.3%, urban hospital: 57.4%).

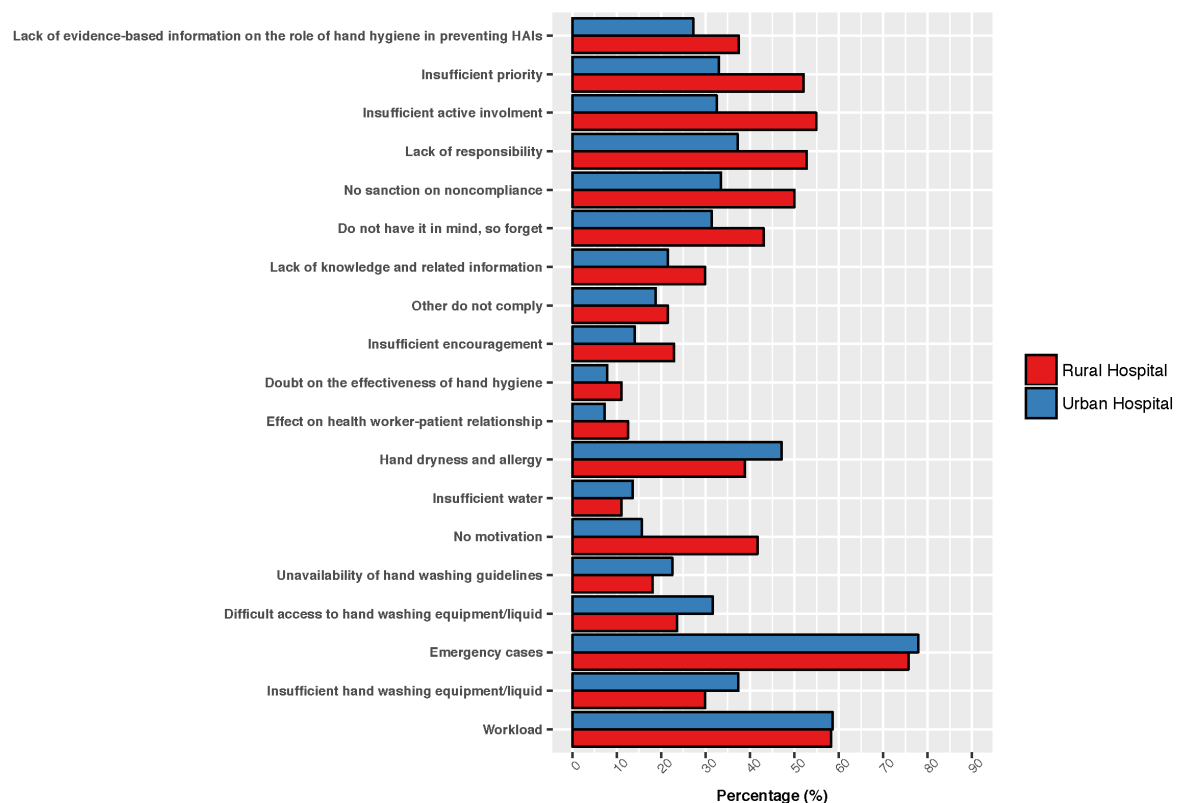


Figure 5. Reported reasons for non-compliance with hand hygiene

Similarly, high workload was strongly emphasised by participants in interviews and group discussions. The doctors viewed large numbers of patients every day as a dominant obstacle for them in following the hand washing procedures.

“Examining 100-150 patients per day, we do not even find time to raise our head to see the faces of patients, not to mention carrying out hand-washing procedures.” (hospital manager – urban hospital)

“If you count 30 seconds per patient to wash the hands, for 100 patients – 50 minutes excluding time for moving and wiping hands. It is impossible to follow.” (doctors – rural hospital)

The rural hospital had poorer conditions for infection control compared to the urban one. The rural hospital merely had a group in charge of infection control, which mainly took care of laundry, drying and steaming clothes, linen and waste management. The hospital did not have a microbiological laboratory.

“We cannot culture and test susceptibility of bacteria due to the absence of a microbiological laboratory, so we cannot make conclusions about suspected cases if they are HAIs or not.” (nurses – rural hospital)

4.1.3 Comparison of knowledge and practices across occupation groups (Papers I and II)

Among occupational groups, cleaning workers had lower knowledge as well as practice scores than doctors and nurses in both hospitals. The differences were statistically significant in the case of the urban hospital (Table 6).

Table 6. Comparison of knowledge and practice scores across occupation groups

Comparison	Knowledge score		Practice score	
	OR	p-value	OR	p-value
Rural hospital				
Nurses vs. Physicians	0.76	0.99	1.33	0.99
Cleaning workers vs. Physicians	0.14	0.09	0.48	0.99
Cleaning workers vs. Nurses	0.18	0.17	0.37	0.71
Urban hospital				
Nurses vs. Physicians	1.14	0.99	1.30	0.99
Cleaning workers vs. Physicians	0.13	0.001*	0.19	0.005*
Cleaning workers vs. Nurses	0.12	<0.001*	0.15	<0.001*

During the FGD, cleaners in the rural hospital were not aware about HAIs and their role in preventing HAIs in the hospital. They supposed that the possibility of people getting infections when staying in hospitals was the hospital's responsibility, not their responsibility. They merely cared about their own risks of getting infections as a result of their work.

“We are mainly afraid that we get infections, so we wear gloves and protection masks, pay attention to needles...Our work is just cleaning and throwing away the waste. Regarding HAIs, that is the hospital's responsibility.” (cleaning workers – rural hospital)

4.1.4 Staff awareness of the hospital situation (Paper II)

Poor awareness of the hospital situation among the staff was a challenge for effective infection control. In the rural hospital, whilst some participants in FGDs believed that there were no, or very few, cases of HAIs in their hospital, others argued that there could be some or even many cases. The hospital did not have adequate laboratory capacity for microbiological analysis, and the hospital staff did not know about any specific data on HAIs.

“In fact, in our hospital there are almost no HAIs. The evidence is that the rate of surgical site infections is very low.” (doctors – rural hospital)

“I don’t think that we have few cases of HAIs. We couldn’t detect them [HAIs] and we do not have statistical data.” (doctors – rural hospital)

In the urban hospital, the staff believed that the HAI situation in their hospital was serious. They were of the opinion that the risk of getting HAIs was extremely high if the patients were admitted for a long time. However, staff were not informed about any specific HAI related data.

“Almost every patient staying for a long time, more than five days, gets a HAI, for example patients with cerebrovascular accidents, respiratory infections, catheter placement and patients who receive mechanical ventilation for a long time.” (nurses – urban hospital)

“We cannot get data from anywhere...In the hospital there might be many HAIs in reality.” (doctors – urban hospital)

In both the hospitals, it emerged that certain efforts to improve infection control were in place, for example, annual survey of HAIs, training on infection control, and availability of standard protocols. In spite of that, the staff were not aware of the impact of their own practices. They thought of infection control as someone else’s issue not their own, yet their practices were satisfactory.

“Sometimes liquid for hand washing [alcohol based hand-rub or soap] is available, but they do not use it.” (doctors – urban hospital)

“Sometimes the oxygen generator is free, but they do not sterilise the tube.” (doctors – rural hospital)

It emerged that there is a need to make all hospital staff, starting from the heads of wards, understand the HAI situation in their hospital and view infection control as their responsibility. The main theme identified was: ‘Making data on HAIs available for health workers can improve their awareness and motivate them to put their existing knowledge into practice and to make use of the facilities they already have for infection control’.

“Providing specific figures on HAIs to the hospital staff could ‘wake them up’ to change their behaviour.” (a head – rural hospital)

4.2 Antibiotic concentrations and antibiotic-resistant bacteria in hospital wastewater (Papers III and IV)

4.2.1 Antibiotic concentrations in wastewater and the relationship to hospital antibiotic use (Paper III)

In both the hospitals, total concentrations of the studied antibiotics per month after wastewater treatment were lower than before treatment. The reductions in total concentrations of the studied antibiotics per month after wastewater treatment were 49% and 67% for the rural and urban hospitals respectively.

In the rural hospital, differences in concentrations of studied antibiotics per month after wastewater treatment ranged from 35% to 81%. Ciprofloxacin was detected at the highest concentration (mean concentration: 42.8 µg/L in WBT and 21.5 µg/L in WAT). Ceftazidime was rarely detected over the one year period. Decreases in antibiotic concentrations in wastewater after treatment were statistically significant except for ceftazidime and sulfamethoxazole (Figure 6).

In the urban hospital, differences in concentrations of studied antibiotics per month after wastewater treatment ranged from 51% to 80%. Metronidazole was detected at the highest concentration (mean concentration: 36.5 µg/L in WBT and 14.8 µg/L in WAT). Ceftazidime was not detected in any of the wastewater samples over the one year period. When comparing concentrations before and after wastewater treatment the differences were statistically significant for all six detected antibiotics (Figure 7).

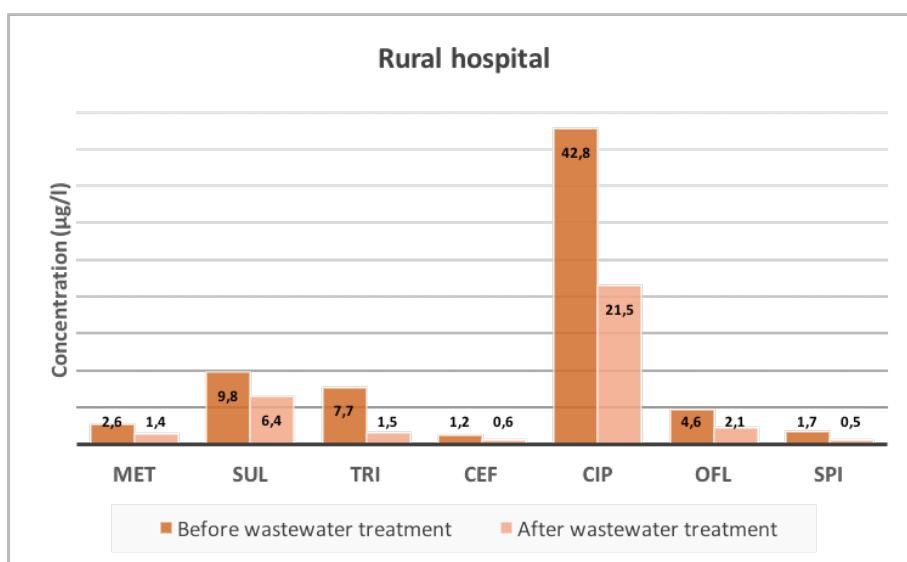


Figure 6. Concentrations of studied antibiotics per month in wastewater of the rural hospital (24-hour sampling) [MET: Metronidazole ($p=0.01$); SUL: Sulfamethoxazole ($p=0.06$); TRI: Trimethoprim ($p=0.002$); CEF: Ceftazidime ($p=0.16$); CIP: Ciprofloxacin ($p=0.002$); OFL: Ofloxacin ($p=0.003$); SPI: Spiramycin ($p=0.004$)]

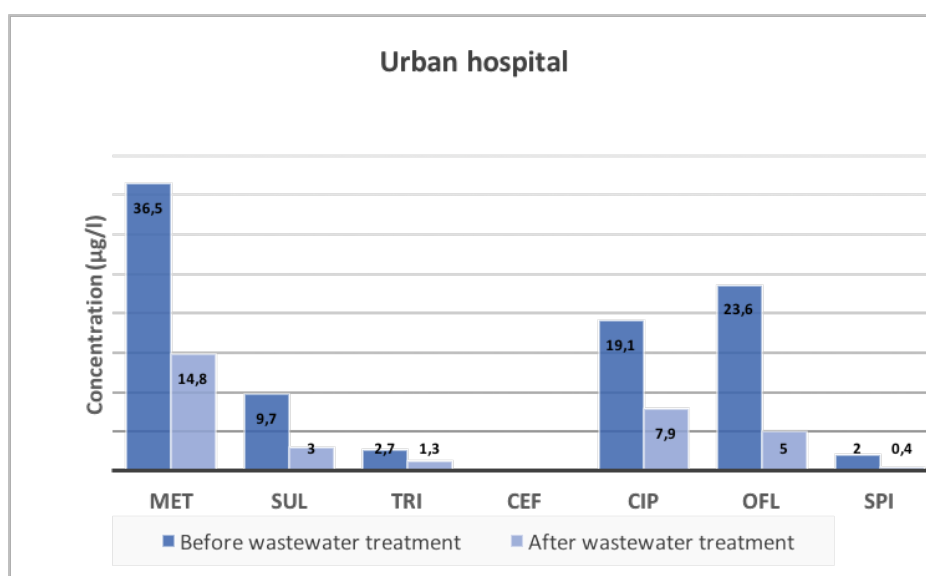


Figure 7. Concentrations of studied antibiotics per month in wastewater of the urban hospital (24-hour sampling) [MET: Metronidazole ($p=0.03$); SUL: Sulfamethoxazole ($p=0.02$); TRI: Trimethoprim ($p=0.01$); CEF: Ceftazidime; CIP: Ciprofloxacin ($p=0.008$); OFL: Ofloxacin ($p=0.009$); SPI: Spiramycin ($p=0.04$)]

Significant correlations between the quantities used in the rural hospital and the concentrations detected in wastewater before treatment were found in case of ciprofloxacin ($r=0.99$; $p<0.001$) and metronidazole ($r=0.78$; $p=0.01$) (Figure 8).

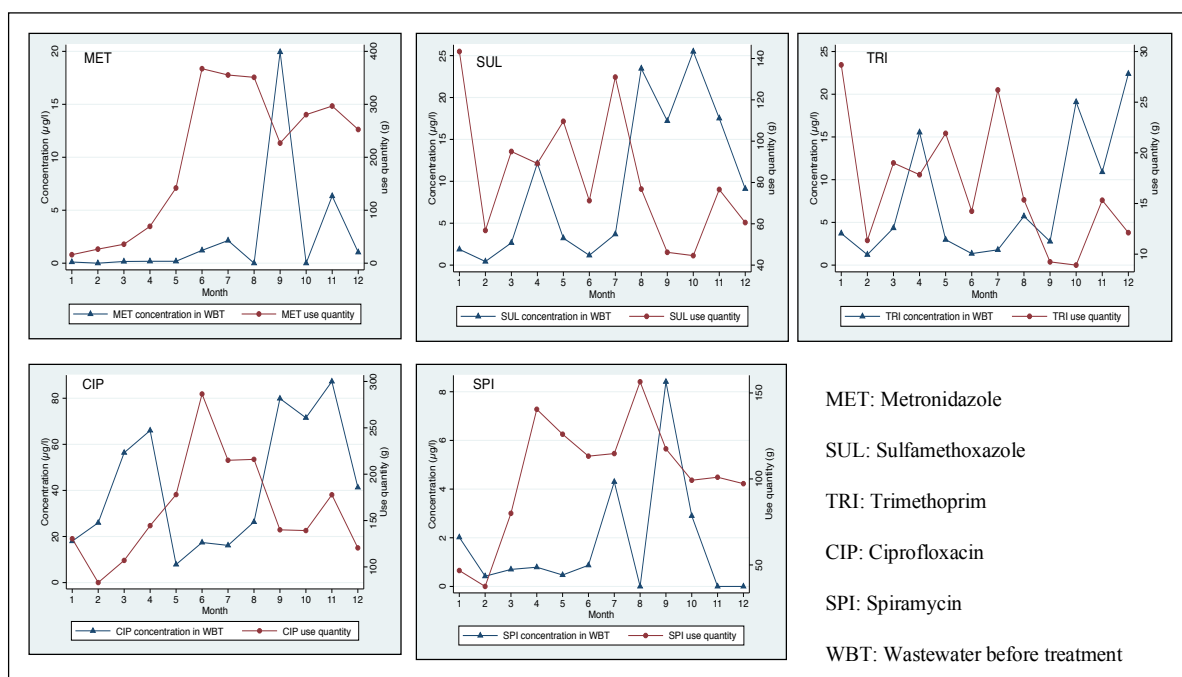


Figure 8. Relationship between quantities of antibiotics used and residues of studied antibiotics in wastewater before treatment in the rural hospital

4.2.2 Antibiotic resistance and antibiotic-resistance genes in *Escherichia coli* isolates from hospital wastewater (Paper IV)

Prevalence of resistance to the studied antibiotics among *E. coli* isolates in both the hospitals is presented in Table 7.

In the rural hospital, 85% of *E. coli* isolates were resistant to at least one of the tested antibiotics. Resistance was most common towards co-trimoxazole (70% of isolates), followed by ceftriaxone resistance (49% of isolates). Resistance to ceftazidime, gentamicin, and amoxicillin/clavulanic acid was around 40%, respectively. Thirty percent (30%) of the isolates were resistant to ciprofloxacin and 2% to fosfomycin. Resistance to imipenem was only detected in one isolate (1%). MDR was found in 35% of isolates.

In the urban hospital, 79% of *E. coli* isolates were resistant to at least one of the studied antibiotics. Co-trimoxazole resistance was again most common (71% of isolates), followed by ceftriaxone resistance (39%). Resistance to gentamicin and ceftazidime was found in 29% and 28% of isolates respectively, followed by amoxicillin/clavulanic acid (24%) and

ciprofloxacin (21%). Fosfomycin resistance was least common (8% of isolates). MDR was found in 27% of isolates.

Table 7. Prevalence of resistance to studied antibiotics in *Escherichia coli* isolates found in hospital wastewater

Studied antibiotics	Rural hospital (n = 158)				Urban hospital (n = 107)				Both hospitals (n = 265) Overall (%)
	WBT (%) n = 84	WAT (%) n = 74	p-value	Overall (%)	WBT (%) n = 60	WAT (%) n = 47	p-value	Overall (%)	
Amoxicillin/clavulanic acid	51	24	0.001*	39	28	19	0.36	24	33
Ceftazidime	42	36	0.52	39	32	23	0.40	28	35
Ceftriaxone	55	42	0.11	49	45	32	0.23	39	45
Ciprofloxacin	25	35	0.22	30	23	17	0.50	21	26
Co-trimoxazole	86	53	<0.001*	70	80	60	0.03*	71	70
Fosfomycin	1	3	0.60	2	15	0	0.005*	8	4
Gentamycin	51	31	0.02*	42	33	23	0.30	29	37
Imipenem	1	0	1.00	1	0	0	N/A	0	1
At least one antibiotic	94	74	0.001*	85	88	68	0.02*	79	83
MDR	44	26	0.02*	35	32	21	0.30	27	32

NOTE. MDR: multidrug resistance; N/A: not applicable; WBT: wastewater before treatment; WAT: wastewater after treatment

*Differences in prevalence of resistant *Escherichia coli* strains isolated from WBT and WAT are significant

In both the hospitals, significant differences in resistance among isolates from wastewater before versus after treatment was observed, with the resistance after treatment being less. However, the increase in the number of *E. coli* resistant to ciprofloxacin and fosfomycin separately after treatment was also found in the rural hospital.

The distribution of MIC values for ceftazidime and ciprofloxacin are presented in Figure 9. The number of *E. coli* isolates with high MIC values is large compared to the number of isolates with lower MIC values, indicating high levels in addition to high proportions of resistance. Decreased susceptibility was found when comparing to ECOFF values for amoxicillin/clavulanic acid (45% of isolates), ceftazidime (39% of isolates), ceftriaxone (48% of isolates), ciprofloxacin (29% of isolates), and imipenem (2% of isolates).

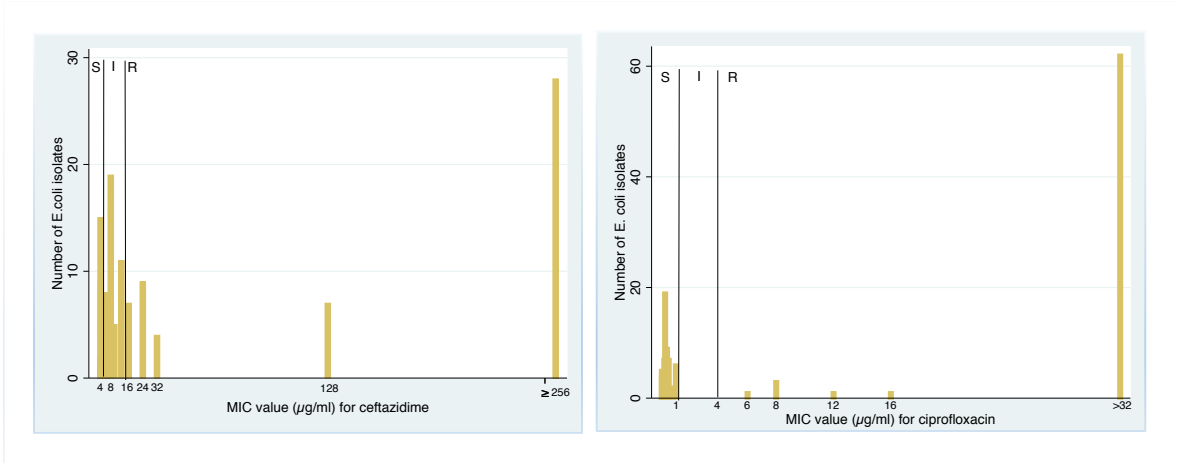


Figure 9. Distribution of minimum inhibitory concentration (MIC) values for ceftazidime and ciprofloxacin susceptibility testing.

NOTE.S: susceptible; I: intermediate; R: resistant

In the rural hospital, ESBL-production was detected in 48% of *E. coli* isolates. Among them, *bla*_{TEM} was detected in 97% of isolates and *bla*_{CTX-M} in 76%. Both *bla*_{CTX-M} and *bla*_{TEM} were detected in 75% of isolates. The quinolone-resistance gene (*qepA*) was detected in 72% of ciprofloxacin-resistant isolates.

In the urban hospital, ESBL-production was detected in 36% of *E. coli* isolates. Among them, *bla*_{TEM} was detected in 95% of isolates and *bla*_{CTX-M} in 41%. Both *bla*_{CTX-M} and *bla*_{TEM} were detected in 41% of isolates. The quinolone-resistance gene (*qepA*) was detected in 86% of ciprofloxacin-resistant isolates.

5 DISCUSSION

5.1 Hospital infection control (Papers I and II)

The staff were generally knowledgeable of hospital infection control (median knowledge score: rural hospital = 11.8/15; urban hospital = 12/15). However, the score range was wide (1.4-14.5) and the staff were not aware of the situation in their own hospital. Practices of infection control in the hospitals seemed to remain poor although self-reported practices by the staff were good or adequate. Self-reported practices in the urban hospital were better than in the rural one ($p=0.003$). Overall, cleaning workers had lower scores than physicians as well as nurses. Reported challenges for infection control were lack of resources, poor awareness and patient overload. The main theme identified was: ‘Making data on HAIs available for health workers can improve their awareness and motivate them to put their existing knowledge into practice and to make use of the facilities they already have for infection control’.

5.1.1 The need for continued training and tailor-made education for hospital staff and cleaning workers

Findings of this thesis indicate adequate or good knowledge towards infection control among the majority of hospital staff. This is in contrast with a range of studies previously published from different LMICs, where knowledge deficit has been observed as one of the main barriers for effective infection control [23, 26, 27, 80-82].

There was a prevailing belief among healthcare workers at a Ugandan hospital that infection control was mainly important for occupational safety. The nurses and support staff in the hospital were less likely to perceive that their practice can cause disease transmission, resulting into HAIs in patients [80]. Similarly, Ghadamgahi et al. (2011) concluded that most nursing staff participating in their study in Iran do not have a good knowledge about HAIs [82]. Another study from Iran found that nearly half of the nurses investigated (42%) in a teaching hospital had poor knowledge of standard precautions for HAIs [81]. Only one third of the healthcare workers knew the definition of HAIs and one fifth were aware of the main factors that increase the risk of HAIs in a study from Burkina Faso, West Africa [83].

Adequate hand hygiene is an important preventive measure against HAIs. Sethi et al. (2012) found that nurses considered hand washing as a means of self-protection rather than a mean to prevent patient-to-patient transmission [80]. Limited knowledge on hand hygiene practices

among healthcare professionals was also reported at a community hospital in the United States [84]. A study from India indicated that knowledge of hand hygiene was adequate, but a significant deficiency in the knowledge of other infection control practices was observed among intensive care nurses in a tertiary care hospital [85].

In this study, the hospital staff were generally aware of the definition of HAIs, the transmission pathways of HAIs and the importance of hand hygiene in preventing HAIs. This can be explained by the Vietnamese MoH's policies which have recently been implemented to update and popularize documents for staff training in hospital infection control [70, 86, 87]. Nevertheless, the knowledge score range was wide and their practices were not yet satisfactory. A number of respondents still showed poor knowledge in both hospitals, which indicates a need for continuing professional development.

In Vietnam, infection control has not been included in the curricula of medical and healthcare programmes; a standard national curriculum on infection control is still lacking. Standard training centres with infection control specialists in the three main regions across the country are in the pipeline however none have been established [67]. Continuing training on infection control for hospital staff at individual hospitals or at local health departments can also be a good way toward improve infection control practice.

Among occupation groups, cleaning workers showed lower knowledge score than both doctors and nurses. This difference was statistically significant for the urban hospital. In case of the rural hospital, nonsignificant results can be due to the fact that a small number of cleaning workers participated in the study (n=4). The role of cleaning workers in hospital infection control is usually underestimated although they themselves and their work can be a mean of infection transmission. In fact, adequate cleaning can be an important intervention in preventing and controlling HAIs in hospitals [88]. Thus, there is a need for tailor-made education on this topic for cleaning workers on this topic.

5.1.2 Possible discrepancies between self-reported and 'actual' practices

The study showed good or adequate reported practice scores for the majority of respondents. However, staff reported various reasons for noncompliance with hand hygiene, an important component in infection control. At the same time, findings of the qualitative study revealed poor infection control practices, including inadequate compliance with hand hygiene procedures in both the hospitals. Low compliance with hygiene protocols among hospital staff was reported from other LMICs [83].

In a recent workshop on infection control by MoH, it was emphasised that the infection control practice in Vietnamese hospitals is of low-quality and does not properly comply with the MoH regulations [89]. Generally, effective infection control is difficult for many Vietnamese hospitals due to high workload resulting from patient overload and inadequate staff, and poor infrastructure for infection control [59]. There is no standard monitoring system for hospital infection control, no monitoring and reporting criteria have yet been established, and no tools and monitoring software have been developed or applied. Therefore, a possible difference between the staff's self-reported practice and their real practice needs to be paid appropriate attention in the future infection control strategies of the studied hospitals.

5.1.3 Lack of resources for infection control: poorer conditions in the rural versus the urban setting

Lack of financial resources for infection control is one of the major challenges for resource-limited settings [21, 90-92]. Like many other LMICs, in Vietnam the overall investment for healthcare is limited. While high-income countries spent up to 17% of the GDP for healthcare in 2013, the Government of Vietnam spent only 6.0% of the GDP (this was reduced from 6.4% in 2010) [93]. Moreover, budget allocation for infection control has been given less priority compared to other activities in hospitals such as clinical activities or medicines. This may explain the lack of facilities, and insufficient equipment and supplies for infection control reported in the studied hospitals.

Inadequate equipment and facilities for infection control are the case in many Vietnamese hospitals. A recent review on infection control activities in health facilities by the Vietnamese MoH indicated that, nationwide, nearly 40% of hospitals lack at least one isolation room in wards, nearly half of the hospitals do not have sterilisation units, 34% of hospitals do not have a dirty utility room per ward, 40% of hospitals do not have one sink per 10 inpatient beds as per recommended standards, and more than half of the hospitals do not have access to soap or alcohol hand rub [94].

Lack of human resources is another challenge for infection control in many Vietnamese hospitals. The majority of the hospital staff working with infection control management are not properly trained or specialised. The MoH has estimated that about half of the infection control staff lack adequate training; about half of the staff working with disinfection and sterilisation are unqualified, and almost all the hospitals do not meet the recommended one infection control manager per 150 inpatient beds [94]. The hospitals included in this study

face similar challenges. In both the hospitals, no staff received specific education on infection control. In fact the staff would rather choose not to work with infection control. In some hospitals, the leaders may not fully understand the importance of infection control, thus, the hospitals may not have appropriate policies in place, such as encouraging staff to work with infection control management [67].

Comparing the two studied hospitals, the rural district hospital had poorer conditions for infection control than the urban provincial one, which was highlighted by the lack of a separate infection control department and a microbiological laboratory. The rural hospital had a working group in charge of infection control, which mainly took care of laundry, drying and steaming clothes and bed linen, and waste management. This may explain the higher practice scores in the urban hospital.

Vietnam is now in the process of implementing major healthcare reforms with a shift from a centrally-planned system, where healthcare services were provided to the population free of charge, to a decentralised and contracted social health insurance model. Nonetheless, many public hospitals are still dependent on the state's budget. Central or provincial hospitals located in wealthier regions normally receive more investment and are better equipped with medical technology [95].

A survey on HAIs conducted in 51 Vietnamese hospitals at all administrative levels (central, provincial and district) indicated that there are big differences between the central and local hospitals, especially district hospitals, in many medical aspects including infection control. Such differences are one of the existing problems of medical care in Vietnam [96].

5.1.4 Infection control practices: the know-do gap

Although good knowledge is a pre-requisite for effective infection control, it does not necessarily guarantee good practice. The know-do gap in infection control practice has previously been reported in various studies [24, 25, 97, 98]. A systematic review indicated that despite adequate knowledge on hand hygiene and the introduction of many initiatives, adherence to hand hygiene remains poor. Hand hygiene was considered to be an acquired habit rather than a reasoned process. This area is currently understudied and effective methods for changing health workers behaviours are needed to reduce HAIs and improve patients' safety [99].

Egwuenu et al. (2014) showed that nurses in selected primary healthcare centres in Nigeria had good knowledge of infection control, but did not fully reflect on their own level of practice. The majority of participants knew that washing hands with soap and water (98%) and wearing gloves (99%) prevents HAIs. However, in practice compliance was below average for glove use (48%) [97]. According to Iliyasu et al. (2016), a gap was also identified between knowledge and practice of infection control among studied doctors and nurses. Most of the respondents correctly identified hand washing as the most effective method to prevent HAIs, but not all of them always practised hand hygiene between patients [98].

Contradictions between what physicians knew and what they actually did were also indicated in Sweden, a country with a well-developed healthcare system [100]. According to that study, doctors were knowledgeable about proper hand-hygiene practices, but failed to bear in mind that their own practices might be harmful to patients.

In this study, hospital staff were aware of the importance of hand hygiene in preventing HAIs. However, even when the necessary facilities were in place, they did not seem to comply with recommended hand hygiene procedures. Doctors complained that patient overload was a dominant barrier for their compliance. However, if alcohol-based hand rub was available, it would be feasible for them to use it after each patient [101]. One determinant of this know-do gap might be the staff's poor awareness of the HAI problem in their own hospitals.

5.1.5 Improving infection control practices: making data on healthcare-associated infections available for hospital staff

Hospital staff suggested various measures to improve infection control practices such as surveillance, training, access equipment and facilities, and frequent audit and control. Undoubtedly, a comprehensive approach with an investment of financial and human resources is needed to optimise the practice. Nevertheless, it has been difficult to implement in the studied settings which have limited resources like many other Vietnamese hospitals.

Findings of this thesis indicate that if the hospital staff were aware of the HAI problem in their hospital, they would 'become awake', that is they will become aware of the need for behaviour change, and the know-do gap would eventually be diminished. Thus, making data on HAIs available for healthcare workers can improve their awareness on infection control, motivate them to put into practice the existing knowledge and make use of facilities they already have. That would be a feasible intervention to improve infection control practices in

settings which have constraints in optimising their facilities and systematic approaches for infection control due to limited resources.

As aforementioned, adequate infection control practices can mitigate the spread of infectious bacteria including antibiotic-resistant bacteria in healthcare facilities and beyond, thus reducing the use of antibiotics to treat HAIs, and thereby reducing overall use of antibiotics in hospitals.

5.2 Dissemination of antibiotic residues and antibiotic resistant bacteria from hospitals (Papers III and IV)

In the two hospitals, antibiotic residues were present in the wastewater both before and after wastewater treatment. In the rural hospital, ciprofloxacin was detected at the highest concentrations (before treatment: mean = 42.8 µg/L; after treatment: mean = 21.5 µg/L). In the urban hospital, metronidazole was detected at the highest concentrations (before treatment: mean = 36.5 µg/L; after treatment: mean = 14.8 µg/L). A significant correlation between quantities of antibiotics used in the rural hospital and the antibiotic concentrations in the hospital wastewater before treatment was found for ciprofloxacin ($r = 0.78$; $p = 0.01$) and metronidazole ($r = 0.99$; $p < 0.001$). Resistance to at least one of the antibiotics investigated was detected in 83% of the *E. coli* isolates from the hospital wastewater. Multidrug resistance was found in 32% of the isolates. The highest resistance rate was found for co-trimoxazole (70%) and the lowest for imipenem (1%). Forty-three percent (43%) of isolates were ESBL-producing, with the *bla*_{TEM} gene being more common than *bla*_{CTX-M}. Gene *qepA* was detected in 77% of the ciprofloxacin-resistant isolates. Among them co-harboring of the *bla*_{CTX-M}, *bla*_{TEM} and *qepA* genes was found in 46% of the isolates.

5.2.1 Relationship between antibiotic concentrations in hospital wastewater and hospital antibiotic use

Although very few studies explore the correlation between antibiotic residues in hospital wastewater and quantities of antibiotics used in the same hospital, antibiotic residues in hospital wastewater are logically expected to be dependent on the quantity of antibiotics used in the same hospitals. A “good accuracy” was demonstrated for the predicted concentrations of ciprofloxacin, metronidazole and sulfamethoxazole in hospital wastewater based on drug consumption data of a Swiss university hospital [102]. Similarly, findings of this thesis show significant correlations between the concentrations of ciprofloxacin and metronidazole in

wastewater and the quantities used in the rural hospital. Both the antibiotics are chemically stable and were detected at high concentrations in the hospital wastewater [103].

In the case of other antibiotics, there could be other factors influencing their residue levels in hospital wastewater such as metabolism rates or/and their stability in the aquatic environment. Ceftazidime was only detected in four water samples (out of 24) in the rural hospital and not detected in any samples from the urban hospital, although it was normally prescribed in the hospitals. Ceftazidime is a third-generation cephalosporin with a high metabolic rate and is easily degraded due to decarboxylation of the β -lactam ring [104]. In contrast, ofloxacin was found in water samples from the rural hospital when it was not on the antibiotic use data of the hospital. It is possible that the antibiotic had been purchased from outside and used by patients whilst being admitted in the hospital, or that patients had used it prior to admission.

5.2.2 Reduction of antibiotic concentrations in hospital wastewater after wastewater treatment

In both the studied hospitals wastewater treatment plants resulted in concentration reductions of the tested antibiotics. The average reduction in concentrations of ciprofloxacin (58.6% in the rural hospital and 49.8% in the urban hospital) was less compared to a study from another Vietnamese hospital (86%) [42]. In that study, water samples were collected using grab sampling over 2 days, while in the thesis study the water samples were collected using continuous sampling for 24 hours once a month over a period of one year.

The reductions in the concentrations of ciprofloxacin found in the thesis study were also relatively smaller compared to figures reported from some high-income countries: 59-76% in the United States, 92% in Switzerland, and 94% in Sweden [105-107]. Due to the variation in sampling techniques as well as the frequency of sampling, it is difficult to say whether the results are in fact comparable. Nevertheless, it could be expected that in LMICs, wastewater treatment plants might be less up-to-date and function less effectively than in high-income countries.

5.2.3 Antibiotic residues in hospital wastewater may promote the selection of resistant bacteria

Antibiotics were still detected at significant concentrations in the effluent of the studied hospitals. Among tested antibiotics, ciprofloxacin, a 2nd-generation fluoroquinolone, was found at high concentrations. Its high concentrations in hospital effluents were previously

reported in other studies [108-110]. Like other fluoroquinolone antibiotics, ciprofloxacin is chemically stable and it is also one of the most prescribed antibiotics in hospitals [111]. This might explain its high residues in the hospital wastewater.

Metronidazole, an imidazole derivative, was also detected at high concentrations compared to other studied antibiotics. It is widely used to treat infections caused by anaerobic bacteria as well as protozoa. It is highly soluble in water and has low biodegradability, which makes metronidazole a difficult contaminant to remove by traditional wastewater treatment methods [112].

Antibiotic residues can contribute to the development of antibiotic resistance in bacteria through selective pressure. Specifically, maybe even the exposure of a bacterium to a single molecule of an antibiotic can favour natural selection for resistance, or a mutation developing resistance [15]. The selection of resistant bacteria can occur even when antibiotics were present at very low concentrations below MICs [113-116].

Predicted no-effect concentrations (PNECs) for resistance selection have been suggested for various antibiotics including antibiotics tested in this study: ceftazidime 0.5µg/L, ciprofloxacin 0.064µg/L, metronidazole 0.125µg/L, ofloxacin 0.5µg/L, spiramycin 0.5µg/L, sulfamethoxazole 16µg/L, and trimethoprim 0.5µg/L [117]. For ciprofloxacin, the minimal selective concentration (MSC) was postulated to be 0.1µg/L in another study [116]. Compared to the concentrations detected in this study, in many cases they were higher than the reported PNECs and MSC, indicating that resistance selection among bacteria in the hospital wastewater can occur [118].

5.2.4 High prevalence of antibiotic-resistant *Escherichia coli* in hospital wastewater both before and after wastewater treatment

Findings of this thesis indicates high prevalence of *E. coli* isolates resistant to commonly used antibiotics in hospital wastewater even after treatment. Prevalence of *E. coli* resistant to at least one of the studied antibiotics was 83%, higher than reported by Kwak et al. (2015) in their study investigating antibiotic resistance among *E. coli* in wastewater in Stockholm, also over a one-year period (55%) [119]. This is, to the best of my knowledge, the first time the presence of resistant *E. coli* in hospital effluent has been reported in Vietnam. Prior to the thesis study, Duong et al. reported that all *E. coli* strains isolated from treated wastewater of another Hanoian hospital were susceptible. As mentioned above, in that study, grab sampling method was used and the number of isolates tested was small (15 isolates including 3 isolated from the treated water sample). In our study, continuous sampling of water samples for 24

hours was applied and a total of 265 *E. coli* isolates were tested.

Resistance to co-trimoxazole was most common in both the hospitals (around 70% of isolates). This prevalence rate was comparatively higher than in other studies previously conducted. An Indian study found that 55% of enteric bacteria isolated from hospital wastewater during 2004-2005 were resistant to co-trimoxazole [120]. Another study from Poland indicated a co-trimoxazole resistance rate of 20% among *E. coli* isolates from hospital wastewater [121].

Co-trimoxazole has been extensively used to treat urinary tract infections as well as respiratory tract infections in clinical settings, which was also the case in the hospitals investigated in the thesis. This might explain the high occurrence of co-trimoxazole resistance in the *E. coli* isolates [122]. At the same time, the residues of sulfamethoxazole and trimethoprim, the two active ingredients in co-trimoxazole, persistently found in the same hospital wastewater could play a role in the development of co-trimoxazole resistance in bacteria [123].

Resistance to ceftazidime and/or ceftriaxone, both 3rd-generation cephalosporins, was also found in higher proportions than other antibiotics tested (around 50%). Chagas et al. (2011) showed similar prevalence rates of cephalosporin resistant bacteria in the effluent of a Brazilian hospital [124]. Mechanisms of cephalosporin resistance vary, with ESBL production being the most important [125].

Fosfomycin resistance and imipenem resistance was also found among the *E. coli* isolates. Fosfomycin is a broad-spectrum antibiotic. Due to increasing resistance to other commonly used antibiotics for urinary tract infections, such as co-trimoxazole and ciprofloxacin, fosfomycin is recommended as one of the first-line drugs to treat uncomplicated urinary tract infections in many countries [126]. Imipenem is the first carbapenem, a last-line antibiotic which is used to treat infections caused by β -lactamase-producing bacteria [127]. High prevalence of carbapenem-resistance in clinical isolates has been reported from several Vietnamese hospitals [127]. The detection of resistance to these antibiotics in *E. coli* isolates from the hospital wastewater in this study, even at low prevalence rates, is of concern, as these resistant bacteria can be spread in the environment.

5.2.5 Detection of extended-spectrum beta-lactamases-producing *Escherichia coli* isolates

ESBLs were first isolated in the 1980s [128]. These enzymes are capable of inactivating cephalosporins as well as other beta-lactam antibiotics by hydrolysing the beta-lactam ring. Genes coding ESBLs are often plasmid-mediated and thus can be transferred between different bacterial strains within or between species (horizontal gene transfer). This facilitates the spread of antibiotic resistance [129]. Besides, ESBL-producing bacteria have showed co-resistance to quinolones, sulphonamides, and aminoglycosides [8]. Multidrug resistance in ESBL-producing *E. coli* isolates from hospital wastewater has been demonstrated [124]. Resistance to up to 9 non-beta-lactam antibiotics was found in ESBL-producing *E. coli* isolates from hospital wastewater [130].

Detection of ESBL-producing bacteria in hospital effluents has been earlier reported. Chandran et al. (2014) showed a very high prevalence of ESBL-producing *E. coli* in the wastewater of an urban and a rural hospital in central India (96%) [131]. Alam *et al.* (2013) also indicated common presence of ESBL-producing enteric bacteria the hospital wastewater [120]. In this study, the prevalence of ESBL producing *E. coli* isolates in hospital wastewater was around 40%, relatively higher than the figures reported by Diwan et al. (2012) (25%), Korzeniewska et al. (2013) (37%) and Abdulhaq et al. (2015) (25%) [121, 132, 133].

5.2.6 Detection of antibiotic resistance genes: genetic evidence of antibiotic resistance

TEM and CTX-M are the most common ESBL-coding genes [134]. Genes *bla*_{CTX-M} group 1, *bla*_{CTX-M} group 9, *bla*_{SHV} and *bla*_{TEM} were found in ESBL-producing *Enterobacteriaceae* in hospital effluents by Korzeniewska et al (2013) [135]. In this study, both genes *bla*_{CTX-M} and *bla*_{TEM} were detected with *bla*_{TEM} being predominant. Varela et al. (2015) reported similar results where *bla*_{TEM} was the most prevalent ESBL-encoding gene, followed by *bla*_{CTX-M} [136]. In contrast, Chandran et al. (2014) found higher prevalence of *bla*_{CTX-M} than *bla*_{TEM} among *E. coli* isolates from hospital wastewater in India [131]. However, not all *bla*_{TEM} genes are responsible for ESBLs. Further sequencing is needed to show the exact frequency of the ESBL *bla*_{TEM} in this study.

Ciprofloxacin resistance was found with gene *qepA* coding for large proportions of the resistant strains. Gene *qepA* is plasmid-mediated and capable of horizontal gene [137]. In this

study, co-existence of *qepA* together with *bla_{CTX-M}* and *bla_{TEM}* was investigated, genetically demonstrating co-resistance in *E. coli* isolates. The prevalence of MDR detected phenotypically was up to 35%, and co-resistance to six out of eight antibiotics has been found.

5.2.7 Public health implications

Release of antibiotic residues and antibiotic resistant bacteria into the environment by hospitals has become a growing concern for public health [138-145]. This study for the first time indicate the combined presence of antibiotic residues, antibiotic-resistant bacteria along with ARGs before and even after wastewater treatment in Vietnam. Hospital wastewater treatment plants themselves can harbour antibiotic residues and also antibiotic-resistant bacteria [146-148]. Various studies showed that wastewater treatment plants are ‘hotspots’ for the release of antibiotics and antibiotic-resistant bacteria into the environment [32, 149-152]. Iweriebor et al. (2015) found that 100% of enterococcus species isolated from the final hospital effluent in South Africa were resistant to vancomycin and concluded that wastewater treatment plants could act as a reservoir of antibiotic-resistant bacteria [148]. Several studies found the enrichment of antibiotic-resistant *E. coli* in wastewater treatment plants, which was also the case at the rural hospital in the thesis study for ciprofloxacin and fosfomycin [153, 154].

Certain amounts of antibiotic residues, antibiotic-resistant bacteria and ARGs are discharged into the ambient environment. In the environment, the presence of antibiotics in turn can lead to the selection of new antibiotic-resistant bacteria and the resistant genes can be transferred between environmental bacterial strains. Resistant bacteria harbouring resistance genes can then enter water bodies which can be used for agriculture, farming or household purposes (Figure 10). Potentials for horizontal gene transfer between environmental antibiotic-resistant bacteria and the pathogenic clinical bacteria has been demonstrated [155, 156].

The problem can become even more aggravated when hospitals do not have wastewater treatment plants and wastewater is discharged directly into the environment. In Vietnam as well as in many other LMICs, this is still common practice, particularly in rural areas [32]. The investigated hospitals had conventional wastewater treatment plants using filtering, chemical and biological mechanisms. It has been reported that advanced wastewater treatment processes such as ozone, ultraviolet (UV) and ultrafiltration significantly reduce the prevalence of antibiotic-resistant bacteria [157, 158]. Hospitals must invest in effective

wastewater treatment plants with treatment processes that completely eliminate antibiotic residues and antibiotic-resistant bacteria.

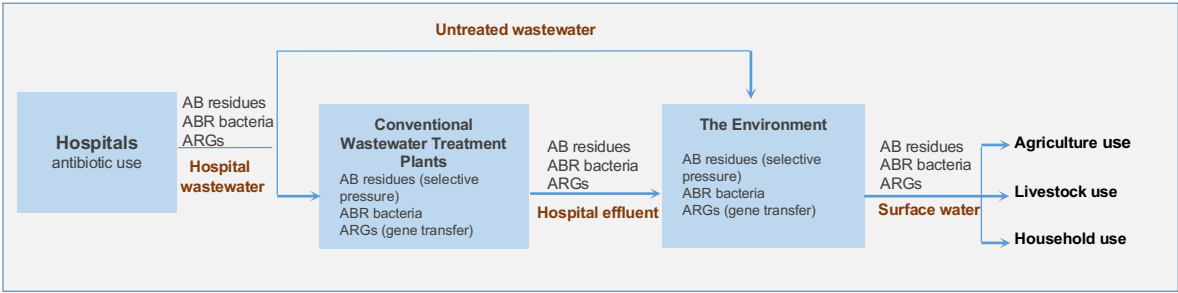


Figure 10. Potential routes for the transmission of antibiotic residues, antibiotic-resistant bacteria and antibiotic resistance genes from hospital wastewater to humans

(NOTE. AB: antibiotics; ABR: antibiotic resistance; ARGs: antibiotic resistance genes)

The presence of antibiotic-resistant bacteria and ARGs in the environment can lead to their transmission to humans [159], however direct evidence for this is still scarce [160]. Further studies are needed to identify links between the discharge of antibiotic-resistant bacteria by the hospital wastewater treatment plants, their occurrence in the ambient environment, and their acquisition by humans via environmental exposure.

5.3 Factors influencing hospital infection control and hospital dissemination of antibiotic residues and antibiotic resistant bacteria

It can be seen that factors influencing hospital infection control and hospital dissemination of antibiotics and antibiotic-resistant bacteria are complex as presented in Figure 11. Putting these factors in the context of the healthcare system, economy, culture and society may help to gain a deeper understanding of the problems.

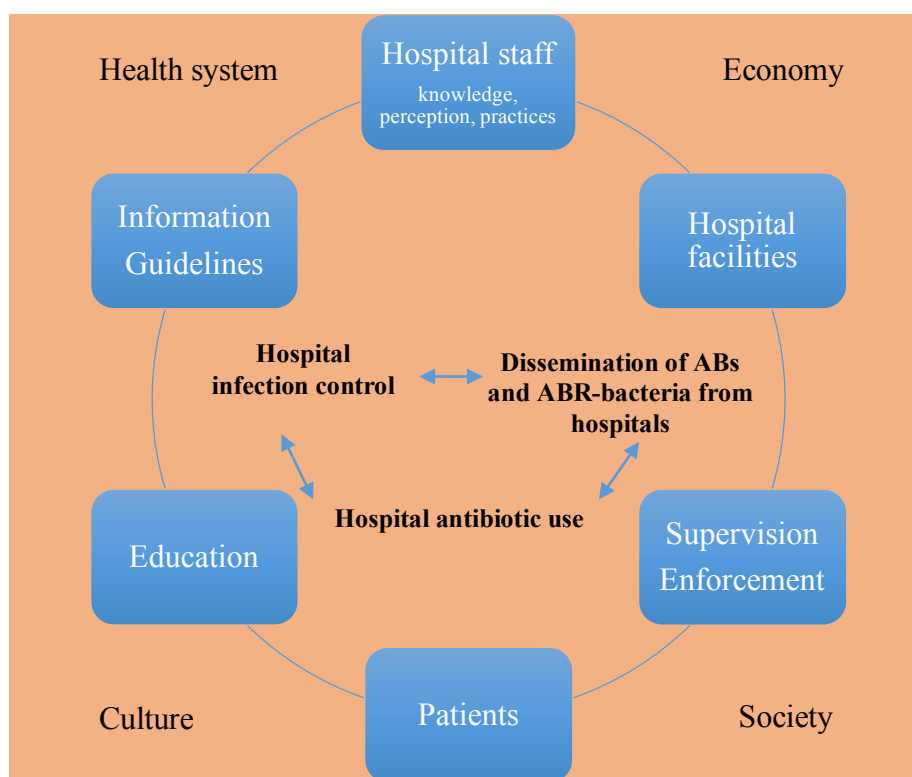


Figure 11. Factors influencing infection control and dissemination of antibiotics and antibiotic resistant bacteria in hospitals

(NOTE. ABs: antibiotics; ABR: antibiotic-resistant)

As aforementioned, in Vietnam, healthcare expenditure is limited with inadequate investment in the healthcare system for infection control in healthcare facilities. In fact, HAI control has been relatively new for the Vietnamese healthcare system. The first regulation on organisation and implementation of infection control in healthcare facilities was issued by the Vietnamese MoH in 2009, where hospitals were required to set-up an organisation system for infection control. The system should include infection control committees, infection control

departments and infection control networks [86]. In high-income countries, similar regulations has been in place for many years, for example since 1988 in France [161].

During the recent years, the Vietnamese MoH has been putting effort in improving infection control practices in healthcare facilities by issuing regulations, guidelines and training documents [70, 86, 87, 162]. A system for infection control has been gradually set-up in hospitals. In 2015, about 90% of the hospitals had infection control committees, 85% had infection control networks, and about 80% of the hospitals with 150 beds or more had infection control departments [94]. However, the core issue is whether these committees, departments and/or networks are indeed effective.

Infection control practices in Vietnamese hospitals are still of low quality [89]. Recently, in November 2017, four infant babies died on the same day in a provincial obstetrics-paediatrics hospital. "It can be due to HAIs" – that was the conclusion of the professional medical committee set-up by the MoH to investigate the case [*Vietnamese television*]. An experienced medical doctor working for the MoH heartbreakingly expressed that, "That was our fault" [*Personal communication*]. This sad case may have been avoided if all hospital managers were aware of the importance of infection control and all healthcare workers were aware of their role in effective infection control. There has been an issue, to some extent like a 'culture' in Vietnamese hospitals, that healthcare workers have not been willing to work with infection control. Instead of encouraging their staff, some hospitals even provided sanctions by transferring them to work at infection control units.

There has been no standard monitoring system for infection control as well as antibiotic resistance in healthcare facilities [163]. National systems for monitoring infection control and antibiotic resistance need to be established. It needs to consist of national committees in charge of developing national plans for surveillance and prevention, coordinating committees and centres at regional and local levels, and collaborating activities with other national agencies. Standard monitoring/reporting criteria and tools or software need to be developed and applied. Rates of HAIs and antimicrobial resistance including antibiotics are considered as indicators of the quality of care in healthcare facilities. These figures need to be specified in the related national action plans as one of the reporting and monitoring criteria.

At health facility level, infection control groups need to be qualified. They should consist of clinical physicians, nurses and microbiologists. The selection of the teams should be based on their experience in infectious diseases, hygiene, epidemiology, and microbiology. Small

hospitals with fewer resources can collaborate with bigger hospitals which have an infection control group [161].

In the context of a general lack of capitals for upgrading healthcare facilities, an adequate investment towards healthcare waste management including wastewater treatment is difficult. About half of the hospitals in Vietnam do not have any wastewater treatment plant. Providing wastewater treatment plants with advanced treatment processes to eliminate antibiotic residues and antibiotic-resistant bacteria may not be feasible in the near future. Thus, adequate infection control practices in healthcare facilities as well as in the community are crucial in preventing the unnecessary use of antibiotics, thereby, mitigating the development and spread of antibiotic resistance.

5.4 Methodological considerations

The thesis was conducted in one rural and one urban hospital in Hanoi, using both qualitative and quantitative approaches. The staff's knowledge and practices of infection control investigated in the quantitative paper (Paper I) was explored further in the qualitative paper (Paper II). A repeated cross-sectional design in papers III and IV was applied to simultaneously investigate levels of antibiotic residues, its relationship with hospital antibiotic use, and prevalence of antibiotic-resistant *E. coli* along with ARGs in the same hospital wastewater. Methodological considerations for the constituent papers are presented below.

Papers I and II

Questionnaires as well as qualitative data on hospital infection control were conducted with hospital staff of various occupation groups (doctors, nurses and cleaning workers), capturing a broad and diverse perspective on the topic. To collect qualitative data, both individual interviews and FGDs were conducted. Interviews with hospital managers provided views from the management aspect. At the same time, FGDs with the staff, who were directly involved in daily hospital activities, helped for open-minded and flexible discussions and ensured a detailed picture. In addition, the research team has varied professional backgrounds with experience in qualitative research from several settings.

A limitation of the questionnaire study lies in the fact that participants completed the questionnaires without supervision. Thus, a number of questionnaires lacked good quality information and were therefore excluded from the final analysis. In the case of the qualitative

study, since not all the researchers understand Vietnamese, the data analysis was first performed in Vietnamese. Final codes and selected quotations were then translated into English and the analysis continued in English, which could potentially introduce bias. To avoid that, the coding results and quotation selection were checked by a senior researcher who is fluent in both Vietnamese and English. Another limitation of the qualitative study is that member check of the interview and discussion transcripts was not done with the participants.

Paper III and IV

Few studies globally have attempted to concurrently identify antibiotic residues, antibiotic-resistant bacteria and ARGs in hospital wastewater. Continuous sampling is considered to be a method of choice to collect water samples for determining pharmaceutical residues. In the studies, continuous constant sampling with a pump of a fixed speed was used. This water sampling method has shown less sampling uncertainty than grab sampling. However, continuous flow-proportional sampling would be the optimal method to minimise the sampling error [43,44].

It has not yet been established yet which is a standard method to investigate correlation between antibiotic consumption in a hospital and antibiotic concentrations in the hospital wastewater. Efforts have been made to calculate predicted environmental concentrations based on annual data of hospital antibiotic consumption. In these thesis studies, only monthly antibiotic consumption data from the rural hospital were available to analyse the correlation with antibiotic concentrations in the hospital wastewater. Measuring antibiotic concentrations daily was beyond the financial means of this project. Therefore, 24 hour measured concentrations of antibiotic residues in the last week of the month were taken to represent one month.

Physicochemical and microbiological testing applied in these studies were adapted based on the literature and conducted by experienced technical staff and microbiologists in a qualified drug testing centre and microbiological laboratory. Water samples were stored, protected and transferred for analysis using appropriate standard procedures.

Antibiotic susceptibility testing was conducted following CLSI guidelines [75]. CLSI breakpoints are standardised for clinical isolates. There have been no standardised methods directly applicable to environmental samples [164]. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), ECOFF values, which separate resistance-

acquired bacteria (non-wild type) from the wild-type bacteria (having no resistance), can be used for environmental isolates [76]. We found decreased susceptibility in the *E. coli* isolates when comparing to ECOFF values.

A limitation of the studies is that water samples could not be collected during three months from June to August in the urban hospital due to refurbishment. Besides, a detailed description of the wastewater treatment plants was not available to the researchers from the hospitals. In addition, the resistance patterns among the *E. coli* isolates presented might not be representative for the whole *E. coli* population in the hospital wastewater because of the small number of the isolates tested from each water sample. Furthermore, not all genes responsible for cephalosporin resistance and quinolones resistance were tested due to financial constraints.

Credibility

Credibility is the quality of being believable in the selection of study settings, study participants and research methods to gathering data in relation to the study's aim [67]. In order to improve credibility/trustworthiness of the study, triangulation of data collection, data analysis, and investigators were used.

For data collection in the qualitative study, both FGDs and individual interviews were applied. In FGDs, interactions between participants were useful to generate information without personal biases. The participants were from different wards of the hospitals, different occupation groups, age, sex, and years of experience. Individual interviews with hospital managers gave deeper insights into the topic.

To improve the credibility of the findings, triangulation and member checks were applied during data analysis as well as during result interpretation. Both manifest and latent analysis were used to analyse qualitative data. The researchers have different backgrounds with extensive experience in their own fields, bringing various perspectives which is important when analysing qualitative data. This also helps in understanding the results of the quantitative studies. Although checking the interview and discussion transcripts was not done by the participants, cross-checking by two researchers could minimize misinterpretation.

Validity and reliability

In order to improve the validity/reliability of the studies, instruments for data collection were developed in consultation with experienced professionals and were pilot-tested. The

questionnaire was piloted with 20 respondents, following which few modifications were made. Water samples were pilot-collected and tested for two months prior to the main study to check the feasibility of the study. Instruments to report results from chemical and microbiology analysis of water samples were tested. Collection of water samples every month over a year-long period was also helpful to increase the validity of the study. The chemical and microbiological analyses were performed in qualified laboratories using standard laboratory methods. The inputted data were checked and the researchers got back to the laboratories in case of any confusion or doubt.

Reflexivity

Reflexivity in qualitative research refers to “the recognition that the researcher is part of the process of producing the data and their meanings, and to a conscious reflection on that process” [165]. In this study, none of the researchers were employed at the studied hospitals. The researchers and research assistants conducting FGDs and interviews had no direct relationship to the participants, making the participants less restricted in expressing their opinions. A discussion/interview guide with open-ended questions was used to exclude bias which might be caused by the researchers’ existing knowledge and experience. Researcher triangulation during data analysis helped to minimise biases due to the possible objective interpretation of the results.

Generalisability and transferability

To generalise or transfer the findings, it would be important to have representative samples. For the thesis topic, the feasibility of conducting studies in hospitals of various regions in Vietnam over a one-year period was difficult due to resource constraints. It could be argued that the investigated hospitals were typical of district hospitals located in rural areas and provincial hospitals located in urban areas in Vietnam. The findings would thus be transferable and be relevant to develop interventional strategies in other hospitals in the country and similar settings.

6 CONCLUSIONS

The key conclusions of this thesis are:

1. Hospital staff were generally knowledgeable about hospital infection control, with cleaning workers having poorer understanding. However, the staff were not aware of the situation in their own hospital.
2. Although self-reported practices on infection control were good or adequate for the majority of the hospital staff, in fact, the practices seem to remain poor in the hospitals.
3. Perceived difficulties with the hospital infection control were lack of financial and human resources, and lack of staff awareness about HAI situation.
4. Antibiotic residues were present in hospital wastewater both before and after wastewater treatment at concentrations that can promote the development of antibiotic resistance.
5. Antibiotic-resistant *E. coli* along with genes coding for cephalosporin resistance *bla*_{CTX-M} and *bla*_{TEM}, and a gene coding for ciprofloxacin resistance *qepA* were highly prevalent among *E. coli* isolates from hospital wastewater both before and even after wastewater treatment.

7 POLICY IMPLICATIONS AND FUTURE RESEARCH

1. A multipronged approach is crucial to optimise hospital infection control practices including prioritising infection control in hospital management, provision of adequate infrastructure, inclusion of infection control in medical curricula, continuing training for health professionals, as well as patient education.
2. Training healthcare workers in order to improve their awareness of infection control, including HAI situation in the hospitals, can be a feasible intervention to improve their practices in existing conditions of the hospitals. There is also a need for tailored education for cleaning workers.
3. There is a need for development and inclusion of hospital wastewater treatment plants which are effective at eliminating antibiotic residues, antibiotic-resistant bacteria and antibiotic resistance genes.
4. Further studies should aim to investigate links between hospital discharge of antibiotic residues and antibiotic-resistant bacteria, their occurrence in the nearby environment, their acquisition by humans when exposed to this environment and its impact on public health.

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10 APPENDICES

APPENDIX 1

Questionnaire on knowledge and self-reported practices of infection control among hospital staff

Knowledge items

1. **What do you understand by HAIs?**
 - Patients have symptoms of infection upon admission
 - Infections occurring within 24 hours after admission
 - Infections occurring more than 48 hours after admission
 - Re-admission due to surgical site infections within 1 month after severe operations
 - Surgical site infections developing within 10 days after surgery
 2. **From the items listed below, what are symptoms of HAIs?**
 - Fever upon admission
 - Urinary tract infection due to catheterisation
 - Drainage from a surgical wound
 - Pneumonia upon admission
 - Fever after surgery
 - Diarrhoea 4 days after admission
 - Drainage at an injection site 5 days after admission
 - Umbilical infections 5 days after admission
 - Infections after ophthalmic surgery
 - Upper respiratory tract infections 4 days after admission
 3. **Which of the following are transmission pathways for HAIs?**
 - Via hospital staff
 - Via hospital environment (air, water, food, etc.)
 - Via medical equipment
 - Via patients
 - Via visitors
 4. **Is infection prevention and control important? (Yes/No/Do not know)**
 5. **What are the possible consequences of HAIs?**
 - Increased morbidity and mortality rates
 - Increased treatment costs
 - Increased financial burden for hospitals and patients
 - Increased use of antibiotics
 - Prolonged treatment course
 6. **Can practising infection control reduce the rate of HAI? (Yes/No/Do not know)**
 7. **What are the possible measures for infection prevention and control?**
 - Hand washing
 - Extensive antibiotic use
 - Use of gloves
 - Use of prophylactic vaccines
 - Antiseptic conditions in operating rooms
 - Maintaining hygiene of the hospital environment
 8. **What do you understand by cross-infection?**
 - Infections transferred from patient to patient
 - Infections upon admission
 - Infections transferred from hospital staff to patients
 - Infections transferred from patients to hospital staff
 - Infections transferred within hospitals
 9. **Isolation rooms are necessary for?**
 - Patients with contagious diseases
 - Patients with acquired multidrug-resistant bacteria
 - Patients with acquired Methicillin-resistant *Staphylococcus aureus* (MRSA)
-

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- HIV patients
 - Hepatitis B patients
 - Patients with measles, rubella or mumps
10. **What are possible measures for prevention of HAIs related to catheterisation in hospitals?**
- Treatment using antibiotics
 - Aseptic technique at insertion
 - Avoiding catheterisation when unnecessary
 - Limiting the duration of catheterisation
 - Longer duration of catheterisation
11. **Which types of waste are so-called 'healthcare waste'?**
- Waste from diagnosis and treatment processes
 - Waste from sub-clinical testing
 - Waste from research activities
 - Waste causing toxic effects or disease spread from hospitals
 - Waste from the administration areas of hospitals
12. **What are the risks of improper hospital waste management?**
- Increased incidence of HAIs
 - Environmental pollution
 - Increased health risks for the healthcare workers
 - Violation of hospital practice management
 - Creating a bad image for the hospital
13. **Choose the correct answers for proper waste classification.**
- Red bags/boxes are for sharp objects such as injection needles and surgical knives
 - Blue or white bags/boxes are for water cans, juice cans, packages
 - Yellow bags/boxes are for gauzes/cloths which are blood-stained or contain bodily fluids
 - Black bags/boxes are for tubes, conductor lines, plastic catheters
 - Green bags/boxes are for placentas, body tissues and organs
14. **When do you think is the proper time to classify healthcare waste? (choose only one)**
- After all the waste in the ward is collected
 - Immediately at the waste bin
15. **When do you think is the proper time to collect waste bags? (choose only one)**
- When the bags are full
 - When the bags are half full
 - When the bags are $\frac{3}{4}$ full
-

NOTE. HAI: Healthcare-associated infection

Practice items

1. Are data on HAIs routinely collected at your hospital? (Yes/No/Do not know)
2. Did you ever receive information on HAIs in your hospital? (Yes/No/Do not know)
3. Does your hospital have an infection control department? (Yes/No/Do not know)
4. How many times do you wash your hands at work per day? (Choose only one)
 - Less than five times
 - Five times or more
5. At any one time, for how long do you wash your hands?
 - Less than one minute
 - About one minute or more
6. With what do you wash your hands?
 - Water only
 - Water and soap/hand washing liquid/alcohol hand rub
7. Why do you wash your hands?
 - To prevent myself from getting an infection
 - To prevent myself and my patients from getting an infection
8. Is waste classified at your ward? (Yes/No/Do not know)
9. If yes, when is the waste classified at your ward?
 - After all the waste in the ward has been collected
 - At each waste bin
10. What bags do you have at your ward for waste classification?
 - Red
 - Blue/white
 - Yellow
 - Black
 - Green
 - Not classified
 - No bags/bins for waste
11. What bags do you use for plastic waste such as catheters and infusion lines?
 - Red
 - Blue/white
 - Yellow
 - Black
 - Green
 - Not classified
 - No bags/bins for waste
12. In which bags do you put expired medicines?
 - Black
 - Yellow
 - Blue/white
 - Green
 - Any bag
13. When are waste bags collected at your ward?
 - When the bags are full
 - When the bags are half full
 - When the bags are $\frac{3}{4}$ full
14. How often are waste bags collected at your ward?
 - Within 24 hours
 - Once per day to once per week
 - Longer than once week
15. In the last 3 years, did you participate in any training on hospital infection control? (Yes/No/Do not know)

NOTE. HAI: Healthcare-associated infection

APPENDIX 2

Interview/discussion guide

Aim: To explore hospital staffs' perceptions on infection control practices including healthcare-associated infections, hand hygiene and healthcare waste management.

Preparation:

- Greetings, briefly introduction of the study's aim, operators and secretaries of the discussion/interview
- Ask permission to record the interview/discussion
- Introduction of the participants


Discussion points:

- The definition of healthcare-associated infections (HAIs)
- Types of HAIs
- Transmission pathways of HAIs
- Situation of HAIs in the hospitals
- Difficulties in controlling HAIs
- Importance of hand hygiene
- Reasons for non-compliance with hand hygiene guidelines
- Healthcare waste management
- Situation of healthcare waste management in the hospitals

Thank the participants for their cooperation.

APPENDIX 3

Form for reporting results of analysis of hospital wastewater samples for antibiotic concentration

	<p>Hanoi Drug and Cosmetic Testing Centre</p> <p>RESULTS OF ANALYSIS OF HOSPITAL WASTEWATER SAMPLES</p> <p>Month 2013</p>
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Samples:

Being analysed according to the procedures established

No.	Antibiotics	Concentrations (µg/l)					
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
1	Metronidazole						
2	Sulfamethoxazole						
3	Trimethoprim						
4	Ceftazidime						
5	Ciprofloxacin						
6	Ofloxacin						
7	Spiramycin						

- : Below limit of detection

Date / /2013

Technician

Head of the laboratory

Director

APPENDIX 4

Form for reporting results of antibiotic susceptibility testing in *E.coli* isolates from hospital wastewater

No	Antibiotics	Disk content (µg)	Urban/rural hospital – before/after treatment_Month...2013									
			Number of <i>E. coli</i> isolates:									
			<i>E. coli 1</i> <i>ESBL (+/-)</i>		<i>E. coli 2</i> <i>ESBL (+/-)</i>		<i>E. coli 3</i> <i>ESBL (+/-)</i>		<i>E. coli 4...</i> <i>ESBL (+/-)</i>		<i>E. coli n</i> <i>ESBL (+/-)</i>	
			Zone diameter (mm)	MIC (µg/ml)	Zone diameter (mm)	MIC (µg/ml)	Zone diameter (mm)	MIC (µg/ml)	Zone diameter (mm)	MIC (µg/ml)	Zone diameter (mm)	MIC (µg/ml)
1	Amoxicillin/ clavulanic acid											
2	Ceftazidime											
3	Ceftriaxone											
4	Ciprofloxacin											
5	Co-trimoxazole											
6	Fosfomycin											
7	Gentamycin											
8	Imipenem											

APPENDIX 5

Form for reporting PCR results

Month 2013	<i>E. coli</i> codes	ESBL		Quinolone resistance gene <i>qepA</i>
		<i>blaCTX-M</i>	<i>blaTEM</i>	
January				
February				
March				
April				
May				
June				
July				
August				
September				
October				
November				
December				

+: Positive

-: Negative