MINISTRY OF EDUCATION
AND TRAINING

MINISTRY OF
HEALTH

NATIONAL INSTITUTE OF HYGIENE & EPIDEMIOLOGY

TRAN DAC TIEN

CURRENT STATUS OF ENTEROBACTERIA RESISTANT TO BROAD SPECTRUM BETA-LACTAM ANTIBIOTICS AMONG HEALTHY PEOPLE AT ONE COMMUNE, HA NAM PROVINCE

Major: Public Health
Code: 62720301

SUMMARY OF PhD THESIS ON MEDICINE

HANOI - 2021

THESIS PERFORMED AND COMPLETED AT THE NATIONAL INSTITUTE FOR HYGIENE & EPIDEMIOLOGY

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This doctoral thesis will be defended at the Dissertation Committee of Institutional level at:

National Institute of Hygiene & Epidemiology

on 2021

The doctoral thesis can be found at:

1. The National Library

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LIST OF PUBLISHED SCIENTIFIC ARTICLES RELATED TO THE THESIS

1. Tran Dac Tien, Nguyen Thi Phuong Lien, Tran Huy Hoang, Tran Thi Van Phuong, Nguyen Thanh Duong, Pham Van Thang, Pham Duy Thai, Tran Nhu Duong (2017), “Status of enterobacteria resistant to broad spectrum β- lactam antibiotics among healthy carriers at Thanh Ha commune, Thanh Liem district, Ha Nam province in 2015”, *Journal of Vietnam Preventive Medicine*, Vol. 28, No. 11: 11-19.

2. Tran Dac Tien, Nguyen Thi Phuong Lien, Tran Huy Hoang, Doan Thi Ngan, Nguyen Thanh Duong, Pham Van Thang, Pham Duy Thai, Tran Nhu Duong (2019), “Some factors related to the status of resistance to broad spectrum β - lactam antibiotics of enterobacteria isolated from healthy people at Thanh Ha commune, Thanh Liem district, Ha Nam province in 2015”, *Journal of Vietnam Preventive Medicine*, Vol. 29, No. 10: 30- 37.

3. Tran Dac Tien, Nguyen Thi Phuong Lien, Tran Huy Hoang, Nguyen Thanh Duong, Nguyen Minh Thai, Pham Duy Thai, Tran Nhu Duong (2020), “Some molecular biological characteristics of intestinal bacteria resistant to broad spectrum β - lactam antibiotics isolated from community at Thanh Ha commune, Thanh Liem district, Ha Nam province in 2015’’, *Journal of Vietnam Preventive Medicine*, Vol. 30, No. 7.

INTRODUCTION

Antibiotic resistance is a very serious public health problem that endangers human and animal health in the 21st century. The World Health Organization has warned a major threat posed by the antibiotic resistance to the ability of treatment for common infections in the community and in the hospitals. Antibiotic resistance is projected to be the cause of about 10 million deaths annually by 2050 and the cause of over $ 100 trillion damage worldwide. This situation is not only an urgent problem in hospitals but also in the community due to the improper use of antibiotics in humans and in agriculture.

Enterobacteriaceae is a very large family of bacteria important in medicine because of its capacity in causing disease in humans. However, this group of bacteria is almost resistant to antibiotics, especially to the broad spectrum β-lactam antibiotics because they are capable to produce extended spectrum beta-lactamases (ESBL). A broad spectrum of β- lactam antibiotics, including cephalosporin, is one of the most widely used antibiotics in the past, accounting for more than half of all antibiotics used in the treatment of both humans and animals.

In Vietnam, a community antibiotic resistance surveillance system has not been established or has been available, but it is not continuous; the plans, activities and some recent studies on antibiotic resistance were mostly concentrated in treatment facilities, not many comprehensive studies were conducted in community, especially on the resistance situation of broad-spectrum β - lactam antibiotics of certain enterobacteria in healthy people. Basic and in-depth understanding of this issue, its epidemiology, relevant factors, microbiological and molecular characteristics of broad-spectrum β-lactam resistant enterobacteria, including third generation cephalosporins, is extremely necessary in the current period.

Study objectives

1. Describe the current status of healthy people carrying enterobacteria resistant to broad spectrum β-lactam antibiotics at Thanh Ha commune, Thanh Liem district, Ha Nam province in 2015.
2. Analyse some factors related to status of healthy people carrying enterobacteria resistant to broad spectrum beta - lactam antibiotics in Thanh Ha commune, Thanh Liem district, Ha Nam province, 2015.
3. Determine some biological characters of broad spectrum beta - lactam antibiotic resistant enterobacteria isolated at studied sites.

New contributions in science and practical value of the thesis

Currently, there are not many studies on the situation, factors related to broad spectrum β- lactam antibiotic resistance of intestinal bacteria in healthy people in the community and molecular biological characteristics of the the resistant bacteria isolated from different samples: healthy human feces, animal feces samples, and environmental samples collected from households at the study site. The thesis reveals the new contribution in science and research results have high practical value, provided scientific evidence for management and professional specialists of health sector, agriculture and governments at all levels to develop monitoring strategies on people's use of antibiotics, bacterial resistance and intervention plans, propaganda and prevention of antibiotic resistance in the community.

THE STRUCTURE OF THESIS

Thesis consisted of 129 pages (excluding references and appendices), 36 tables and 14 figures and 5 diagrams.

Of which, 3 pages of the Introduction; 36 pages of Literature overview; 20 pages of Study subjects and methods; 38 pages of Research Results; 29 pages of Discussion; 2 pages of Conclusion and 1 page of Recommendation.

Chapter 1. LITERATURE OVERVIEW

1.1. Antibiotic resistance and public health problems

1.1.1. Antibiotic resistance concept

Antibiotic resistance is the situation when microorganisms are resistant to previously susceptible antibiotics, leading to ineffective treatment, persistent (even fatal) infection, possible to spread it to others. Antibiotic resistance is an inevitable consequence of using antibiotics in treatment and especially increases when the abuse of antibiotics is more and more common.

1.1.2. Mechanism of antibiotic resistance of bacteria: (1) Altering the action target; (2) Creation of enzymes; (3) Reduces the permeability of the protoplasmic membrane and (4) Creates isoenzymes.

1.1.3. The effects of antibiotic resistance on public health

The antibiotic resistance is not only a concern of doctors in treatment, but also a concern of the whole society for public health such as: Impact on treatment costs and burden of disease, management monitoring, management of trading antibiotic production and the use of antibiotics in humans and agriculture.

1.2. The situation of antibiotic resistance to broad spectrum of β-lactamise antibiotics of enterobatcteria isolated from healthy people

1.2.1. **The general situation of antibiotic resistance on the world and in Vietnam**

- *On the world*: According to the previous studies, antibiotic resistant bacteria often appear very quickly after antibiotic is put into use.

- *In Vietnam*: Climatic conditions are favorable for the growth of microorganisms. Therefore, the control of bacterial infection and the management of anbiotic use as well as the control of antibiotic resistance are issues of social concern. Studies also showed that the rate of antibiotic resistant bacteria increased over time and resistant to many antibiotics; not only the antibiotic resistance in humans but also in agricultural products that detected bacteria carrying new antibiotic resistant genes.

Table 1.1. The development of antibiotic resistance

Antibiotic	Year to use	Year to detect antibiotic resistance
Sulfonamid	1930	1940
Penicillin	1943	1946
Streptomycin	1943	1959
Chloramphenicol	1947	1959
Tetracyclin	1948	1953
Erythromycin	1952	1988
Vancomycin	1956	1988
Methicillin	1960	1961
Ampicillin	1961	1973
Cephalosporin	1960	1960

1.2.2. Current situation of broad spectrum β- lactam antibiotic resistance of enterobacteria

- *Family of Enteribacteriaccae*: Enterobacteriaccae is a very large family of microorganisms, widely distributed in humans, animals, plants and in the environment. These are frequenly found in human and animal gut. Enterobacteriaccae is important in medicine because many species can cause disease in humans, including those that cause epidemic outbreaks.

- *Mechanism of antibiotic resistance to broad spectrum β-lactam antibiotic group of enterobacteria:* The synthesis of ESBL is considered as the main resistance mechanism of intestinal bacteria strains to broad spectrum β-lactam antibiotics. When the β-lactam ring is destroyed by the extended spectrum beta-lactamase (ESBL), the antibiotic will form cephalosporic acid and no longer being active.

- *The current situation of resistance to broad spectrum β-lactam antibiotic group of intestinal bacteria in the world*:

One study in Europe discovered the ESBL-born enterobacteriaceae family in 1980 and not long after that, this was detected in America and in some Asian countries with different rates. The difference in the rate of ESBL born bacteria is not only expressed by the countries and areas, but this rate differs by the hospitals and the community. According to the study conducted in Taiwan in 2010, the rate of bacteria producing ESBL found in the hospital was 45.7% while in the community this was 4.1%. One research in India also showed that the rate of bacteria producing ESBL found in the hospital was 85.4% and in the community was 53%. The forecast is that the rate of ESBL produced bacteria increases rapidly over time and especially in densely populated countries such as China, India, this is the transmission source of antibiotic resistant bacteria to other population areas.

- *The situation of broad spectrum β-lactam antibiotic resistance of the intestinal bacteria in Vietnam:* Some studies showed that the trend of resistant intestinal bacteria increases rapidly over time. The study of Vo Chi Mai in 1998 showed that the rate of intestinal bacteria producing ESBL was 6.3%; in 2005, Nguyen Thi Xuan Yen's research showed this rate of 34%; study of De Rosa F.G. in 2011 on gram-negative bacteria conducted in the Asia-Pacific region, showed the rate of intestinal bacteria resistant to antibiotics in Vietnam of over 50%. The study conducted at Military Central Hospital No.108 during 2015 to 2016 showed that the rate of ESBL producing *E.coli* and *K. pneumoniae* rapidly increased, in particular, rate of ESBL producing *E.coli* in 2015 was 26.86 %, in 2016 it was 73.14% and rate of ESBL producing *K. pneumoniae* in 2015 was 32.82% but in 2016 was 67.18%.

1.3. Some factors related to antibiotic resistance and it's transmission of bacteria carried resistant gene

1.3.1. The factors related to antibiotic resistance of bacteria

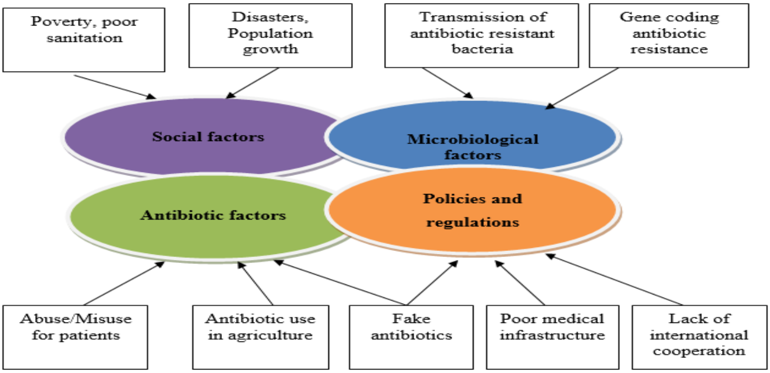

Figure 1.1 Factor groups related to antibiotic resistance of bacteria

1.3.2 The transmission of enterobacteria resistant to broad spectrum β-lactam antibiotics

Studies showed that the spread of antibiotic resistant genes originated in Europe and then spread to Americas and then Asia. A 1990 study showed that in Asia mainly found SHV gene and it recorded in Japan and Korea. The CTX-M gene began to appear in Europe in bacteria of Enterobacteriacea family in 1989 and up to now studies have shown that the CTX-M gene has changed the distribution of ESBL worldwide and is increasingly potential in the gram-negative strains, especially in *E.coli* and *K.pneumoniae*. A study in China in 2009 on *E.coli* and *K.pneumoniae* showed the presence of bacteria strains carrying 06 genes encoding β-lactam at the same time, mainly TEM, SHV, CTX-M, OXA, ... and they are easily passed from bacteria to bacteria through conjugation.

1.3.3 The characters of genes coding resistance of enterobacteria to broad spectrum β - lactam antibiotics

Today, researches have found more than 300 strains producing ESBL enzyme, carrying mainly TEM, SHV, CTX-M and OXA genes. These multidrug - resistant genes are transmitted mainly through plasmids, transposons and intergrons. These genes can be passed between families of gram-negative bacteria, leading to the rapid spread of antibiotic resistance.

1.4. Recent strategy of antibiotic resistance prevention and control

The World Health Organization (WHO) identified the issue of antibiotic resistance as a medical emergency at global level, which would seriously endanger the advancement of modern medicine. Therefore, it is necessary to have more investment in research and development of antibiotics.

The contents of WHO's strategy: (1) Proper use of antibiotics, (2) Access to high-quality antibiotics, (3) Disease prevention and control, good control of hospital infections, (4) Good surveillance on antibiotic resistance rate of each country.

Vietnam: Based on the goals and contents of implementing WHO's strategy to develop national strategy for the prevention of antibiotic resistance with the following solutions: (1) Raise public awareness about antibiotic resistance, (2) Reduce selective pressure causing antibiotic resistance of bacteria, (3) Strengthen infection prevention, (4) Regulate the use of antibiotics in agriculture, (5) Build up the surveillance systems on antibiotic-resistant bacteria, (6) Develope the sustainable economy and increase the investment in health development.

Chapter 2. STUDY SUBJECTS AND METHODS

2.1. Study subjects and time

2.1.1 Study subjects

- Healthy people living at Thanh Ha commune, Thanh Lieem district, Ha Nam province.
- The strains of enterobacteria resistant to broad spectrum β-lactam antibiotic group and bacteria resistant to cephalosphorin of third-generation, isolated from healthy human feces, domestic animal feces, processed food samples, samples of drinking water collected at the study site.

2.1.2 Study site

Thanh Ha commune, Thanh Liem district, Ha Nam province.

2.1.3. Study time frame: From November 2014 to September 2016; The surveillance of study subjects and collection of the samples were conducted in March 2015.

2.2 Study methods

2.2.1 Sample size

- *Sample size for Objective 1 and Objective 2:*

$$n = Z^2_{(1-\alpha/2)} \frac{p(1-p)}{(p.\mathcal{E})^2} \times DE$$

Of which:

- n: number of healthy people living in the community should be investigated

- p = 0.76, is the proportion of patients without gastrointestinal syndrome coming to Cho Ray hospital isolated with enterobacteria of the broad spectrum β-lactam group.

- Ɛ: relative accuracy, in this study Ɛ = 10% was choosen.

- $Z_{1-\alpha/2}$: the corresponding value for reliability, with a confidence level of 95% ($Z_{1-\alpha/2}$= 1.96)

- DE: design factor, DE = 2 was choosen.

The minimum sample size was calculated after adding 10% as 244 people; in reality, 265 healthy people were enrolled in this study.

- *Sample size for Objective 3:*

Apply the entire sampling method, all strains of enterobacteria that isolated from subjects of target 1 was selected for analyzing their phenotypic molecular biology characteristics of those belong to broad spectrum β-lactam group. In fact, 232 strains resistant to broad spectrum β-lactam antibiotic group were selected.

To analyze the genotypic similarity between the strains resistant to broad spectrum β-lactam antibiotic group isolated from different subjects, the strains that resistant to cephalosprin of third generation group (carrying specific genes) isolated from study samples were selected. Actual numbers of bacterial strains selected for analysis: 54 strains isolated from healthy people; 29 strains isolated from domestic animals; 01 strains isolated from processed food samples and 04 strains isolated from drinking/domestic use water samples.

2.2.2 Sample selection

For the subjects of all 3 Objectives, the household sample selection was applied in the study, in particular:

- Household sample selection: According to the systematic random sampling method.
- Selection of the healthy people living in the household: All selected household members were invited to participate in the study when eligible and if they agreed to participate.
- Selection of other subjective samples: animal samples, processed food samples and drinking/domestic use water samples were collected based on the household sample frame. All above described samples from households that meet selection criteria for the study were selected.

2.2.3 Data analysis and treatment

The survey data on household and subjective members selected for study were checked, cleaned and entered into Epi Data 3.1 software; data analysis was done by Stata 14.0 software. Laboratory data were managed by Microsoft Excell 2010 software, analyzed on Stata 14.0 software and Bionumerics 6.6.11 software (Applied Maths). The biomedical statistics were analysed using statistical threshold $\alpha = 0.05$.

2.2.4 Ethics in research

The rights and interests of subjects participated in the study were guaranteed to comply with ethical regulations, approved by the Ethics Council of the National Institute of Hygiene and Epidemiology. The study design complied with the regulations on research ethics, so there were no intervention effects on the health of the subjects participating in the study. The collected variables were common used variables, not subtle variables that need to be kept in secret. The voluntariness and consent of the subject were ensured.

Chapter 3. RESULTS

3.1 Current situation of healthy people carrying bacteria resistant to broad spectrum β- lactam antibiotics at Thanh Ha commune, Thanh Liem district, Ha Nam province

Table 3.1 Proportion of household members carrying enterobateria resistant to antibiotic per household

Rate of household having member carrying antibiotic resistant enterobacteria	**Enterobacteria strains resistant to BSBL antibiotics**		**Enterobacteria strains resistant to cephalosporin of 3rd generation**	
	Number	Rate (%)	Number	Rate (%)
100% of members	54	67.5%	6	7.5%
From 60% to <100% of members	15	18.7%	11	13.7%
From 30% to <60% of members	10	12.5%	23	28.8%
<30% of members	0	0	14	17.5%
Not any member	1	1.3%	26	32.5%
Total	**80**	**100%**	**80**	**100%**

Intestinal bacteria resistant to broad spectrum β-lactam antibiotics were found in most households (79/80 = 98.8%). Of which, 67.5% of households have all members carrying antibiotic resistant enterobacteria strain; 18.7% of households have from 60% to <100% of members carrying these bacteria. The rate of households showed to have from 30% to less than 60% of members infected with enterobacteria resistant to antibiotics was 12.5%; only 1 household (1.3%) was found to have no member carrying resistant strain. The study results also showed the proportion of households having members carried strains resistant to cephalosporin of third generation of 67.5% (54/80 households); of which those having all members carried bateria resistant to cephalosporin of third generation was accounted for 7.5%. 13.7% of households showed to have from 60% to <100% of their members carried enterobacteria resistant to the third-generation cephalosporin group. The households that have from 30% to <60% of their members carrying bacteria resistant to cephalosporin of 3rd generation was rated 28.8%; 32.5% of the households did not have any member carrying these type of strains in the research community.

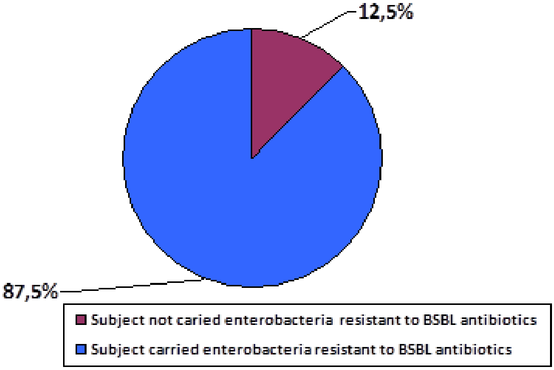

Chart 3.1 Proportion of healthy people carrying enterobacteria resistant to broad-spectrum β-lactam antibiotics in the research community

Out of 265 studied healthy subjects provided fecal samples, 232 people were able to isolate the strains of enterobacteria resistant to broad specrum β-lactam antibiotics (87.5%); only 12.5% of the subjects did not carry any strain resistant to this group of antibiotics.

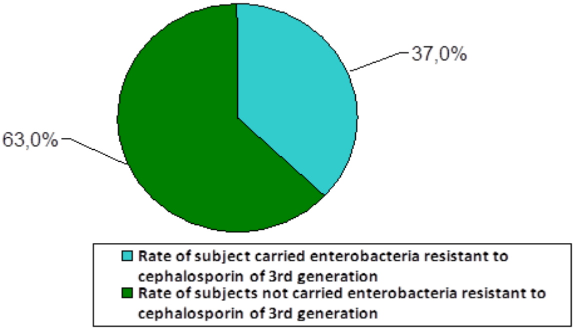

Chart 3.1 Rate of healthy people carrying strains of enterobacteria resistant to cephalosporin of 3rd generation tnresearch community

Out of 265 healthy people enrolled in the study, 98 persons were found with enterobacteria resistant to cephalosprin of 3rd generation, rated 37,0%; those did not isolated with the strains resistant to cephalosprin of 3rd generation was accouted 63%.

Table 3.2 Distribution of healthy people carrying enterobacteria resistant to antibiotics by the gender

Sex	Enterobacteria resistant to BSBL antibiotics		Enterobacteria resistant to cephalosprin of 3rd generation	
	Number	Rate (%)	Number	Rate (%)
Male	107	46.1	43	43.9
Female	125	53.9	55	56.1
Total	**232**	**100**	**98**	**100**

The research results showed 232 healthy people carrying strains of enterobacteria resistant to broad spectrum β-lactam antibiotic group; of which, female subjects rated (53.9%) higher than that of men. Similar results were also found among 98 cases carrying enteribacteria resistant to cephalosporin of third generation with 56.1% of women and 43.9% of men.

Table 3.3 Distribution of healthy people carrying enterobacteria resistant to antibiotics by age groups

Age group (years old)	Enterobacteria resistant to BSBL antibiotics		Enterobacteria resistant to cephalosprin of 3rd generation	
	Number	Rate (%)	Number	Rate (%)
From 1-10	34	14.7	11	11.2
From 11-20	46	19.8	19	19.4
From 21-30	25	10.8	13	13.3
From 31-40	23	9.9	9	9.2
From 41-50	41	17.7	19	19.4
From 51-60	43	18.5	18	18.4
Above 60	20	8.6	9	9.2
Total	**232**	**100**	**98**	**100**

The status of enterobacterial strains resistant to broad spectrum β-lactam antibiotics met in all studied age groups, of which, the age group from 11-20 years old was accounted for the highest rate (19.8%) and the age group over 60 years old was accounted for the lowest rate (8.6%). The same trend was also found in healthy people carried enterobacteria resistant to cephalosporin of third generation. The age group of 11-20 and 41-50 years old showed to have the highest rate (19.4%); followed by the age group of 51-60 years old (18.4%). The age group of over 60 years old and the group from 31-40 years old were accouned for the lowest proportion (9.2%).

Table 3.4 Distribution of healthy subjects carrying antibiotic resistant enterobacteria by the occupation

Occupation	Enterobacteria resistant to BSBL antibiotics		Enterobacteria resistant to cephalosprin of 3rd generation	
	Number	Rate (%)	Number	Rate (%)
Children/students	77	33.2%	26	26.5%

Farmers	108	46.5%	50	51.0%
Workers, Employees.	38	16.4%	20	20.4%
Others	9	3.9%	2	2.0%
Total	**232**	**100%**	**98**	**100%**

Table 3.4 shows that healthy people carrying enterobacteria resistant to broad spectrum β-lactam antibiotics could be detected in all analysed occupational groups, of which, the farmers were accounted for the highest rate (46.5%), followed by children/student group (33.2%). Other occupations (such as trading, freelance ...) were accounted for the lowest rate (3.9%). Among the 98 cases detected to carrying bacterial strains resistant to the third generation cephalosporin group, the farmer group was also accounted for the highest rate (51.0%), followed by children/student group and workers/employees (26.5% and 20.4%, respectively); other occupations showed to have the lowest rate (2.0%) of healthy people carried strains resistant to the third generation cephalosporin.

Table 3.5 Distribution of healthy people carrying enterobacteria resistant to antibiotics by education level

Education level	**Enterobacteria resistant to BSBL antibiotics**		**Enterobacteria resistant to cephalosprin of 3rd generation**	
	Number	Rate (%)	Number	Rate (%)
Secondary school or lower	174	75.0%	77	78.6%
High school or higher education level	58	25.0%	21	21.4%
Total	**232**	**100%**	**98**	**100%**

Amont the study subjects, the people with secondary school education or lower level were accounted for a higher proportion (75%) compared to those with high school education or higher educational level (25%). The same distribution was also seen in 98 cases carrying bacteria resistant to cephalosporin of third generation (78.6% having 21.4%, respectively).

Table 3.6 Antibiotic resistance level of enterobacteria isolated from healthy people in the community (n=232)

Antibiotics	Sensitive (S)		Intermediate (I)		Resistant (R)	
	Number	Rate (%)	Number	Rate (%)	Number	Rate
Ampicilin	0	0,0	0	0,0	232	100,0
Cephalothin	0	0,0	0	0,0	232	100,0
Cefuraxim	1	0,4	2	0,9	229	98,7
Ceftazidim	109	47,0	52	22,4	71	30,6
Ciprofloxacin	70	30,2	3	1,3	159	68,5
Imipenem	228	98,3	3	1,3	1	0,4

100% of healthy people were found resistant completely to ampicillin and cephalothin, followed by the rate resistant to cefuraxime (98.7%), ciprofloxacin (68.5%), ceftazidime (30.65%) and imipenem (0.4%). Rate of ESBL - producing strains sensitive to imipenem was 98.3%, to ceftazidime was 47.0% and to ciprofloxacin was 30.2%.

3.2. Some factors related to status of healthy people carrying enterobacteria resistant to broad spectrum β- lactam antibiotics at thanh Ha commune, Thanh Liem district, Ha Nam province in 2015

Table 3.7. Multivariable analysis of the association between some factors and the status of healthy people carrying enterobacteria resistant to broad spectrum β-lactam antibiotics at the study site

Factors		**Carrying antibiotic resistant enterobacteria**		**Not carrying antibiotic resistant enterobacteria**		**aOR**	**95%CI**
		Number	Rate (%)	Number	Rate (%)		
Education level	Below high school	58	92.1	5	7.9	2.15	(0.15-6.17)
	High school and higher level	174	86.1	28	13.9	1	
Economic status of family	Poor households	5	71.4	2	28.6	5.9	0.73 – 47.7
	Not poor households	227	88.0	31	12.0	1	
Chronic medical history	Yes	20	95.2	1	4.8	7.16	0.57 – 90.38
	No	212	86.9	32	13.1	1	
History of antibiotic use in the past 06 months	Yes	**116**	**95.1**	**6**	**4.9**	4.78*	**1.83 – 12.43**
	No	**116**	**81.1**	**27**	**18.9**	1	
family raising pets	Yes	**212**	**89.1**	**26**	**10.9**	**3.26***	**1.13 – 9.47**
	No	**20**	**74.1**	**7**	**25.9**	1	
Use antibiotic in livestock	**Yes**	**157**	**90.2**	**17**	**9.8**	**2.34***	**1.05 – 5.22**
	No	**75**	**82.4**	**16**	**17.6**	**1**	

Results of multivariable analysis using logistic regression model - stepwise of the factors with $p<0.25$ and factors related to the carriers of enterobacteria that resistant to broad spectrum β - lactam antibiotics obtained by univariate analysis, showed some related factors, such as history of using antibiotics in the past 6 months (aOR = 4.78; 95% CI: 1.83-12.43), household with livestock (aOR = 3.26; 95% CI : 1.13-9.47) and households using antibiotic in livestock (aOR = 2.34; 95% CI: 1.05-5.22), can be able to increase the proportion of healthy people carrying enterobacteria resistant to broad spectrum β-lactam antibiotic group in the community.

Table 3.8 Multivariable analysis of the association between status of healthy people carrying enterobacteria resistant to cephalosporin of 3rd generation and some factors

Factors		Carrying enterobacteria resistant to 3rd generation cephalosporin		Not carrying enterobacteria resistant to 3rd generation cephalosporin		aOR	95%CI
		Number	Rate (%)	Number	Rate (%)		
Occupation	Agriculture	**50**	**41.7**	**70**	**58.3**	**3.95***	**1.18 – 13.17**
	Others	**48**	**33.1**	**97**	**66.9**	**1**	
Gender	Female	43	35.0	80	65.0	0.42	0.12 – 1.40
	Male	55	38.7	87	61.3	1	

Factors that might be related to the status of healthy people carrying enterobacteria resistant to the third generation cephalosporin at the study site obtained by univariate analysis continued to be included in multivariabe analysis (including the factors with p value of <0.25 and the factors related to the status of carrying bacteria resistant to third generation cephalosporin antibiotics in healthy people), using the logistic - stepwise regression model. Results showed that the occupation as agriculture is a related factor that can increase the likelihood of healthy people carrying enterobacteria resistant to third generation cephalosporin antibiotics [aOR = 3.95 (95% CI: 1.18 - 13.17)].

3.3 Some molecular biological characteristics of enterobacteria resistant to broad spectrum β- lactam antibiotics isolated at research site

Table 3.9 Isolation rate of enterobacteria strains resistant to broad spectrum β-lactam antibiotics from healthy human feces samples at the study site

Enterobacteria	**Isolation of enterobacteria resistant to BSBL antibiotics**	
	Number	**Rate (%)**
E. coli	232	100.0
Klebsiella spp.	0	0
Citrobacter spp.	0	0
Enterobacter khác	0	0
Total number of strains	**232**	**100%**

All 232 strains of enterobacteria resistant to broad sectrum β-lactam antibiotics isolated from fecal samples taken from 265 healthy people were *E. coli* (100%).

Data shown in Table 3.10 indicated the isolation rate of enterobacteria resistant to broad spectrum β- lactam antibiotic group isolated from healthy human feces samples (87.5%); samples of animal feces (78.4%); drinking/domestic use water samples (25.3%)

Samples	**Enterobacteria resistant to BSBL antibiotics**		**Enterobacteria resistant to 3rd generation cephalosporin**	
	Number	Rate (%)	Number	Rate (%)
Fecal samples of healthy people (n=265)	232	87.5%	98	37.0%
Fecal samples of domestic animals (n=125)	98	78.4%	57	45.6%
Household's processed food (n=160)	17	10.6%	2	1.3%
Drinking/domestic use water (n=182)	46	25.3%	24	13.2%
Number of households (n=80)	**79**	**98.7%**	**54**	**67.5%**

and from processed foods (10.6%). 79 households showed to have members carrying enterobacteria resistant to broad spectrum β-lactam antibiotic group (98.7%). Accordingly, isolation rate of enterobacteria resistant to cephalosporin group of 3rd generation was 37.0% from healthy people; 45.6% from animal feces samples; 13.2% from waste water/domestic use water and the lowest isolation rate was obtained from processed food samples (1.3%). 54 households showed to have members isolated with enterobacteria resistant to third-generation cephalosporin.

Table 3.10 Identification result of enterobacteria strains isolated from the samples collected at study site

Table 3.11 Rate of isolated enterobacteria strains resistant to broad spectrum β-lactam antibiotics carrying genes encoded TEM, CTX-M, OXA and SHV

Encoded genes	**Human feces**		**Animal feces**		**Proccessed foods**		**Drinking/domestic used water**	
	#	Rate (%)	#	Tỷ lệ (%)	#	Rate (%)	#	Rate (%)
TEM	126	47.6	53	42.4	7	4.4	27	14.8
CTX-M	98	37.0	57	55.6	2	1.3	24	13.2
OXA	18	6.8	3	2.4	2	1.3	3	1.7
SHV	4	1.5	3	2.4	0	0	3	1.7

Healthy people isolated with enterobacteria resistant to broad spectrum β-lactam antibiotics carrying TEM encoding gene accounted for the highest rate (47.6%), followed by those carrying CTX-M gene (37.0%). The strains that carrying gene OXA was accounted for 6.8% and SHV gene accounted for the lowest (1.5%). For animal feces samples, the strains resistant to broad spectrum β-lactam group was found to carry gene encoding CTX-M with the highest rate (55.6%), followed by TEM gene (42.4%), SHV gene (2.4%) and the lowest was OXA gene (2.4%). For the processed food samples, the rate of strains resistant to broad spectrum β-lactam antibiotics carrying TEM-encoded genes was the highest (4.4%), followed by those carrying CTX-M, OXA genes (1.3%); none of strains carrying SHV gene was detected. For drinking/domestic use water samples, rate of isolated bacteria carrying TEM encoding gene was the highest (14.8%), followed by those carrying CTX-M gene (13.2%) and the strains carrying SHV, OXA genes were accounted for the lowest (both 1.7%).

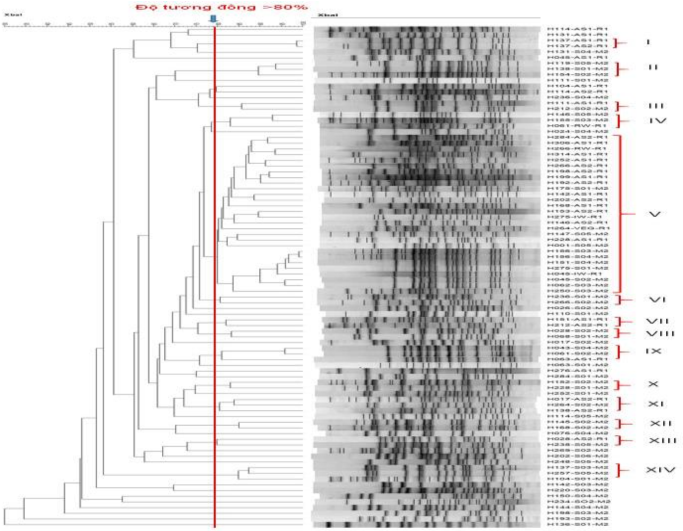

Figure 3.1 Genotyping results of enterobacteria strains resistant to third generation cephalosporin, isolated from samples collected at research sites in 2015

H: Coding households; SO: Code of strains isolated from healthy people; AS: Code of strains isolated from animal feces samples; RW and IW lần are the codes of strains isolated from drinking /domestic use water samples; VEG: Code of strains isolated from proccessed food samples.

Study results showed 80% bacterial strain genotyping similarity was observed in 14 genotypes groups, of which, 12 genotype groups were found among the strains isolated from human feces samples, not included groups I and group VII.

Among animal faecal samples, 7 genotypes were detected, not included groups II, IV, VI, VIII, X, XII and XIV.

All strains isolated from the processed food samples belong to one group (group V) and those isolated from the drinking/domestic use water samples were belong to 02 genotypes (group IV and group V).

The V genotype group composed of 28 strains and distributed in all types of research samples, including 10 strains isolated from healthy human feces, 14 strains from animal feces, 03 strains from drinking/domestic water samples and 01 strain from processed food samples.

Chapter 4. DISCUSSION

4.1. Status of healthy people carrying enterobacteria resistant to broad spectrum β- lactam antibiotics at Thanh Ha commune, Thanh Liem district, Nam Ha province in 2015

The research results showed 87.5% of healthy people at Thanh Ha commune carrying strains of enterobacteria resistant to broad spectrum β-lactam antibiotic group. This rate was much higher than the rates found by some other previous studies in Vietnam. The explaination can be related to the use of the broad spectrum β - lactam antibiotics since many years ago in Vietnam, in particular in Ha Nam province, widely used in humans and in agriculture, creating the antibiotic resistance of bacteria in general and in enterobacteria in particular. On the other hand, it is possible that the strains carrying antibiotic resistance encoding genes that have been transmitted between different objects in the population community, this has also been mentioned by previous studies such as: due to environmental pollution, unsure sanitation conditions, quality of drinking/domestic use water sources, foods,… Results of investigation conducted on healthy people in the community showed that the percentage of subjects resistant to third generation cephalosporin was 37.0%. This rate was higher than data obtained by some previous studies in the world and in Vietnam. The study done by NNIS in 2002 showed the rate of Enterobacter strains resistant to 3rd generation cephalosporin of 34.0%; study counducted by Doan Mai Phuong et al at Bach Mai hospital in 2003 showed that the rate of *K.pneumoniae* strains resistant to cephalosporins with broad spectrum effects such as ceftazidime was 25.0%. Our result was lower than data reported by the Ministry of Health in 2009 on the use of antibiotics and the antibiotic resistance of 15 hospitals in Vietnam from 2008 to 2009. According to this report, the gram-negative bacteria resistant to cephalosporin of 3rd and 4th generations were about 30-40%. The study of Pitt T.L. et al in the UK in 2003 has reported 39% of bacteria resistant to ceftazidime. Ly Ngoc Kinh et al. conducted a research from December 2009 to October 2010 in active treatment units in some medical facilities in Ho Chi Minh City, Hanoi and Hai Phong. Results showed the resistance rate to cephalosporin of 3rd and 4th generation variated from 66% to 83%. Research results of Vo Thi Chi Mai showed that the rate of enterobacteria and E.coli isolated from healthy people in Ho Chi Minh City in 2008 and 2009 resistant to third generation cephalosprin antibiotic group was 82.5%, much higher compared with the results of the our study.

Our study results also showed that people carrying Enterobacter strains resistant to broad spectrum β-lactam antibiotics and those resistant to third generation cephalosporin group met in both men and women and in all age groups, all occupations and of different education levels; however, the proportion of women was higher in our study. This was in accordance with the results obtained by study of Do Duc Phuc et al in 2013 in Ho Chi Minh City on healthy people. In this study, rate of strains resistant to antibiotics was found the highest among subjects ageed from 11-20 years old (19.8%) and the lowest was of age group over 60 years old (8.6%). This might be because the group from 11-20 years old is the age group that has been using many antibiotics, especially, the age group under 10 years old, who often suffers from infection diseases of the respiratory tract, digestive tract,…. During treatment, bacteria have developed resistance mechanisms against antibiotics. For the occupations, the farmer group was accounted for the highest proportion (46.5%); followed by children / student group (33.2%). In general, antibiotic resistance status of bacteria is related to the inappropriate use of antibiotics by people in treatment and in animal husbandry, so occupations are directly related to this type of work such as livestock farmers, especially with the small scale of livestock production by household, sanitary conditions, barns and protective equipment, personal hygiene attitude when handling animals as well as the manner and dosage of used antibiotic. Therefore, the risk of carrying bacteria resistant to antibiotics, especially the broad spectrum β- lactam group, was higher, in which included the enterobacteria resistant to third generation cephalosprin found in people of this occupational group was much higher than that of other groups.

Our study results showed that among 232 strains resistant to the broad spectrum β-lactam antibiotic group, 100% were identified as *E. coli*. Our results is similar to results obtained by the study of Do Duc Phuc et al on the prevalence of ESBL-producing Enterobacteria in the community in Ho Chi Minh City in 2013, that showed 63.3% of isolated strains were *E. coli.* Results obtained by the study of Vo Chi Mai et al in 2010, by the study of Le Kim Ngoc Giao et al 2009 also showed that the isolation rate of *E. coli* bacteria was dominant in the family of Enterobacteriaccae.

Our research results showed that the rate of enterobacteria resistant to broad spectrum β - lactam antibiotic group was very high, especially for some antiobiotics such as ampicillin, cephalothin (100%), cefuraxim (98%), ciprofloxacin (68%), ceftazidime (30.6%) but still low for imipenem (0.4%). These rates were higher than the rates obtained by the study of Vo Chi Mai et al in 2010 at the hospital, in particular, the rate of strains producing ESBL resistant to cephalosprin, gentamicin, ciprofloxacin and Levofloxacin were 82.5%, 22.6%, 65% and 81% respectively. This showed the increasing of antibiotic resistance level to β-lactam group both in hospital and in the community. Ampicillin, cephalothin, Cefuraxim, ceftazidim and ciprofloxacin are the antibiotics that used orally to treat inflammatory diseases of the respiratory, urinary, and gastrointestinal tract and are often recommended for use in the community. People have used it widely for both humans and animals. Therefore, it may increase the resistance among enterobacteria at a high rate. We considered that imipenem belongs to the group of carbamenem, currently used by injection and is often indicated in the hospital, therefor only 0.4% of healthy people carrying enterobacteria strains that resistant to this antibiotic.

4.2. Some factors related to status of healthy people carrying enterobacteria resistant to broad spectrum β-lactam antibiotics at Thanh Ha commune, Thanh Liem district, Nam Ha province

We used univariate and multivariate logictis regression analysis with logistic - stepwise regression model to remove the independent variables when its p value achieved >0.25 to analyze the corelation between some factors and status of healthy people carrying enterobacteria resistant to broad spectrum β-lactam at Thanh ha commune. The analysed factors were gender, age group, occupation, education level, marital status; factors of living conditions such as economic conditions, toilets, water sources used, livestock condition; characteristics of subjects to use antibiotic such as: health status, antibiotic use, type of antibiotic used, usage between the group of people carrying bacteria resistant to broad spectrum β-lactam antibiottics and group do not carry these bacteria; between the group of people carrying enterobacteria resistant to the third generation cephalosporin group and those who did not. The results showed that history of using antibiotics in the past 6 months (aOR = 4.78; 95% CI: 1.83-12.43), household with livestock (aOR = 3.26; 95 % CI: 1.13-

9.47) and households using antibiotics in livestock raising (aOR = 2.34; 95% CI: 1.05-5.22) were the related factors that can be able to increase the rate of healthy people carrying enterobacteria resistant to broad spectrum β- lactam antibiotics in the community. Our research results also showed that the occupational factor as farmer [aOR = 3.95 (95% CI: 1.18 - 13.17)] was a related factor that can increase the likelihood of healthy people carrying bacteria resistant to the 3rd generation cephalosporin group in the research community. This result is similar to the results obtained by Dyar OJ et al among the group of children living in rural areas of Vietnam in 2007 and the study of Ruh et al. conducted in Northern Cyprus in 2019.

4.3. Some molecular biological characteristics of isolated enterobacteria strains resistant to broad spectrum β-lactam antibiotics

Our study results showed that the enterobacteria isolated from healthy people feces samples resistant to broad spectrum β-lactam antibiotics carried TEM encoding gene with the highest rate, and those carried CTX-M, OXA and SHV genes were the lowest. This result is consistent with the results obtained by study of Karen Bush et al. in 2010, indicating the ESBL-producing E.coli strains carrying the TEM-encoding gene accounted for the highest, followed by CTX-M, OXA, and SHV genes.

The rates of enterobacteria resistant to broad spectrum β-lactam antibiotics isolated from animal feces samples, processed food samples, drinking/domestic use water samples that carried TEM and CTX-M encoding genes were high, followed by those carried SHV and OXA genes; it might be due to the transmission of genes encoding antibiotic resistance between the enterobacteria strains or that people here have a habit of using certain antibiotics for a long time.

Currently, the PFGE technique is a molecular biological technique used by many scientists to study and evaluate the transmission of antibiotic-resistant bacteria in hospitals and in the community. This method serves as the golden standard in analyzing genotypic similarity. The technique allows to assess the genotypic similarity between microorganism strains isolated from infected patient’s and animal’s samples,... that difficult to analyse by traditional epidemiological methods. Research results showed that up to 14 different genotypes were formed among the isolated bacteria strains obtained in this study. Each genotype usually included 2 to 3 strains of bacteria. This can be explained by the widespread use of antibiotics in both humans and animals in the communityand this has led to the pressure of natural selection of bacteria, making them resistant to antibiotics. Notably, genotype I group included 02 strains isolated from dog feces and chicken feces in the same household. Genotype group VI included 02 strains isolated from human feces in 02 households of Mau Chu village and genotype XI group included 02 strains isolated from human feces and 01 strain from chicken in the same village Hoa Ngai. In particular, genotype V group included up to 28 strains of enterobacteria with genotypes similar to more than 80% of strains isolated from all type of samples (healthy people in households, feces of dogs, chickens, samples of drinking/domestic use water and processed food samples). These samples were isolated mainly in Duong Xa and Hoa Ngai villages. 01 human and 01 dog feces sample collected at Ung Liem village showed to have the same gennotype V group. Our study results showed that the strains expressed the genotypic similarity of over 80% were isolated from humans, domestic animals, food and water samples taken in the same household, village, geographic area close together. This initially showed the genotype relationship between the strains resistant to third generation cephalosporin, between humans and animals, living environment at the study site. This showed that the spread of 3rd generation cephalosporin-resistant enterobacteria strains in the community.

CONCLUSION

1. Status of healthy people carrying enterobacteria resistant to broad spectrum β- lactam antibiotics at Thanh Ha commune, Thanh Liem district, Nam Ha province in 2015

The rate of healthy people carrying enterobacteria resistant to broad spectrum β - lactam antibiotic group at Thanh Ha commune was very high (87.5%), especially those resistant to 3rd generation cephalosprin antibiotics (37.0%); most of the households (67.5%) in the study have all their members carrying strains resistant to broad spectrum β - lactam antibiotic.

The resistance of bacteria to some common antibiotics of betalactam group was very serious with 100% strains resistant to ampicillin and cephalothin, 98.7% was resistant to cefuraxim. Only imipenem showed highly sensitive to this bacteria (98.3%).

Distribution of healthy people carrying enterobacteria resistant to broad spectrum-lactam antobiotics in the community was not different by gender, age group, and occupation.

2. Some factors related to status of healthy people carrying enterobacteria resistant to broad spectrum β-lactam at Thanh ha commune, Thanh Liem district, Nam Ha province

Subject’s history of using antibiotics in the past 6 months (aOR = 4.78), households with livestock (aOR = 3.26) and use of antibiotics in raising livestock (aOR = 2.34) were the factors that might increase the risk of healthy people carrying enterobacteria resistant to broad-spectrum β-lactam antibiotics. Beside, the farming occupation might also be related to the status of healthy people carrying antibiotic resistant enterobacteria, but only with the third generation cephalosporin group (aOR = 3.95).

No association has been found between status of healthy people carrying broad-spectrum β-lactam resistant enterobacteria and the factors in terms of demographics, living conditions, household economy situation and the households samples such as animal, water, and processed food samples that contaminated with intestinal bacteria resistant to broad spectrum - lactam antibiotics collected at research site.

3. Some molecular biological characteristics of isolated enterobacteria strains resistant to broad spectrum β-lactam antibiotics

All intestinal bacteria strains resistant to broad spectrum β - lactam antibiottics isolated from healthy human in the study community were *E.coli.* These bacteria were found to carry the TEM encoding gene with the highest rate among the 04 genotype groups isolated from human feces simples (47.6%), from drinking / living water samples (14.8%) and from processed food samples (4.4%). Followed by the rate of strains that carrying CTX-M, OXA genes and SHV gene was not detected among intestinal bacteria isolated from processed food samples. For animal feces samples, the proportion of intestinal bacteria resistant to broad spectrum β- lactam antibiotics that carrying CTX-M encoding genes accounted for the highest percentage (55.6%).

14 genotype groups were discovered among enterobacteria resistant to cephalosporin of 3rd generation, of which genotype V was the most common group.

The genotype relationship was detected among enterobacteria strains of genotype V group resistant to the 3rd generation cephalosporin isolated from all types of samples, in particular healthy human feces, animal feces, processed food samples, and samples of drinking/domestic use water collected in the family households and geographic location (Mau Chu village).

RECOMMENDATION

1. Professional and regulatory bodies of the Health, Agriculture sectors and Government at all levels

- Develop a strategy to monitor the use of antibiotics, current situation of antibiotic resistance and prevent antibiotic-resistant bacteria in the community, especially attention need to be paid to the antibiotic resistance of intestinal bacteria and *E. coli* strains that resistant to antibiotic.

-Continue to raise awareness for the community in the prevention of gastrointestinal diseases as well as strictly manage the use of antibiotics in accordance with the issued regulations.

2. Research facilities

Continuing to carry out in-depth studies to investigate the risk factors as well as the transmission of antibiotic-resistant intestinal bacteria in the community.

3. People in the community

- Use of antibiotics for humans and in agriculture in accordance to the instructions of health workers, veterinary staff and follow the instructions of the Ministry of Health, Ministry of Agriculture and Rural Development.
- Practice good personal hygiene, household hygiene, practice food safety and hygiene, use clean water for drinking and domestic use.