LITHUANIAN UNIVERSITY OF HEALTH SCIENCES

VETERINARY ACADEMY

Faculty of Veterinary Medicine

Erik Svensson

CAMPYLOBACTER JEJUNI PADERMIŲ, IŠSKIRTŲ IŠ ĮVAIRIŲ ŠALTINIŲ, ATSPARUMAS ANTIBIOTIKAMS

ANTIMICROBIAL RESISTANCE OF *CAMPYLOBACTER JEJUNI* ISOLATED FROM DIFFERENT SOURCES

MASTER THESIS

Of Integrated Studies of Veterinary Medicine

Supervisor: Professor Mindaugas Malakauskas

KAUNAS 2018

THE WORK WAS DONE AT THE DEPARTMENT OF FOOD SAFETY AND QUALITY

CONFIRMATION OF THE INDEPENDENCE OF DONE WORK

I confirm that the presented Master Thesis "**Antimicrobial resistance of *Campylobacter jejuni* isolated from different sources"**

1. has been done by me;
2. has not been used in any other Lithuanian or foreign university;
3. I have not used any other sources not indicated in the work and I present the complete list of the used literature.

2017-12-11 Erik Svensson

(date) *(author´s name, surname)* *(signature)*

CONFIRMATION ABOUT RESPONSIBILITY FOR CORRECTNESS OF THE LANGUAGE IN THE DONE WORK

I confirm the correctness of the language in the done work.

2017-12-11 Erik Svensson

(date) *(author´s name, surname)* *(signature)*

CONCLUSION OF THE SUPERVISOR REGARDING DEFENCE OF THE MASTER THESIS

2017-12-11 Professor dr. Mindaugas Malakauskas

(date) *(supervisor's name, surname)* *(signature)*

THE MASTER THESIS HAVE BEEN APPROVED IN THE DEPARTMENT/CLINIC

2017-12-14

(date of approbation) *(name, surname of the manager of department/clinic)* *(signature)*

Reviewers of the Master Thesis

1) _____

2) _____

(name, surname) *(signatures)*

Evaluation of defence commission of the Master Thesis:

(date) *(name, surname of the secretary of the defence commission)* *(signature)*

TABLE OF CONTENTS

SUMMARY

*“ **Antimicrobial resistance of Campylobacter jejuni isolated from different sources** ”*
Erik Svensson
Master thesis

The aim of the study was to estimate the antimicrobial resistance of *Campylobacter jejuni* strains isolated from different sources. This master thesis was prepared in the Department of Food Safety and Quality in the Lithuanian University of Health Sciences (LUHS) in Kaunas, Lithuania.

Samples of fresh turkey meat origin were collected from retail shops in Kaunas. The samples were checked for contamination with *Campylobacter* bacteria by plating on selective media subsequently on enrichment media. Incubation was performed in 37° C with microaerophilic gases. Multiplex-PCR was used for the identification of thermophilic *Campylobacter spp*. *Campylobacter jejuni* strains of broiler meat and sick human origin isolated in previous studies were included. These *campylobacter* strains were included for comparison purposes to give a wider perspective of understanding to evaluate the relevance connected to public health. Antimicrobial resistance was tested by the agar dilution method. The four antimicrobial agents tested were: erythromycin, ciprofloxacin, gentamicin and tetracycline. The results were evaluated according EUCAST´s proposed MIC breakpoints.

The study revealed that the contamination with campylobacters of turkey meat from retail shops is very low (1,5%), 2 fresh turkey thighs out of 130 samples tested were contaminated with *Campylobacter jejuni*. The study revealed that both *C. jejuni* strains isolated from turkey meat were resistant to ciprofloxacin and sensitive to gentamicin and erythromycin. However only one strain was sensitive to tetracycline, whereas the second strains was resistant to this antibiotic. Out of 15 *C. jejuni* strains isolated from infected humans 2 were resistant to erythromycin, 3 resistant to gentamicin and all tested strains were resistant to ciprofloxacin and tetracycline. Out of 15 *C. jejuni* strains isolated from broiler meat 2 were resistant to erythromycin, 1 – gentamicin, 13 strains were resistant to tetracycline to all the strains were resistant to ciprofloxacin. In total out of the 32 tested *C. jejuni* strains, we found 31 strains to be sensitive to at least one tested antibiotic. Seven examined strains of *C. jejuni* were multidrug-resistant, as they were resistant to at least 3 tested antibiotics.

Keywords: *Campylobacter jejuni*, turkey meat, broiler meat, campylobacteriosis, antimicrobial resistance

SANTRAUKA

“Campylobacter jejuni padermių, išskirtų iš įvairių šaltinių, atsparumas antibiotikams”
Erik Svensson
Magistro baigiamasis darbas

Šio tyrimo tikslas buvo įvertinti *Campylobacter jejuni* padermių, išskirtų iš įvairių šaltinių, atsparumą antibiotikams. Šio baigiamojo darbo tyrimai atlikti Maisto saugos ir kokybės katedroje, Veterinarijos akademija, Lietuvos sveikatos mokslų universitetas.

Tyrimų metu buvo tirtas *C. jejuni* padermės, išskirtos iš kalakutienos šio tyrimo metu bei padermės, išskirtos iš paukštienos ankstesnių tyrimų metu bei padermės, gautos iš kampilobakterioze užsikrėtusių žmonių. Atliekant tyrimus buvo įvertintas kalakutienos, parduodamos Kauno mažmeninės prekybos įmonėse, užkrėstumas kampilobakterijomis. Kampilobakterijos iš kalakutienos buvo išskiriamos atliekant tiesioginį sėjimą bei sėjimą po pagausinimo selektyviame sultinyje ant selektyvios agarizuotos terpės bei pasėlius inkubuojant mikroaerofilinėmis sąlygomis37 ^{o}C temperatūroje. Išskirtos kampilobakterijos buvo identifikuojamos iki rūšies naudojant dauginės PGR metodą. Kampilobakterijų atsparumas antimikrobinėms medžiagoms buvo įvertintas taikant agaro praskiedimo metodą. Bakterijų atsparumui nustatyti buvo pasirinkti keturi skirtingi antibiotikai: eritromicinas, ciprofloksacinas, tetraciklinas bei gentamicinas. Bakterijų atsparumas įvertintas remiantis EUCAST rekomenduojamomis ribinėmis minimaliomis augimą slopinančiomis koncentracijomis.

Tyrimai parodė, kad kalakutienos, parduodamos Kauno mažmeninės prekybos įmonėse, užkrėstumas kampilobakterijomis yra labai mažas ir tesiekia 1,5%, kadangi tik dvejuose kalakutienos mėginiuose buvo aptiktos *C. jejuni* bakterijos. Šios dvi kampilobakterijų padermės buvo atsparios ciprofloksacinui, tačiau jautrios gentamicinui bei eritromicinui. Tuo tarpu viena padermės buvo atspari tetraciklinui, o kitą – jautri. Iš 15 C. jejuni padermių, išskirtų iš sergančių žmonių, 2 buvo atsparios eritromicinui, 3 – gentamicinui ir visos tirtos atsparios ciprofloksacinui ir tetraciklinui. Iš broilerių mėsos išskirtos kampilobakterijos (viso 15 padermių) taip pat pasižymėjo skirtingu atsparumu: 2 atsparios eritromicinui, 1 – gentamicinui, 13 – tetraciklinui ir visos atsparios ciprofloksacinui. Iš visų tirtų 32 kampilobakterijų padermių 31 pasižymėjo jautrumu bent vienam tirtam antibiotikui, o 7 padermės pasižymėjo dauginiu atsparumu antibiotikams, t.y., buvo atsparios ne mažiau kaip 3 tirtiems antibiotikams.

Raktažodžiai: *Campylobacter jejuni,* kalakutiena, paukštiena, kampilobakteriozė, atsparumas antibiotikams

ABBREVIATIONS

BAB-2	Blood Agar Base no. 2
BHI	Brain Heart Infusion broth
BPW	Buffered Peptone Water
ECOOF	Epidemiological Cut-Off value
EFSA	European Food Safety Authority
EUCAST	European Committee on Antimicrobial Susceptibility Testing
LUHS	Lithuanian University of Health Sciences
mCCDA	modified Campylobacter Charcoal Differential Agar
MIC	Minimal Inhibition Concentration
PBS	Phosphate Buffered Saline
WHO	World Health Organisation

INTRODUCTION

Campylobacteriosis is today the most often reported food borne zoonotic disease in the European Union and worldwide. It is the most frequently reported zoonotic disease, since 2012 the notification rate has an increasing trend. According EFSA's report of zoonotic diseases 2016 based on 27 reporting member states, 246 307 cases of human campylobacteriosis were reported. 19 265 (28.5%) humans were hospitalized and 62 (0.03%) fatal cases were reported. The highest proportion of cases were present in Czech republic, similar to earlier annual reports, 228.2 cases per 100 000 inhabitants followed by Slovakia, 140.5. The least frequent rate were reported by Bulgaria, Cyprus and Latvia. The subspecies *C. jejuni* and *C. coli* are the two most frequently reported species responsible for human campylobacteriosis. Campylobacteriosis in humans are in mild cases asymptomatic, moderate and severe infections give gastrointestinal irritation with symptoms like diarrhoea, fever, abdominal pain and vomiting. [1-3]

The main source of human infections is consumption of broiler meat contaminated with *Campylobacter spp.* and in general poultry products. Human campylobacteriosis is in 20-30% caused by direct contamination through consumption handling and preparation of infected broiler meat. In total 50-80% of human campylobacteriosis are caused by contact with other chicken reservoirs. Other sources of human campylobacteriosis are consumption of beef, pork, turkey meat, raw milk, and water. As well as contact with infected animals, untreated water which is frequently contaminated by birds or farm animals hosting the bacteria [4]

Contamination with *campylobacter spp.* during the slaughtering process is a major issue in the production of broiler and turkey meat. The contaminated flocks brings the bacteria to the environment at the slaughterhouse and contaminates the equipment. Sampling shows that the bacteria survives. Bacterial swab samples from the cloacae of the birds match the bacteria present on the equipment. The control is complicated as contaminated birds usually do not show any clinical signs of sickness. Campylobacteriosis in birds is an asymptomatic disease with the bacteria colonizing in the intestinal tract of what is seen as a healthy bird. Surveillance programs aiming to see which flock is contaminated and not is performed by sampling of bacterial swabs collected from the cloacae. [5, 6]

Most often human campylobacteriosis does not cause clinical symptoms. In case of campylobacteriosis caused diarrhea, treatment with antibiotics is usually not needed. In individuals with an underlying disease, old or very young patients the infection can be prolonged and relapsing.

In such cases antimicrobial treatment is indicated, the treatment of choice is commonly macrolides or fluoroquinolones. To increase the efficiency and minimize the use of antibiotics, antimicrobial resistance testing should be performed. Erythromycin is the most commonly prescribed antibiotic, in case of empirical administration ciprofloxacin is prescribed until an antibiogram is evaluated. In surveillance programs the resistance to ciprofloxacin and tetracycline have been reported to be high in human and animal. The resistance to erythromycin and gentamicin is reported as low in most surveillance programs. [7]

The aim of the study: To estimate the antimicrobial resistance of *Campylobacter jejuni* strains isolated from different sources

Objectives:

1. To analyze research data about the prevalence and antimicrobial resistance of *Campylobacter spp*.
2. To evaluate contamination with Campylobacter *spp.* of turkey meat cuts, including breast filé, cured and light smoked breast filé, thighs and wings, sold at retail in Kaunas city.
3. To identify the species diversity of *Campylobacter* isolated from turkey meat samples.
4. To estimate the antimicrobial resistance of *Campylobacter* isolated from turkey meat samples.
5. To estimate the antimicrobial resistance of *Campylobacter* isolated from poultry meat and humans

1. LITERATURE REVIEW

1.1 Campylobacter and its prevalence

Campylobacter spp. are small spiral or curved shaped rods measuring 0.2–0.8μm × 0.5–5μm. The bacteria is gram negative, oxidize positive and microaerophilic. C*ampylobacter* is the most common food borne disease in the world today. The family of *Campylobacteriaceae* involves over 20 species of bacteria, they cause various infections in humans and animals. *Campylobacter spp*. are growing in temperature in between 37 and 42°C, the optimal temperature for growing is 41.5°C. [4] In Europe the two most commonly identified species isolated from poultry and other foodstuffs are *C. jejuni* and *C. coli*. *Campylobacter* is found in milk and improperly cooked meat, but also in the environment including faeces and water in the environment around the infected animals. [8] In developing countries *Campylobacter* is most commonly spread to humans by drinking contaminated water. In developed countries the most common infection source for human is consumption of contaminated fresh poultry meat. [9]

According the reports of the European Food Safety Authority (EFSA) *Campylobacter* is most prevalent in broilers and egg laying hens. Statistics is collected from these animals within the contributing countries around in Europe. Samples are collected from the cut product or during the slaughter process. Neck skin, cloacae swabs and caecal contents are the sites of sampling. Environmental samples of livestock are taken by faecal samples from in the birds' housing area. Presence in humans is evaluated from stool samples of humans with gastrointestinal symptoms with suspicions of campylobacteriosis. Independently of the samples origin, it is further evaluated in bacteriological laboratories. [1] The prevalence of C*ampylobacter* in broilers differs widely depending on season, geographical location and biosecurity routines in the stalls. The reported rate varies from as low as 3 % up to 90 %. [10]

The presence of *Campylobacter* is seen from day seven in hatching facilities due to contaminated environment. [10] The spread from one flock to the next cycle in the same housing area is aimed to be eliminated by cleaning and disinfection of the stalls and surrounding area. There is a range of certified methods and disinfecting agents around in Europe, the most efficient elimination is seen after treatment with quaternary ammonium compounds combined with glutaraldehyde. For success the application routine is the major influencing factor, the concentration is of lower importance. [11]

The spread of C*ampylobacter* at farm facilities and in between farms is seen as a major problem in the surveillance of the spreading. Often feed mills, breeding facilities, hatching houses and abattoirs are in close relation to each other. The same workers visit different facilities and spreading is a fact in case of insufficient biosecurity routines. The major contamination risk is in time of thinning process and distribution of feed. [10]

The correlation between C*ampylobacter* prevalence within flocks and in general higher prevalence in ready to eat products have been studied by following individual birds from farm to fork. The major source of contamination was traced to cross contamination of flocks with low or *Campylobacter* free flocks from contaminated flocks at the abattoir. Minor contamination was seen from bird to bird. Because of this, the recommended routines at abattoirs is to slaughter non-contaminated flocks in the beginning of the day, before a contaminated flock enter the production line. The main reservoir for spreading is intestinal products during evisceration and defeathering. [10] Irrespective of the routines to slaughter non infected herds in the morning, the contamination is still high in abattoirs equipment. Defeathering machines with its rubber finger is constructed for maximum efficiency and primarily not for its hygienic properties. Evaluation of samples taken before and after cleaning and disinfection according to hygienic standards shows that the equipment is equally contaminated before and after. The presence of organic material allows *Campylobacter* to freely survive and cross contaminate along the slaughter line. [5]

The presence of *Campylobacter* at retail level have been studied by sampling of fresh meat and ready to cook products. An Italian study from Toscana showed that 61.6% from 73 samples of poultry meat were positive with *Campylobacter*. The prevalence found in fresh broiler meat was 70.6 % and in fresh turkey meat 33.3 %. *C. coli* 57.9 % and *C. jejuni* 42.1 % were the two identified subspecies. In 12.8 % of the samples both subspecies were identified. [12] In another study also from Italy and the same region as already described, 209 samples were collected. Samples were taken from fresh poultry meat at retail level over the years 2011 to 2014. In 12 samples out of 209 (5.7%) *Campylobacter* was identified. 11 broiler samples out of 162 total samples were positive (6.8%). The last positive samples was collected from a mixed meat sample. None of the 34 tested turkey samples were positive for *Campylobacter.* The influence of package was noticed, in ready packed samples the prevalence was 10.7 % in unpacked meat 2.7 %. The highest prevalence was seen in thighs of chickens and the lowest in minced meat, the correlation with high prevalence in the thighs were explained by its anatomical location and contamination by contact at evisceration. [8]

The study in Sweden on spread of *Campylobacter* included sampling of broiler and turkey meat at retail level. In total 458 samples were collected for investigation, 61 samples originating from turkey and 397 from broiler. The poultry meat samples were raw without any heat treatment. The presence of *Campylobacter* in broiler was as high as 25.4% (101 positive samples out of 397). The prevalence in turkey meat was much lower with only 1 positive sample out of 61 (1.6%) examined. The contaminated turkey meat sample was positive for *C. jejuni.* The prevalence over time varied in broiler samples from 20% to 44%. [13]

1.2 Campylobacter infections in humans

The main infectious agent for human campylobacteriosis is *C. coli* and *C. jejuni.* The study in Wales and England revealed that *C. jejuni* accounts for 90 % and *C. coli* for the other 10 % of human campylobacteriosis cases. Other infectious *Campylobacter spp.* do exist but are only sporadically present in humans. The infectious dosage can be as a few as 500 cells, though individual factors do affect. Humans are infected with C*ampylobacter* from a wide diversity of sources. *Campylobacter* is present in the surrounding nature from wild and domestic animals. The bacteria are transmitted both by consumption of contaminated food and by environmental contamination. For human campylobacteriosis the main symptoms are enteritis with diarrhoea abdominal pain followed by dehydration and fever. In case of mild infections with campylobacteriosis antibiotics are not indicated. The usage of antibiotic treatment in mild cases decrease the sickness period by average only 1.32 days. The non self limiting cases are mostly correlating with elderly people, young children or immunosuppressed patients. [9] In case of not self limiting campylobacteriosis antibiotics are indicated. The antibiotics used for human campylobacteriosis treatment are macrolides and fluoroquinolones. [14] The antimicrobial resistance is a growing problem all over the world. Resistance against certain antimicrobials are regionally up to 100 %. [9] Though it is seen that the resistance rate is lower in isolates of human origin compared to those of animal origin, this according to a study with collected data from Austria, Germany, Hungary and Slovenia 2006-2007. [15]

1.3 Antibiotic interaction and susceptibility

The use of antibiotics in case of human campylobacteriosis is limited to severe infections. World Health Organization (WHO) recommend to use erythromycin, tetracycline and fluoroquinolones. At the same time bacteriostatic and bacteriocidic treatment is uninterruptedly used along the production of poultry meat, also the use of antibiotics functioning as growth promoter is practiced in many regions. The routines of high usage of antibiotics in the production

line have contributed to worries about antimicrobial resistance and multiple drug resistance have been identified. [16] K. Wieczorek *et al* (2015) made a study over longer time, instead of over a single growing session as with conventionally raised broilers. The study over five years showed how the resistance for the tested antibiotics develops. The antibiotic susceptibility of *C. coli* and *C. jejuni* was checked for ciprofloxacin, erythromycin and tetracycline during the years 2009-2013. *C. jejuni* resistance increased significantly for ciprofloxacin from 59.6 % to 85.9 % and tetracycline from 23.2 % to 70.4 % respectively. The resistance of *C. jejuni* to erythromycin was though relatively constant and low over all the studied years. The resistance for *C. coli* was investigated for the same antibiotics and only small changes was seen over the five years. The study concluded that the treatment strategy of campylobacteriosis in humans need to be aware and consider the outcome of the increasing resistance to antibiotics in *C. jejuni*. [17] The use of enrofloxacin for treatment of zoonotic diseases is strictly regulated in some regions of the world. In the USA its completely forbidden and in UK it is restricted, only allowed to use in situations when there is no other possible option for treatment. [18]

The usage of bacteriocidic drugs in poultry is well known, the usage of fluoroquinolones such as ciprofloxacin is widely used even though restrictions and limiting rules of antibiotic policy have started to apply in some regions. It have also directly affected the development of antimicrobial resistance to fluoroquinolones as ciprofloxacin and enrofloxacin. [14]

1.3 Characterization of antimicrobial resistance

The Minimal Inhibition Concentration (MIC) is established by research groups around the world according standardized methods. In Europe six national groups are working with the subject united by the European committee on Antimicrobial Susceptibility Testing (EUCAST). The MIC is used to decide whether the tested isolate is susceptible, intermediate or resistant to a certain antibiotic. A breakpoint value is established to describe resistance for the relation in between each bacteria and antibiotic agent. By EUCAST the definitions are: Clinically resistant: the bacteria is resistant to a higher dosage of antibiotics than clinically used, treatment with the tested antibiotics is expected to be non successful. Clinically susceptible: the bacteria is susceptible to the clinical dosage of antibiotics used, treatment with the tested antibiotics is expected to be successful. Clinically intermediate: the bacteria is on the limit to be resistant or susceptible to the clinical dosage of antibiotics, treatment outcome depends on other circumstances. The outcome of the treatment depends on the practitioner’s experience of evaluating the circumstances, the dosage,

administration route and time of treatment influence the success rate. The MIC of an antibiotic drug is determined by the spread of susceptibilities, pharmacodynamics of the drug, pharmacological characteristics and the clinical outcome. [19]

There are multiple methods to decide whether a bacteria is resistant or susceptible to a certain antibiotic: disk diffusion, broth micro dilution and agar dilution are some of today's methods for determination. The agar dilution method is standardized in USA and Europe. It is by EUCAST standardized for 13 antibiotics including ciprofloxacin, erythromycin. Mueller-Hinton agar diluted with 5% defibrinated sheep blood is used to evaluate antimicrobial resistance in combination of mixing with an antimicrobial solution in two fold series. The agar is prepared with a standardized solution of the bacteria and incubated in 36° C for 48 h alternatively 42° C for 24 h with microaerophilic gases. The evaluation of growing bacteria decide whether the bacteria is resistant or susceptible to the tested antibiotic agent, using MIC as the breakpoint. [7] The epidemiological Cut-Off value (ECOFF) are used to get guidelines for the clinical outcome. ECOFF is an outcome of the compilation of three factors: the tested isolate MIC, the drug's pharmacodynamic and pharmacokinetic properties and last, the successful rate measured in clinical trials. [20]

1.4 Antimicrobial resistance

As the prevalence of *Campylobacter* continue being a problem as a zoonotic disease and the use of antibiotics in poultry production continue development of the antimicrobial resistance this issue will only increase as a problem in the future. The reason is the wide usage of fluoroquinolones and macrolides in poultry production. [14] The usage of antimicrobial drugs are often administrated as feed additives therefore reaching all birds not only the ones that are sick. The routine is especially a problem in the production of meat where the birds are in big groups. The increased consumption of poultry meat and high prevalence of *Campylobacter* in this meat contributes to public health issues. [15] The usage of fluoroquinolones is widely used worldwide in the veterinary sector especially in poultry. Hence the antimicrobial resistance increases for *Campylobacter* independent whether the bacteria originates from the alive bird or the sick human. The main used fluoroquinolones when treating campylobacteriosis are enrofloxacin and ciprofloxacin. [14] Today it is well known that the use of antibiotics in livestock animals raised for human consumption and aqua agriculture progress into resistance in humans either directly or indirectly. The bacteria susceptible to the administrated antibiotic dies at livestock level, the remaining alive ones are the surviving resistant bacteria. The bacteria that survived multiplies without competition, disseminating as the only dominating bacteria. [21]

Primarily macrolides are used as the 1st choice of antibiotics. As second line antibiotics, fluoroquinolones are regionally efficiently used as they have the ability to gain a high tissue concentration. Isolates of *C.coli* and *C. jejuni* resistant to these two antibiotics are spread by consumption of contaminated food and environmental contamination. [22]. The prevalence of resistant macrolides and fluoroquinolones is well known in the neighbouring country of where this study took place. In Poland a study was performed just recently and presented results in 2017, where the situation of high prevalence of macrolid and fluoroquinolone resistant *C. jejuni* and *C. coli* is confirmed by both PCR and matrix assisted laser desorption-ionization time-of-flight. This study was performed on strains of broiler and turkey origin. [23] Besides macrolides and fluoroquinolones researches *in vitro* conclude that gentamicin could be a competent antibiotic treating campylobacteriosis. [24]

The study presented by Patrick F. McDermott *et al* (2001) showed that the resistance to the use of fluoroquinolones is quickly developing when antibiotics are used uninterruptedly. Collected chicken isolates were tested for ciprofloxacin and sarafloxacin *in vivo* on newly hatched chicks. After 5 days of treatment the resistance was seen to be persistent and developing. The 1st inoculated samples all had a MIC of 0.25 mg/L. At the end of the *in vivo* study the MIC was as high as 32 mg/L. This study confirms, that the use of fluoroquinolone resistance in *C. jejuni* of chicken origin persist for extended time. [25] In *Campylobacter* there are two well known concepts described, how the bacteria gain resistance against fluoroquinolones. The inactivation of the target for the antimicrobial drug and the efflux pump, the two characteristics interact synergistically together. The antimicrobial resistance is developed by evolutional mutations in the bacteria as antibiotics have been administrated in unrestricted manners over time. The expected complex formation when fluoroquinolones are active decreases the DNA replication and transcription which leads to cell death. The resistance happens due to enzymatic changes in the intracellular structure of the bacteria. Subunits of the bacteria that fluoroquinolones normally form a complex with when acting properly are encoded. The expected DNA replication and transcription mechanism of the bacterial cell cannot occur. [14]

The resistance to macrolides in C*ampylobacter* is of great importance since it is the drug group of choice when treating severe campylobacteriosis in humans. Macrolides used and studied are erythromycin and azithromycin in the investigation from H. Bolinger *et al* (2017). The resistance is not as widespread as against fluoroquinolones for *C. jejuni*, the resistance have though been seen higher when treating infections from *C. coli.* [14, 26] According an American study the

resistance have maintained low since the beginning of the testing, since 1997 and 2001 correspondingly to isolates of human and chicken origin. The American research have applied MIC to ≥ 8 mg/L for erythromycin claiming that the resistance have persisted below 4% since the start. In isolates originating from human campylobacteriosis in Spain, using MIC ≥32 mg/L for erythromycin, the resistance was seen to be on equally low levels as the American results. *C. coli* isolates from chickens and turkeys collected in slaughter houses, were resistant to macrolides in 11% and 6.7%, respectively. In other investigations with isolates originating from pigs the macrolid resistance have been up to 22%. As long as the resistance remains in levels below 4% in chickens and humans it is concluded to not be a problem to public health. The results of increased resistance of *Campylobacter spp*. to macrolides originating from other sources is though a warning signal indicating that the usages of antimicrobial drug have to remain restricted. [26]

The resistance against erythromycin for *C. jejuni* is dependent on the ribosome's ability to evade at three base pairs by mutations. The base pairs mutation is mainly focused to A2075G, A2074G and A2074C. The mutation in one of the three base pairs above mentioned, leads to erythromycin resistance in *C. jejuni* strains and will have a lower ability to plant. Similar fitness cost of the bacteria is not seen when observing the resistance of *C. coli* against erythromycin. This explains why erythromycin resistance in *C. coli* is more prevalent, than in *C. jejuni*. Anyhow the detailed description of this cellular mechanism is outside the frames of this work, therefore accordingly not further explained. [26]

C. jejuni resistance to tetracycline is not of as great importance in campylobacteriosis in today's medicine as erythromycin and ciprofloxacin since it is not as widely used for treatment. M. Carev *et al (2017)* states that most isolates resistant to tetracycline are coresistant to ciprofloxacin. The research was based on human originating isolates, 24 % were resistant to tetracycline and from those 89 % was coresistant to ciprofloxacin. [27]

The prevalence of C*ampylobacter spp*. was checked in an American study with the aim to check the usability of gentamicin. Samples of human and retail level meat origin were collected. The meat samples were form chicken, turkey, pork and beef. Resistance against gentamicin of obtained isolates were found in 151 samples, 79 of human origin and 72 of retail chicken. Coresistance was seen in a high percentage in the isolates of human origin. 98.7% of the isolates resistant to gentamicin were coresistant to tetracycline and 58.2% were coresistant to ciprofloxacin. Retail chicken originating isolates resistant to gentamicin were 98.6% coresistant to tetracycline and only 1.4% were coresistant to ciprofloxacin. [24] In a Polish investigation, 1335 isolates of

Campylobacter were collected and examined. Out of these 1335 isolates 20 were resistant to erythromycin. Coresistance to erythromycin was seen in 19 of the 20 isolates. Coresistance to tetracycline was seen in 17 out of the 20 isolates. Further coresistance to gentamicin was not identified in any of the ciprofloxacin resistant isolates. [28]

There is a shortage in knowledge of how to handle multiresistant infections. Multiresistant *Campylobacter* are present in the environment due to the wide usage of antibiotics used and the selection of surviving bacteria it results into. In a Finish investigation the alternative drugs for multiresistant *Campylobacter* were evaluated. The investigation focused on macrolid resistant bacteria. The prevalence of *Campylobacter* resistant to macrolides was seen to be relatively low, complemented by the problem to be multiresistant. The Finish investigation showed that a high percentage of multiresistant isolates were susceptible to two antibiotics. The susceptible antibiotics were carbapenem and tigecycline. As the investigation was performed *in vitro*, it is suggested to perform clinical trials to evaluate the two antibiotics usefulness when treating multiresistant bacterial infections. [29]

2. METHODOLOGY

2.1 Study design

The study was designed to evaluate the antimicrobial resistance of *C. jejuni* strains isolated from different sources (Fig. 2.1.). In addition to the *C. jejuni* strains available at the culture collection of the department of Food Safety and Quality, we have sampled fresh turkey meat at the retail shops to evaluate contamination with campylobacters.

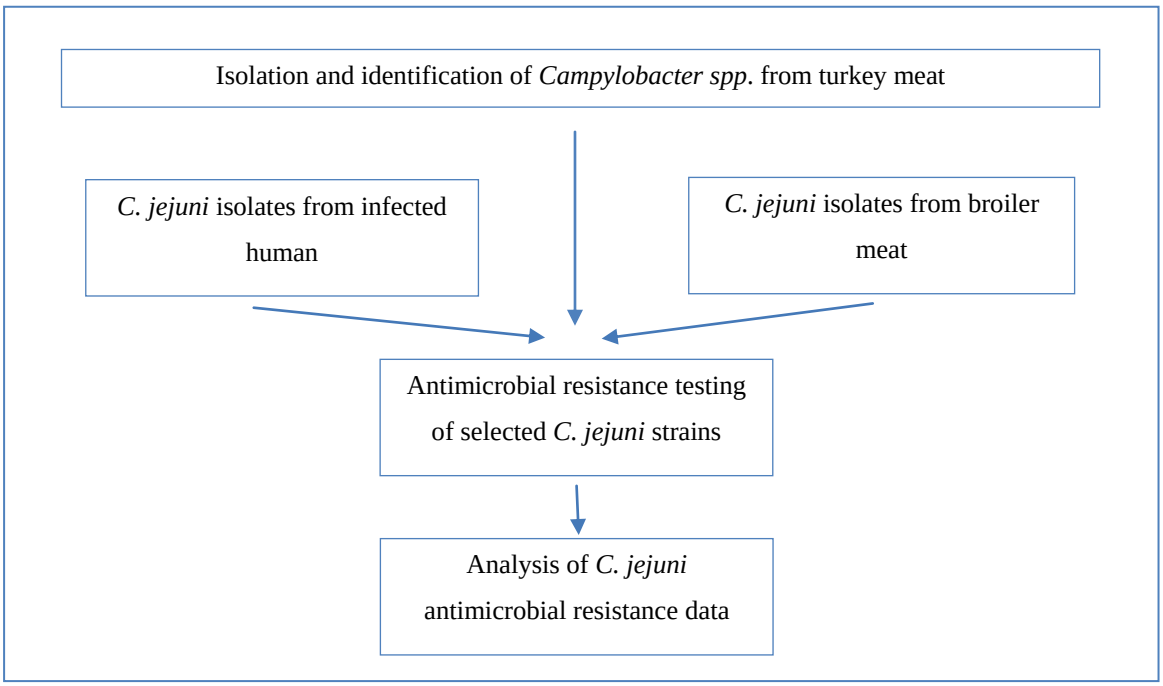

Fig. 2.1. *The design of the study*

2.2 Location and sampling

The samples for this study were collected at retail level stores at the shopping area URMAS and "X" private company store, located in "Viljampole market" in Kaunas. The sampling was carried out within a period of six months. The examination of collected samples was performed at the laboratory of the department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania.

The sampling of fresh turkey meat was carried out in average two times per month from April to May and September to December 2016. Purchased fresh turkey meat samples were placed in separate bags offered by the seller. Depending on the availability of fresh turkey meat, the meat samples have varied in size from 162 to 343 g. Samples were always collected the same day and

within 4 h delivered to the laboratory for further examination. Samples were purchased from retail shops the same day as a new delivery was given.

2.3 Isolation of *Campylobacter spp*. from turkey thigh meat samples

The study was performed according the ISO standard "Horizontal method for detection and enumeration of *Campylobacter spp*.", 10272-1: 2006. During the laboratory examination media and chemicals used were purchased from Thermo Scientific, Oxoid Microbiology Products. Agars and other media were prepared and sterilised according manufacture instructions.

After delivery to the department laboratory, the fresh turkey thigh meat samples were transferred to separate sterile plastic bags by adjusting weight to be approximately 100 g. Each meat sample was mixed with 100 ml of sterile prepared Buffered Peptone Water (BPW) to prepare an initial dilution. The content of the each bag was throughout properly mixed by for 30 seconds.

Using micropipettes ten folded dilutions were made with the BPW as diluent. The dilutions were prepared in 1.0 ml plastic tubes. The dilutions were properly mixed by vortex mixer for 30 seconds, followed by planting on separate sterile prepared modified Campylobacter Charcoal Differential Agar (mCCDA). The ten µl sample was thoroughly spread over the mCCDA using glass bubbles. The mCCDA plates were incubated at microaerophilic atmosphere gases for 48 h in 37° C. A 1 ml sample from each plastic bag was collected in a 15 ml glass tube for enrichment with 9 ml modified Bolton broth selective supplement. The test tubes were incubated in an enclosed chamber with a CampyGen sachet for 24 h in 42° C.

Following incubation, the enrichment broth was mixed by vortex for 30 seconds and a 10 µl plastic loop was used for planting on mCCDA plates. The subsequent routines for enriched samples were the same as for non enriched samples. Inoculated mCCDA plates were visually evaluated after incubation for presence of presumable *Campylobacter* colonies. Suspected C*ampylobacter* colonies were collected using a one µl plastic loop and cultured on sterile prepared Blood Agar Base No. 2 (BAB-2) plates. The BAB-2 plates were incubated under microaerophilic atmosphere for 48 h in 37° C.

After incubation on BAB-2 plates, evaluation of bacteria colonies was done visually in combination with microscopic examination. A one µl plastic loop was used to collect a colony mixed with ten µl of sterile water prepared on a microscope slide under a cover slip. Microscopic examination was performed with 1000x magnification and immersion oil. Motile and curve shaped

rods were identified as presumptive *Campylobacter*. Each positively evaluated colony was separately replated on BAB-2 plates to get non contaminated pure samples.

From pure colony plates bacteria were collected using a 10 µl plastic loop, distributed into 1.5 ml plastic tubes mixed with 1.0 ml sterile prepared Brain Heart infusion broth (BHI) with glycerol functioning as a cryoprotectant to the isolate. Mixed with Vortex and put into deep freezer for storage at -80° C till the further examination. Subsequently plastic loops of 1 µl was used for collection of colonies for extraction of DNA using PrepMan^{TM} Ultra. The bacterial culture was mixed by vortex for 30 seconds. Then dissolved and lysed at 100° C for 10 minutes. The samples were centrifuged at 16000xg for 3 minutes. The supernatant was collected and transferred to plastic tubes, stored in freezer at -20° C.

2.4 Multiplex PCR for *Campylobacter* species identification

Multiplex-PCR was used for the identification of thermophilic *Campylobacter spp*. The method was performed according the multiplex PCR assay represented by Wang *et al*. (2002) with minor adaption. *Campylobacter spp*. 23S rRNA *C. jejuni* (*hip*O) and *C. coli* (*glyA*) were the used primers for identification of present strains in this study. The total solution for PCR was 25µl divided into the 1 µl DNA and the prepared solution of agents. The 24 µl solution was composed of 15.75 µl of bidistilled water, 2.0 µl of a 2 mM deoxynucleoside triphosphate mixture, 2.5 µl of 10X reaction buffer, 2.5 µl of 25 mM $MgCl_2$, 0.25 µl of HotStart *Taq* DNA polymerase (Thermo scientific), 1 µl of a 100 µM primer mixture containing 0.5 µM of each 23S rRNA and *glyA* primers and 1 µM of *hipO* primer. A thermocycler was used for the amplification of DNA of the studied samples. The amplification started with initial denaturation for 6 min in 95°C, followed by amplification for 0.5 min in 95° C, repeated sequentially 30 times. Annealing was done for 0.5 min in 53°C followed by extension for 0.5 min in 72°C. The thermocycler finalized the DNA amplification by extension for 7 min in 72°C. After the DNA amplification the samples were moved into 1.3% TopVisio LM GQ agarose (Thermo Scientific, Waltham, USA) gel supplemented by 0.05 µl/ml of ethidium bromide solution and analyzed by gel electrophoresis. For justification of the processed samples, they were displayed on an UV board. For molecular size marker GeneRuler 100 bp DNA Ladder (Thermo Scientific) was used. [30]

2.5 Pre isolated samples

In this study we used isolates obtained from broiler meat and infected humans isolated in previous studies. These campylobacter strains were included for comparison purposes to give a wider perspective of understanding to evaluate the relevance connected to public health. The isolates of broiler meat origin were isolated according the same procedure as described previously for the isolates of turkey meat origin. Inoculation was done on BAB 2 of both broiler and human isolates from stored in BHI with glycerol at -80° C. Followed by the antimicrobial resistance investigation which was performed in the period of March to April 2017. The origin, date isolated and product can be seen below (Table 2.5.1). Samples were chosen with the criteria to be *C. jejuni* equally distributed over the sampling period to correspond the performed sampling of turkey meat. As the research work focused on broiler meat was performed a few months earlier there is a shift in periods. A total 15 isolates of broiler origin were chosen for further investigation. The infected children isolates used in this study were obtained from the Microbiological Laboratory of Kaunas Clinical Hospital during January to September 2016. The specific distribution of sampling is presented in (Table 2.5.2.). The criteria for selection of isolates were humans infected by *C. jejuni*. In total 15 isolates of human origin were chosen for further antimicrobial resistance investigation.

Table 2.5.1. *Broiler isolates included in this study*

No.:	Date isolated:	Product:
3	2016-01-25	Wing
4	2016-02-08	Wing
5	2016-02-08	Wing
6	2016-02-08	Drumstick
7	2016-02-22	Wing
8	2016-02-22	Wing
9	2016-02-22	Wing
10	2016-02-22	Drumstick
11	2016-05-17	Drumstick
12	2016-05-17	Wing
13	2016-05-17	Wing
14	2016-05-24	Wing
15	2016-06-08	Wing
16	2016-09-05	Drumstick
17	2016-09-05	Wing

Table 2.5.2. *Human isolates included in this study*

No.:	Date isolated:
18	2016-01-07
19	2016-01-13
20	2016-01-29
21	2016-02-03
22	2016-02-03
23	2016-02-26
24	2016-02-26
25	2016-05-12
26	2016-05-30
27	2016-06-01
28	2016-06-02
29	2016-06-06
30	2016-06-13
31	2016-06-13
32	2016-09-19

2.6 Evaluation of antimicrobial resistance by agar dilution method

Antimicrobial resistance was tested by the agar dilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2006a). The following four antimicrobial agents were tested: erythromycin (ERY), ciprofloxacin (CIP), gentamicin (GEN) and tetracycline (TET), (all Sigma-Aldrich, MO, USA). In total, 32 *C.jejuni*, 2 isolated from turkey meat at retail level, 15 isolated from broiler meat samples at retail level and 15 isolated from clinically ill humans. The isolates were examined on Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, UK). The range of prepared and tested dilutions varied depending on the antibiotic agent. The dilutions were prepared independently for each antibiotic: erythromycin from 0.25 to 8 mg/L, ciprofloxacin from 0.25 to 128 mg/L, gentamicin from 0.25 to 4 mg/L and tetracycline from 0.25 to 256 mg/L. The samples were adjusted to equal concentration by diluting into a set and controlled optical density, OD_{600}=0.1 and for each isolate and 5 µl of approximately 1 x 10^7 CFU/ml bacterial suspension dissolved in PBS (Phosphate-buffered saline, Oxoid, Basingstoke, Hampshire, UK) The prepared solution was plated to antimicrobial agent-containing Mueller-Hinton agar in the prepared dilution stated above. The plated samples were incubated for 24 h in 37ºC. The experiment for all isolates was performed until two identical results were obtained, two subsequently alternatively a third incubation confirming one of the two first incubations. [31]The result after incubation was evaluated by determining MIC, defined as the lowest concentration of

antibiotic agent needed for complete inhibition of growth of *C. jejuni* strains. The breakpoints for MIC of the antimicrobial agents used were those set by EUCAST according there web based database. [32]

2.7 Statistical analysis

The statistical processing of data was done using the program Microsoft office excel 2007 by Windows. The significance of results was evaluated by Fisher's-exact test for validation of the obtained results originating from the different groups. Fisher's exact test was chosen as it is the only exact way to reach a correct p-value. To evaluate whether the value is statistically significant or not, a p-value was calculated and applied: P-values < 0.05 are statistically significant, p-values >0.05 are statistically insignificant. The adjusted Wald method was used to determine the representability of the obtained answer. The confidence interval was set to 95 % for the evaluation.

3. RESULTS

3.1 Contamination of turkey meat with *Campylobacter spp*.

The study revealed that contamination of turkey meat samples purchased at the retail level shops is very low. We were able to isolate only bacteria *Campylobacter jejuni* species and only from 2 fresh turkey thighs out of 130 examined turkey meat samples purchased at the Urmas market (other tested samples breast file (n=12), cured and light smoked breast file (n=18) and fresh wings (n=49). Any of tested samples were contaminated neither with *C. coli* nor other *Campylobacter spp. bacteria.* The two contaminated samples were collected during one visit to Urmas market and purchased from the same fresh thighs batch.

3.2 Antimicrobial resistance of *C. jejuni* strains

3.2.1. Antimicrobial resistance of *C. jejuni* isolated from fresh turkey thighs

The antimicrobial resistance of two *C. jejuni* strains isolated from fresh turkey thighs was similar (Fig. 3.2.1.). Both *C. jejuni* strains are susceptible to erythromycin and gentamicin and resistant to ciprofloxacin, respectively. However one strain was susceptible and one resistant to tetracycline.

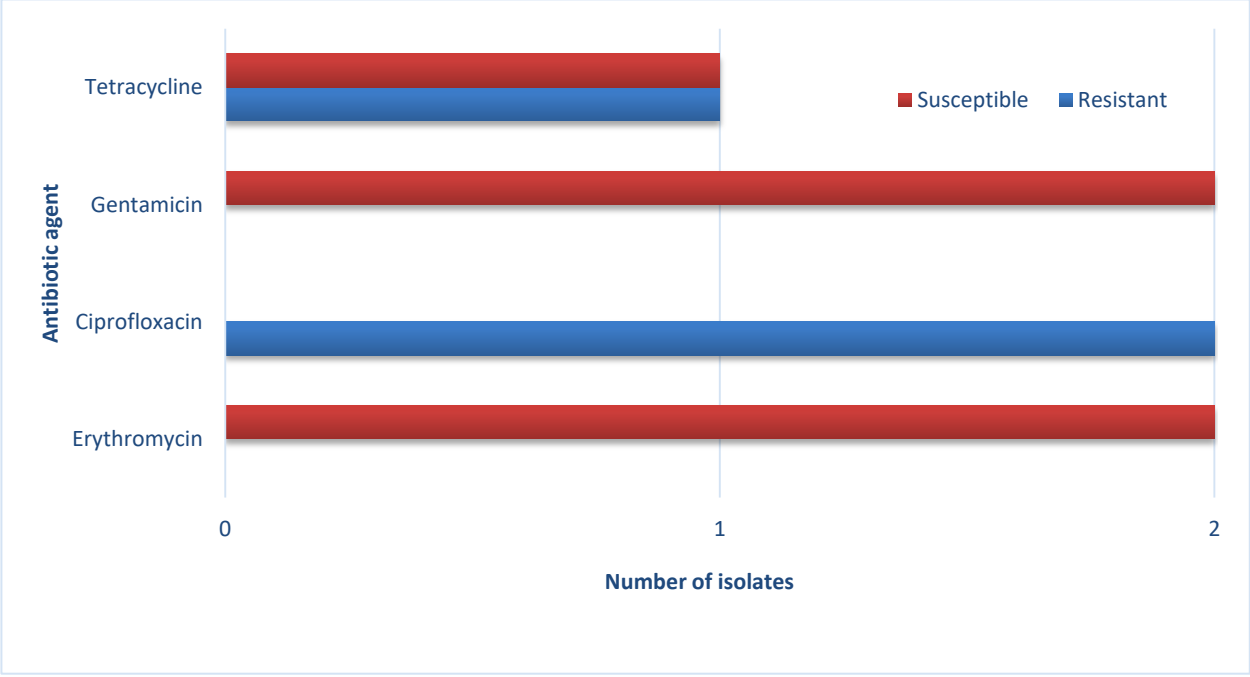

Fig. 3.2.1.1. *Antimicrobial resistance of C. jejuni strains isolated from turkey meat*

The study revealed that minimum inhibitory concentrations (MIC) were similar for both *C. jejuni* strains except for tetracycline (Fig.3.2.1.2.). The growth of both campylobacter strains was inhibited by 32 mg/L of ciprofloxacin. The same MIC of tetracycline inhibited growth of one resistant strain. Even both strains were sensitive to erythromycin, one strain was inhibited by twice lower MIC of this antibiotic. Low concentrations of gentamicin (0.25 mg/L) were effective to prevent growth of tested *C. jejuni* strains.

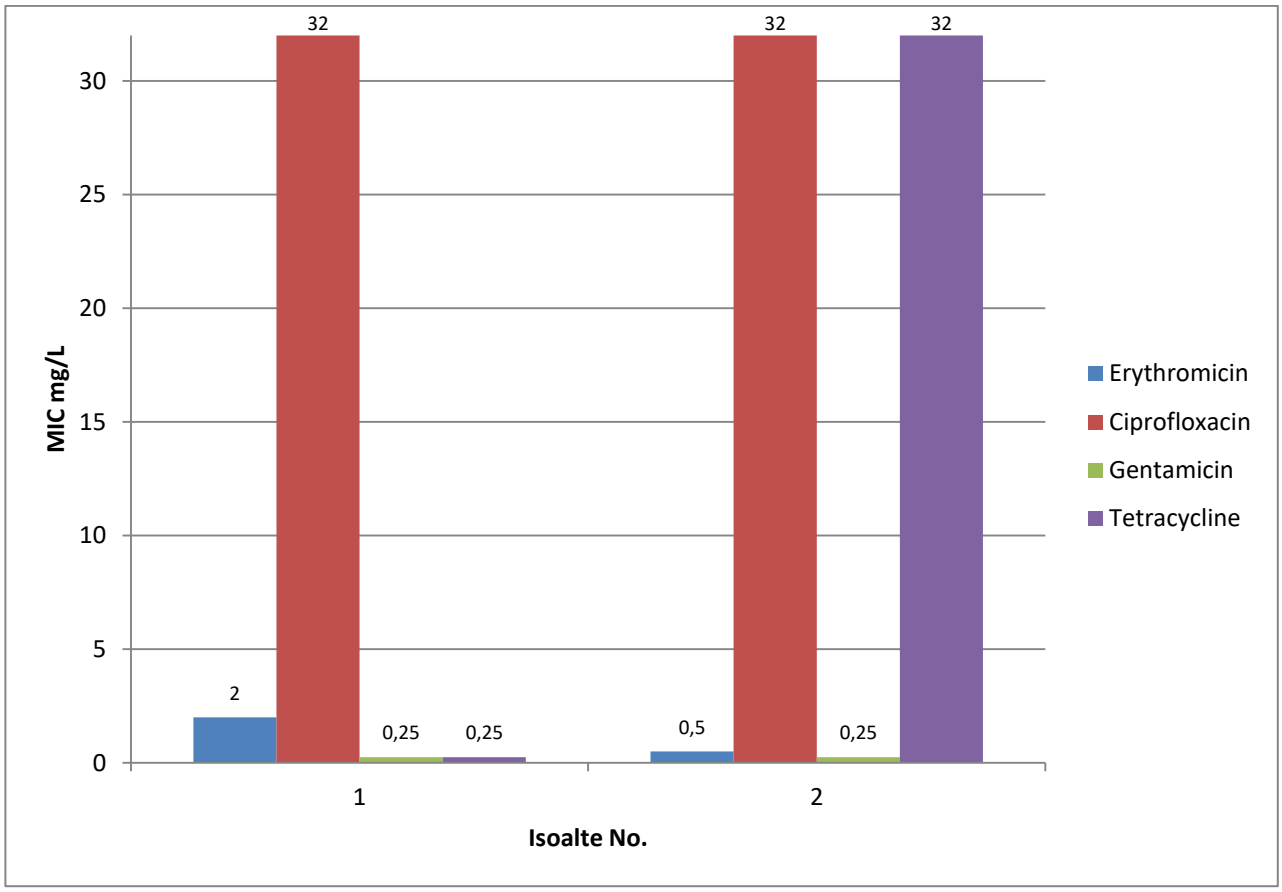

Fig. 3.2.1.2 *Antimicrobial resistance of C. jejuni strains isolated from fresh turkey thighs*

3.2.2 Antimicrobial resistance of C. jejuni isolates of broiler meat

The strains of broiler meat origin showed much higher diversity of phenotypic resistance to tested antibiotics compared to those of turkey meat origin (Fig. 3.2.2). The complete growth inhibition of 10 of 15 tested *C. jejuni* strains on Mueller-Hinton agar was achieved by higher tested concentration of tetracycline, ≥16 mg/L. Only one strain No.7 was susceptible for the lowest tested concentration of tetracycline (0.25 mg/L). The strain No.16 showed the highest resistance to tetracycline from among all tested strains with MIC of 128 mg/L. The strain No.17 showed the

highest resistance to gentamicin among all tested strains with MIC of 4 mg/L, whereas all other strains were susceptible to as low as 0.25 - 1 mg/L MIC of gentamicin. Strain No.16 was also the strain that showed the highest resistance to ciprofloxacin from among all tested strains with MIC of 128 mg/L. Strain No.15 showed the lowest resistance to ciprofloxacin from all tested strains with MIC of 2 mg/L. The complete growth inhibition was achieved for 8 of 15 *C. jejuni* strains when tested on the lowest concentration of erythromycin (0.25mg/L). Strain No.9 and No.10 were the most resistant *C. jejuni* strains to erythromycin, 8 mg/L of the antibiotic was required to achieve complete growth inhibition.

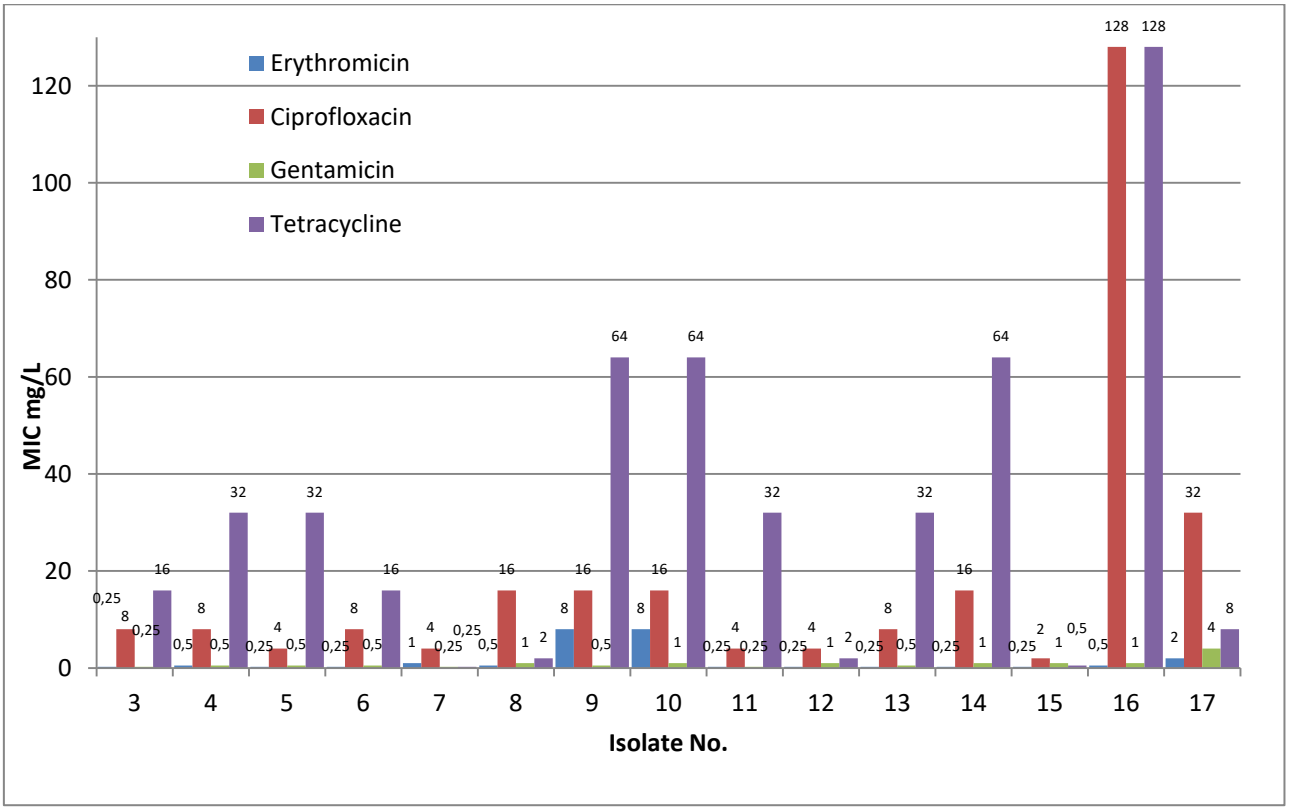

Fig. 3.2.2. *Antimicrobial resistance of C. jejuni strains isolated from broiler meat*

3.2.3 Antimicrobial resistance of *C. jejuni* isolates of human origin

The strains of C. jejuni isolated from infected humans showed high diversity of phenotypic resistance to tested antibiotics (Fig.3.2.3.). The complete growth inhibition of 10 out of 15 tested *C. jejuni* strains on Mueller-Hinton agar was achieved only by higher tested concentration of tetracycline, ≥32 mg/L, No. 25 required 256 ml/L of the antibiotic to achieve complete inhibition of growth. Strain No. 23 was the one that required the lowest concentration of the antibiotic to achieve complete inhibition of growth, 2 ml/L was required. For gentamicin 4 ml/L was the highest

concentration needed for complete inhibition of growth, strain No 21, 29 and 32. 5 out of the 15 strains were susceptible to the lowest concentration of gentamicin tested (0.25 ml/L). To achieve complete inhibition of growth with ciprofloxacin a concentration of 32 ml/L and 16 ml/L was required for 7 strains each. The 15th strain, No. 22 required 8 mg/L of ciprofloxacin to achieve total inhibition of growth. Four strains were susceptible to the lowest tested concentration of erythromycin, 0.25 mg/L. Strain No. 27 and 32 required the highest concentration of erythromycin to achieve complete inhibition of growth.

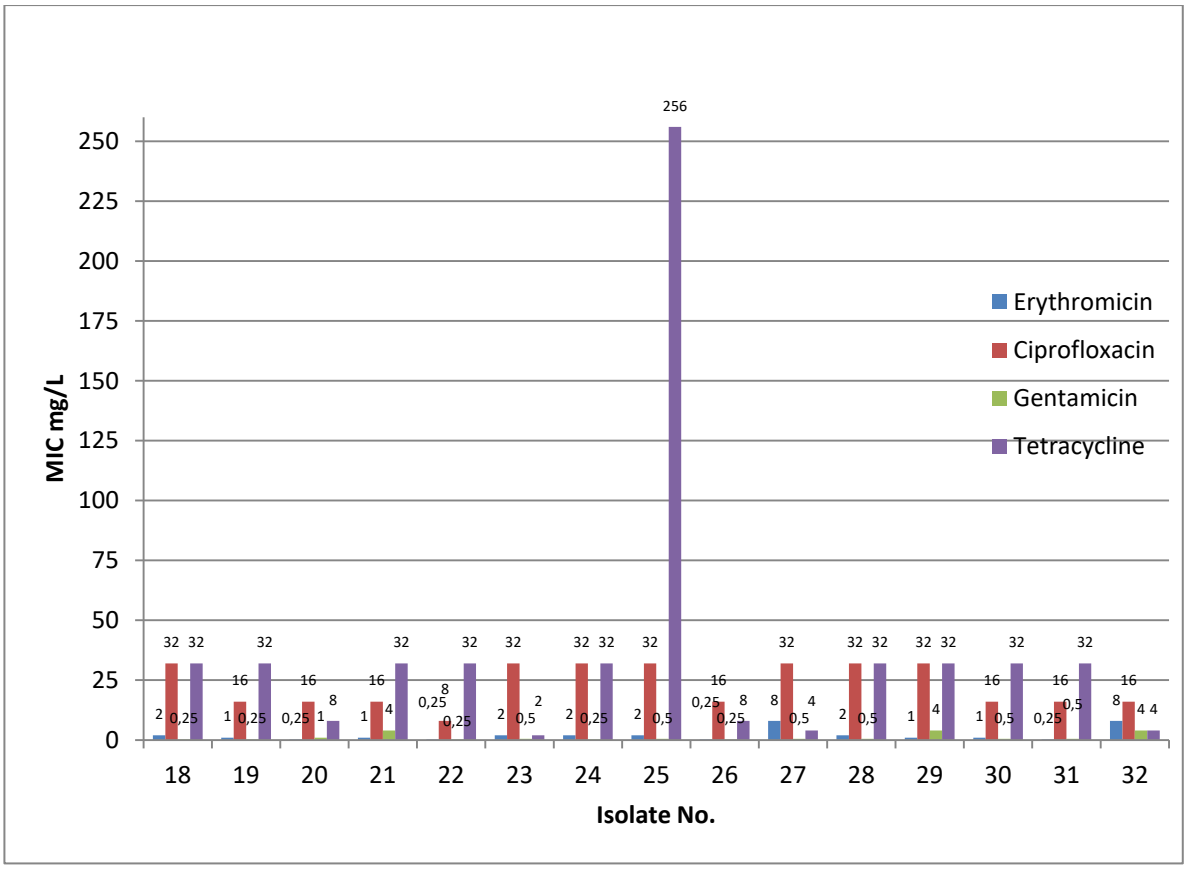

Fig. 3.2.3. *Antimicrobial resistance of C. jejuni strains isolated from infected humans*

3.2.4 Resistance of examined *C. jejuni* strains isolated from different sources

The resistant strains of *C. jejuni* from the different sources based on the break points of MIC is seen in (Fig. 3.2.4.). The study revealed that most often resistant *C. jejuni* strains were isolated from infected humans clinical samples and broiler meat. The strains were more resistant to ciprofloxacin and tetracycline than erythromycin and gentamicin. Two strains of broiler meat and human origin each were resistant to erythromycin. All of the tested strains were resistant to ciprofloxacin. One strain of broiler origin and three strains of human origin were resistant to gentamicin. One of the

strains of turkey origin, 13 of the broiler origin strains and all the 15 strains of human origin were resistant to tetracycline.

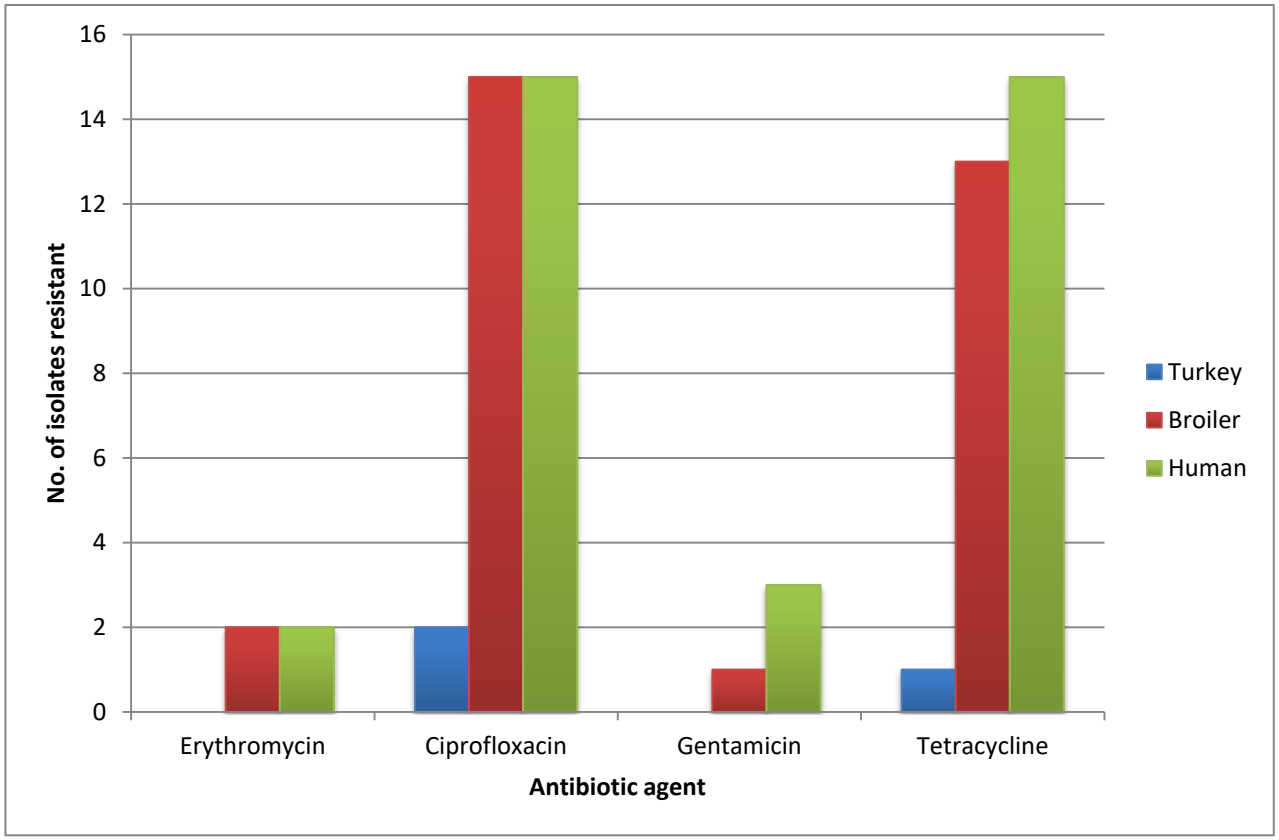

Fig. 3.2.4. *Resistance to tested antibiotics of C. jejuni strains isolated from different sources*

4. DISCUSSION

The study revealed, that the prevalence of C*ampylobacter* bacteria in turkey meat sold at the retail level shops is very low, as only 2 out of 130 (1.5%) samples were contaminated with campylobacters. Two comparable studies were performed in Italy, then one of the studies showed 33% contamination of the examined samples. The second investigation had found no positive samples for C*ampylobacter spp.* A study in Sweden showed 1.6% of the samples to be positive for C*ampylobacter*. In the two Italian and Swedish studies samples of broiler meat from retail level have been studied parallelly. The prevalence of C*ampylobacter* in samples of broiler origin was 70.6 %, 6.8 % and 25.4 % accordingly. The prevalence of *Campylobacter* in turkey meat was lower than in broiler meat in all the parallel studies with samples of both broiler and turkey meat origin. The infection rate varies widely in between the studies performed but they all accordingly agree that the contamination rate is lower in meat of turkey origin in comparison to meat of broiler origin. [8] [12, 13] The statistical significance of the obtained result in this study was evaluated by the Adjusted Wald method. According the adjusted Wald method the confidence interval is in range from 0.0007 to 0.0579, covering 95% of the total population, based on 2 out of 130 positive isolates.

In the neighbouring countries Poland and Latvia investigations have shown that the degree of poultry meat contamination with *Campylobacter* varies depending on geographical origin and season of sampling. The peak of infection in other investigations is seen in the summer months, when the temperature is higher and more humid. [17, 33] The two *Campylobacter* strains identified in this study were collected from turkey meat samples purchased 2016-10-05, which is after the season with the highest contamination probability what is seen in other studies. The possibility that a higher contamination pressure was present during the missed months of sampling cannot be ignored. It can be speculated in weather a higher infection pressure was present during the missed summer months.

The occurrence of contaminated meat is affected by hygiene routines along the production, from farm to selling place. The risk of contamination is present at every step where campylobacters are present in the production line. The birds are exposed to contamination risk from day seven in the broiler house where they are kept for growing. Newly hatched birds are not as susceptible to campylobacter infection. The first major risk to become contaminated is when the very young birds are moved into the growing houses. The critical control point is dependent on whether cleaning and

disinfection of the growing facility was done properly since the previously infected flock was slaughtered. [10] Further the possibility that the flocks are infected depends on the hygiene routines throughout the production, all the way to the final product. At the slaughter facility a major control point is to apply the routine to take in known none contaminated flocks for slaughter in the beginning of the day and known contaminated flocks or flocks with unknown status for slaughter later. If such system is followed at a slaughter facility proper cleaning and disinfection is a measure to be taken each day. [11] The equipments material type is of greatest importance, any organic material is proven non-hygienic and disinfection of such is an insufficient measure for the control. [5] The meat samples purchased at the shopping area URMAS were of unknown Lithuanian production as the labelling was clearly insufficient at the selling place. The possibility that these samples were exposed to cross contamination was predominant as broiler meat was offered at the same selling place. Hence the risk of cross contamination from the broiler meat was possible. As seen in multiple studies, the prevalence of *Campylobacter* contamination is higher in meat of broiler origin than meat turkey origin. Concluding that the risk of cross contamination is big from one to another meat type in the meat samples purchased at the shopping area URMAS. Meat purchased from "X" company could be expected to have a higher and even quality standard. Only products of turkey are offered in the private label store and a new disposable plastic glove was used by the salesman for every new type of meat cut handled.

In (table 4) the MIC are presented for the investigated strains. MIC_{50} is used to describe which concentration of antibiotic that is required to be bactericidal for 50% of the studied strains, respectively MIC_{90} for 90% of the studied strains. To decide whether an antibiotic is clinically usable, the epidemiological cut-off value (ECOFF) was used as the breakpoint to determine if a strain is resistant or susceptible according EUCAST recommendations. [32] The ECOFF is indicated in (table 4) by a bold vertical line in the row of each antibiotic, all strains to the right of the ECOFF line have a higher MIC than ECOFF. The ECOFF is used to describe which part of the studied strains are clinically susceptible to each antimicrobial drug. [34] The tested concentration of antimicrobial is seen as the white zone in the tested concentrations, grey zone is used as an indicator for concentrations not tested. MIC_{50} and MIC_{90} for turkey strains are questionable due to the limited amount of strains found. It is to worth o mention, that all 32 tested strains were resistant to ciprofloxacin. 13.33 % of the strains of broiler and human origin are resistant to erythromycin.

Table 4. *MIC of investigated isolates*

Source	Agent	No.	MIC mg/L											MIC_{50}	MIC_{90}	Resistance (%)
			0.25	0.5	1	2	4	8	16	32	64	128	256			
Turkey	ERY	2		1		1								0.5	2	0
	CIP	2								2				32	32	100
	GEN	2	2											0.25	0.25	0
	TET	2	1							1				0.25	32	50
Broiler	ERY	15	8	3	1	1		2						0.25	8	13.33
	CIP	15				1	4	4	4	1		1		8	32	100
	GEN	15	3	5	6		1							0.5	1	6.67
	TET	15	1	1		2		1	2	4	3	1		32	64	73.33
Human	ERY	15	4		4	5		2						0.25	8	13.33
	CIP	15						1	7	7				16	32	100
	GEN	15	5	6	1		3							0.5	4	20
	TET	15				1	2	2		9			1	32	32	100

Source	Agent	No.	MIC mg/L											MIC_{50}	MIC_{90}	Resistance (%)
			0.25	0.5	1	2	4	8	16	32	64	128	256			
All	ERY	32	12	4	5	7		4						0.5	8	12.5
	CIP	32				1	4	5	11	10		1		16	32	100
	GEN	32	10	11	7		4							0.5	4	12.5
	TET	32	2	1		3	2	3	2	14	3	1	1	32	64	90.63
															Average:	53.91

ERY-Erythromycin CIP-Ciprofloxacin GEN-Gentamicin TET-Tetracycline

The epidemiological cut-off value (ECOFF) of resistance is indicated as the bold vertical line in the row of each antibiotic; all strains to the right of the ECOFF are considered as resistant to particular antibiotic

The antimicrobial resistance to fluoroquinolones among *C. coli* and *C. jejuni* have been studied in other studies, in this study represented by testing MIC of ciprofloxacin. In another study developing resistance is proven *in vivo*, after five days of administration with ciprofloxacin the resistance increased. [25] The antimicrobial resistance develops due to inactivation of target cells in the bacteria where fluoroquinolones interact in combination with the interrupted efflux pump within the cytoplasmic membrane. [14] In this study the interaction with ciprofloxacin to *C. jejuni* is studied and evaluated by the EUCAST established breakpoint of 0.5 mg/L. [32] The set breakpoint value is lower than all tested samples in this study, meaning that all the strains are resistant to ciprofloxacin. Notable is that the MIC 90 value, 32 mg/L of ciprofloxacin for the studied isolates correlates with Patrick F. McDermott *et al* (2001) results for the required concentration needed after five days of treating isolates *in vivo*. [25] Only speculations can be made to decide why the antibiotic´s MIC of the tested strains are as high as seen in this study. Influencing factors such as previous antimicrobial interaction and origin of contamination along the production line is unknown. Concluding that the antimicrobial resistance is high against ciprofloxacin both in the studied strains as well as in the reviewed articles. The use of fluoroquinolones such as ciprofloxacin should be more restricted to maintain ciprofloxacin and other fluoroquinolones adequacy when treating human campylobacteriosis. Groups of production animals contaminated with fluoroquinolone resistant *Campylobacter spp.* should be properly controlled to limit the spread and prevent further contamination. Growing facilities where such flocks have been kept should be properly cleaned and disinfected to prevent the risk surviving resistant *Campylobacter* bacteria.

The antimicrobial resistance to macrolides was studied by measuring the MIC of erythromycin in the collected strains. Resistance to macrolides is of absolutely highest interest for the medical field as it is the drug of choice when treating human campylobacteriosis. Multiple studies have been reviewed on the topic and all confirm that the resistance level against macrolides is low in various *Campylobacter spp.* The reviewed studies show that the rate of resistance is dependent on specie of the host. Strains isolated from turkeys has the lowest resistance rate from the tested species, highest is found in strains of pig origin. [14, 26] The antibiotic policy along the production is highly suspected as a major factor together with the ongoing developing resistance against fluoroquinolones. The cellular mechanism in the bacteria results in macrolid resistant bacteria dependent on a mutation in at least one of the three base pairs of the bacteria where the antibiotic interacts. The resistance rate in the studied strains to erythromycin is comparable to values obtained in the reviewed studies. This study revealed that 12.5% of the strains are resistant to erythromycin which is higher than the recommended 4% for strains of broiler and human origin.

[26] From the 32 strains in this study 28 were susceptible to treatment with erythromycin, using the breakpoint 4 mg/L set by EUCAST. The four isolates resistant, were susceptible to 8 mg/L of erythromycin, meaning that the deviation was lowest possible in this study. According to this study and the reviewed studies, it is possible to conclude that the resistance against macrolides tested by erythromycin is still mostly low. The local deviating value with a resistance in 12.5% of the isolates is an indication to a developing risk. As the samples size in the this study is lower than in the reviewed studies and that the resistant isolates are susceptible to the next following concentration of erythromycin it is not of that big matter compared to the situation with fluoroquinolones. Nonetheless it is of great importance to maintain low macrolid resistance to maintain the benefit and efficiency of the drug. Strict antibiotic policies include limitations in the usage such as susceptibility testing and to only prescribe drugs when needed. The application of routine metaphylaxis with macrolides or any other antibiotic should be strictly prohibited, such as antibiotics mixed into water and or animal feed. Preventive measures against contamination is of greatest importance.

Gentamicin is not commonly used when treating campylobacteriosis until today, it is though seen as an efficient antimicrobial against *Campylobacter* seen *in vitro*. Though it was seen that the coresistance to other antibiotics is high, primarily with the clinically used antimicrobials when treating human campylobacteriosis, fluoroquinolones and macrolides. The coresistance is more prevalent isolates of human origin than those of broiler origin. [24] This study revealed that the resistance against gentamicin was relatively low, 12.5% were resistant to gentamicin. The coresistance was though seen high, all four isolates resistant to gentamicin were coresistant to ciprofloxacin and tetracycline. Similar results of coresistance were revealed in the reviewed studies. The last checked strain, No. 32 was resistant to all the tested antimicrobials.

The usage of tetracycline in case of campylobacteriosis is not as widespread as for macrolides and fluoroquinolones, it is though recommended as a drug of choice by WHO. [16] The quick developing resistance after some time of use makes it an unprofitable drug. The resistance increased from 23.2 % to 70.4 % during a study of 5 years. [17] Coresistance is seen as a problematic factor, in a study 89% of the tested strains were coresistant to ciprofloxacin. [27] This study revealed that 29 out of 32 strains were resistant to tetracycline. The strains resistant to tetracycline were 100 % coresistant to ciprofloxacin. Worth to note is that all tested strains were resistant to ciprofloxacin.

The study revealed that antimicrobial multi-resistance were seen in 7 of 32 strains, meaning hence resistant against 3 of the 4 tested antimicrobials. Strain No. 32, a strain of human origin was

resistant to all of the four tested antimicrobials. Antimicrobial multi-resistance is an increasing problem for the public health. The opportunity to use other antimicrobial drugs have been evaluated as positive, until now there is no drug that all bacteria are susceptible. The biggest issue is, how to treat macrolide resistant campylobacters commonly coresistant to fluoroquinolones. According the reviewed Finish study, the use of co-amoxiclav, imipenem, meropenem and tigecycline were successfully evaluated *in vitro*. [29] As the development of new antimicrobials is limited especially for the treatment of enterotoxic bacteria, the question of developing multiresistant bacteria is of greatest importance.

The antimicrobial resistance of strains varies of depending on the specie of origin, whether the strain is sampled from stools of a human sick in campylobacteriosis or from a meat sample. The reviewed studies from various European countries agree that the antimicrobial resistance is higher in strains of poultry origin compared to strains of human origin. [15] The data plotted in (Fig. 3.2.4.) is statistically evaluated by fisher's exact test and measured by p-value. The p-values given are as follows: erythromycin: 1.00, ciprofloxacin: 1.00, gentamicin: 0.32 and tetracycline: 0.23. in this study. This study reveal that strains of human origin have a higher resistance ratio to gentamicin and tetracycline. The level of resistance to erythromycin and the resistance to ciprofloxacin is higher of strains of meat origin. The received p-values are though statistically insignificant as 0.05 is the upper limit for significance. Reason that the results are not statistically significant could be explained by the low number of tested strains and very high diversity of antimicrobial resistance of the tested strains.

CONCLUSIONS

1. The contamination with campylobacters of turkey meat purchased at the retail level shops is very low (1,5%) as only 2 fresh turkey thighs out of 130 samples tested were contaminated with *Campylobacter jejuni* bacteria. Any of tested turkey fresh wings (n=49), breast file (n=12) or light smoked breast file (n=18) samples were contaminated with other *Campylobacter spp.*
2. The study revealed that both C. jejuni strains isolated from turkey meat were resistant to ciprofloxacin and sensitive to gentamicin and erythromycin according EUCAST proposed MIC breakpoints. One strain was sensitive to tetracycline, thereas another strains was resistant to tetracycline.
3. *C. jejuni* strains (n=15) isolated from infected humans were resistant to erythromycin (n=2), sensitive to erythromycin (n=13). All the isolated strains of human origin (n=15), were resistant to ciprofloxacin and tetracycline. Three strains were resistant to gentamicin and twelve were sensitive to gentamicin.
4. C. *jejuni* strains (n=15) isolated from broiler meat were resistant to erythromycin (n=2), sensitive to erythromycin (n=13). All the isolated strains of broiler meat origin were resistant to ciprofloxacin. 1 strain was resistant to gentamicin and 14 were sensitive to gentamicin. 13 strains were resistant to tetracycline and 2 were sensitive to tetracycline.
5. In total out of 32 tested C. *jejuni* strains, we found 31 strains to be sensitive to at least one tested antibiotic.
6. Seven examined strains of *C. jejuni* were multidrug-resistant, as they were resistant to at least 3 tested antibiotic.

AKNOWLEDGEMENT

I would like to thank you the Department of Food Safety and Quality at the Veterinary Academy of LSMU for providing equipment, laboratory apparatus and set up suitable for this study.

Further I would like to thank you for the support, guidance and help in the laboratory throughout the study given by the Department of Food Safety and Quality. Especially represented by assistant Jurgita Aksomaitiene and Neringa Kasnauskytė.

Finally the deepest appreciation to my supervisor Professor dr. Mindaugas Malakauskas for supporting me throughout the study, providing supervision, guidance and feedback to be able to complete this master thesis successfully.

REFERENCES

1. European Food Safety Authority, European Centre for Disease Prevention and Control. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA Journal 2017;15(12):5077
2. C. Vinueza-Burgos, M. Wautier, D. Martiny, M. Cisneros. Prevalence, antimicrobial resistance and genetic diversity of Campylobacter coli and Campylobacter jejuni in. 2017 Poultry Science no. 0 p.1–9
3. European Food Safety Authority, European Centre for Disease Prevention and Control. EU summary report on zoonoses, zoonotic agents and food-borne outbreaks 2015. EFSA Journal 2016;14(12):4634
4. J. Silva, D. Leite, M. Fernandes, C. Mena, P. Anthony-Gibbs, P. Teixeira. Campylobacter spp. as a foodborne pathogen: a review. Frontiers in Microbiology. vol, 2. no. 200, 2011, p. 1-12
5. L. García-Sanchez, B. Melero, I. Jaime, M-L- Hänninen, M. Rossi, J. Rovira. Campylobacter jejuni survival in a poultry processing plant environment. Food Microbiology no. 65, 2017, p. 185-192
6. Hansson, L. Plym Forshell, P. Gustafsson, S. Boqvist, J. Lindblad, E. Olsson-Engvall, Y. Andersson, I. Vågsholm. Summary of the Swedish Campylobacter Program in Broilers, 2001 through 2005. Journal of Food Protection, Vol. 70, No. 9, 2007, p. 2008–2014
7. B. Ge, F. Wang, M. Sjölund-Karlsson, P. F. McDermott. Antimicrobial resistance in Campylobacter: Susceptibility testing methods and resistance trends. Journal of Microbiological Methods, vol. 95, No. 1, 2013, p. 57-67
8. Z. Mezher, S. Saccares, R. Marcianò, P. De Santis, E. -M. Flores Rodas, V. De-Angelis, R. Condoleo. Occurrence of Campylobacter spp. in poultry meat at retail and processing plants' levels in Central Italy. Italian Journal of Food Safety; vol. 5:5495, 2016, p. 47-49.
9. T. J. Johnson, J.M. Shank J. G. Johnson. Current and Potential Treatments for Reducing Campylobacter Colonization in Animal Hosts and Disease in Humans. Frontiers in Microbiology, vol. 8, 2017, article 487
10. F. Marotta, G. Garofolo, G. Di-Donato, G. Aprea, I. Platone, S. Cianciavicchia, A. Alessiani, E. Di-Giannatale. Population Diversity of Campylobacter jejuni in Poultry and Its Dynamic of Contamination in Chicken Meat. BioMed Research International, vol. 2015, 2014, Article ID: 859845

11. T. Battersby, D. Walsh, P. Whyte, D. Bolton. Evaluating and improving terminal hygiene practices on broiler farms to prevent Campylobacter cross-contamination between flocks. Food Microbiology no. 64, 2017, p. 1-6
12. F. Pedonese, R. Nuvoloni, B. Turchi, B. Torracca, E. Di-Giannatale, F. Marotta D. Cerri. Prevalence, phenotypic and genetic diversity of Campylobacter in poultry fresh meat and poultry products on retail sale in Tuscany (Italy). Veterinaria Italiana no. 53 (1), 2017, p.29-37
13. Livsmedelsverket (National food agency Sweden). Campylobacter: Smittspårning, epidemiologi och typning. Report no, 15/2005.
14. Y. M. Sierra-Arguello, G. Perdoncini, R. B. Morgan, C. T. P. Salle, H. L. S. Moraes, Marcos J. P. Gomes, V. P. do Nascimento. Fluoroquinolone and macrolide resistance in Campylobacter jejuni isolated from broiler slaughterhouses in southern Brazil. Avian Pathology. vol. 45, no. 1, 2015, p. 66–72
15. S. S. Mozina, M. Kurincic, A. Klancnik, A. Mavri. Campylobacter and its multi-resistance in the food chain. Trends in Food Science & Technology. vol. 22, 2011, p. 91-98
16. C. Vinueza-Burgos M. Wautier, D. Martiny, M. Cisneros, I. Van Damme, L. De Zutter. Prevalence, antimicrobial resistance and genetic diversity of Campylobacter coli and Campylobacter jejuni in Ecuadorian broilers at slaughter age. Poultry Science vol. 0, 2017, p.1–9
17. K. Wieczorek, J. Osek. A five-year study on prevalence and antimicrobial resistance of Campylobacter from poultry carcasses in Poland. Food Microbiology vol. 49, 2015, p. 161-165
18. L. Randall, A. Ridley, F. Lemma, C. Hale, R. Davies. In vitro investigations into the use of antimicrobials in combination to maintain efficacy of fluoroquinolones in poultry. Research in Veterinary Science, vol. 106, 2016, p. 47-53
19. A. P. MacGowan, R. Wise. Establishing MIC breakpoints and the interpretation of in vitro susceptibility tests. Journal of Antimicrobial Chemotherapy, vol. 48, 2001, p. 17-28
20. J. Turnidge D. L. Paterson. Setting and Revising Antibacterial Susceptibility Breakpoints. Clinical Microbiology Reviews, vol. 20 no. 3, 2007, p. 391–408
21. M. Lekshmi, P. Ammini, S. Kumar M. F. Varela. The Food Production Environment and the Development of Antimicrobial Resistance in Human Pathogens of Animal Origin. Microorganisms, vol. 5, no. 11, 2017

22. A. Gibreel, D. E. Taylor. Macrolide resistance in Campylobacter jejuni and Campylobacter coli. Journal of Antimicrobial Chemotherapy vol. 58, 2006, p. 243–255
23. A. Woz´niak-Biel, G. Bugla-Płoskon´ska, A. Kielsznia, K. Korzekwa, A. Tobiasz, A. Korzeniowska-Kowal, A. Wieliczko. High Prevalence of Resistance to Fluoroquinolones and Tetracycline Campylobacter Spp. Isolated from Poultry in Poland. Microbial Drug resistance, vol. 00, no.00, 00, 2017
24. S. Zhao, S. Mukherjee, Y. Chen, C. Li, S. Young, M. Warren, J. Abbott, S. Friedman, C. Kabera, M. Karlsson, P. F. McDermott. Novel gentamicin resistance genes in Campylobacter isolated from humans and retail meats in the USA. Journal of Antimicrobial Chemotherapy, vol. 70, 2015, p. 1314–1321
25. P. F. McDermott, S. M. Bodeis, L. L. English, D. G. White, R. D. Walker, S. Zhao, S. Simjee, D. D. Wagner. Ciprofloxacin Resistance in Campylobacter jejuni Evolves Rapidly in Chickens Treated with Fluoroquinolones. The Journal of Infectious Diseases, vol. 185, 2002, p. 837–40
26. H. Bolinger, S. Kathariou. The Current State of Macrolide Resistance in Campylobacter spp.: Trends and Impacts of Resistance Mechanisms. Applied and Environmental Microbiology, vol. 83, 2017
27. M. Carev, A. Kovačić, A. Novak, M. Tonkić, A. Jerončić. Campylobacter jejuni strains coresistant to tetracycline and ciprofloxacin in patients with gastroenteritis in Croatia. Infectious Diseases, vol. 49, no. 4, 2017, p. 268–276
28. K. Wieczorek, I. Kania, J. Osek. Erythromycin-resistant Campylobacter coli from slaughtered animals as a potential public health risk. Veterinarni Medicina, vol. 58, 2013, p. 352–358
29. M. Lehtopolku, U. M. Nakari, P. Kotilainen, P. Huovinen, A. Siitonen, A. J. Hakanen. Antimicrobial Susceptibilities of Multidrug-Resistant Campylobacter jejuni and C. coli Strains: In Vitro Activities of 20 Antimicrobial Agents. Antimicrobial Agents and Chemotherapy, vol. 54, 2010, p. 1232–1236
30. G. Wang, C. G. Clark, T. M. Taylor, C. Pucknell, C. Barton, L. Price, D. L. Woodward, F. G. Rodgers. Colony Multiplex PCR Assay for Identification and Differentiation of Campylobacter jejuni, C. coli, C. lari, C. upsaliensis, and C. fetus subsp. fetus. Journal Of Clinical Microbiology, vol. 40, 2002, p. 4744–4747

31. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard – seventh edition. 2006.
32. European committee on antimicrobial susceptibility testing. Available from: https://mic.eucast.org/Eucast2/SearchController/search.jsp?action=performSearch&BeginIndex=0&Micdif=mic&NumberIndex=50&Antib=-1&Specium=204.
33. K. Kovalenko, M. Roasto, E. Liepins, Mihkel Mäesaar, A. Hörman. High occurrence of Campylobacter spp. in Latvian broiler chicken production. Food control, vol. 29, 2013, p. 188-191
34. S. Olkkola, S. Nykäsenoja, S. Raulo, A.-K. Llarena, S. Kovanen, R. Kivistö, A.-L. Myllyniemi, M.-L. Hänninen. Antimicrobial Resistance and Multilocus Sequence Types of Finnish Campylobacter jejuni Isolates from Multiple Sources. Zoonoses and Public Health, vol. 63, 2016, p. 10–19

ANNEXES

Annex 1. *Complete list of collected turkey samples*

Planting date	Product	Weight (g)	No.	Plate no.	Campylobacter isolated	Buying place
2016-04-05	Thigh	245	1	1	No	Urmas market
2016-04-05	Thigh	255	2	2	No	Urmas market
2016-04-05	Thigh	230	3	3	No	Urmas market
2016-04-05	Breast file	245	4	4	No	Urmas market
2016-04-05	Breast file	265	5	5	No	Urmas market
2016-04-05	Breast file	280	6	6	No	Urmas market
2016-04-19	Thigh	240	7	1	No	Urmas market
2016-04-19	Thigh	250	8	2	No	Urmas market
2016-04-19	Thigh	255	9	3	No	Urmas market
2016-04-19	Breast file	205	10	4	No	Urmas market
2016-04-19	Breast file	200	11	5	No	Urmas market
2016-04-19	Breast file	210	12	6	No	Urmas market
2016-04-19	Wing	250	13	7	No	Urmas market
2016-04-19	Wing	260	14	8	No	Urmas market
2016-04-19	Wing	235	15	9	No	Urmas market
2016-05-03	Thigh	245	16	1	No	Urmas market
2016-05-03	Thigh	255	17	2	No	Urmas market
2016-05-03	Thigh	240	18	3	No	Urmas market

Planting date	Product	Weight (g)	No.	Plate no.	Campylobacter isolated	Buying place
2016-05-03	Breast file	235	19	4	No	Urmas market
2016-05-03	Breast file	215	20	5	No	Urmas market
2016-05-03	Breast file	220	21	6	No	Urmas market
2016-05-03	Wing	260	22	7	No	Urmas market
2016-05-03	Wing	275	23	8	No	Urmas market
2016-05-03	Wing	280	24	9	No	Urmas market
2016-05-24	Thigh	220	25	1	No	Urmas market
2016-05-24	Thigh	230	26	2	No	Urmas market
2016-05-24	Thigh	245	27	3	No	Urmas market
2016-05-24	Breast file	200	28	4	No	Urmas market
2016-05-24	Breast file	205	29	5	No	Urmas market
2016-05-24	Breast file	210	30	6	No	Urmas market
2016-05-24	Wing	265	31	7	No	Urmas market
2016-05-24	Wing	270	32	8	No	Urmas market
2016-05-24	Wing	240	33	9	No	Urmas market
2016-09-12	Thigh	240	34	1	No	Viljampole market
2016-09-12	Thigh	255	35	2	No	Viljampole market
2016-09-12	Thigh	235	36	3	No	Viljampole market
2016-09-12	Thigh	245	37	4	No	Viljampole market
2016-09-12	Thigh	250	38	5	No	Viljampole market

Planting date	Product	Weight (g)	No.	Plate no.	Campylobacter isolated	Buying place
2016-09-12	Thigh	230	39	6	No	Urmas market
2016-09-12	Thigh	235	40	7	No	Urmas market
2016-09-12	Thigh	240	41	8	No	Urmas market
2016-09-12	Thigh	255	42	9	No	Urmas market
2016-09-12	Thigh	235	43	10	No	Urmas market
2016-09-12	Thigh	240	44	11	No	Viljampole market
2016-09-12	Wing	245	45	12	No	Viljampole market
2016-09-12	Wing	255	46	13	No	Viljampole market
2016-09-12	Wing	260	47	14	No	Viljampole market
2016-09-12	Wing	255	48	15	No	Viljampole market
2016-09-12	Wing	275	49	16	No	Urmas market
2016-09-12	Wing	260	50	17	No	Urmas market
2016-09-12	Wing	265	51	18	No	Urmas market
2016-09-12	Wing	270	52	19	No	Urmas market
2016-09-12	Wing	265	53	20	No	Urmas market
2016-09-12	Wing	270	54	21	No	Urmas market
2016-09-12	Wing	265	55	22	No	Urmas market
2016-09-26	Wing	336	56	1	No	Urmas market
2016-09-26	Wing	284	57	2	No	Urmas market
2016-09-26	Wing	296	58	3	No	Urmas market

Planting date	Product	Weight (g)	No.	Plate no.	Campylobacter isolated	Buying place
2016-09-26	Wing	304	59	4	No	Urmas market
2016-09-26	Thigh	298	60	5	No	Urmas market
2016-09-26	Thigh	295	61	6	No	Urmas market
2016-09-26	Thigh	280	62	7	No	Urmas market
2016-10-05	Thigh	236	63	1	No	Urmas market
2016-10-05	Thigh	184	64	2	No	Urmas market
2016-10-05	Thigh	188	65	3	No	Urmas market
2016-10-05	Thigh	292	66	4	Yes	Urmas market
2016-10-05	Thigh	196	67	5	Yes	Urmas market
2016-10-05	Wing	176	68	6	No	Urmas market
2016-10-05	Wing	194	69	7	No	Urmas market
2016-10-05	Wing	208	70	8	No	Urmas market
2016-10-05	Wing	221	71	9	No	Urmas market
2016-10-05	Wing	208	72	10	No	Urmas market
2016-10-18	Wing	283	73	1	No	Viljampole market
2016-10-18	Wing	287	74	2	No	Viljampole market
2016-10-18	Wing	269	75	3	No	Viljampole market
2016-10-18	Wing	300	76	4	No	Viljampole market
2016-10-18	Wing	343	77	5	No	Viljampole market
2016-10-18	Thigh	205	78	6	No	Viljampole market

Planting date	Product	Weight (g)	No.	Plate no.	Campylobacter isolated	Buying place
2016-10-18	Thigh	265	79	7	No	Viljampole market
2016-10-18	Thigh	193	80	8	No	Viljampole market
2016-10-18	Thigh	200	81	9	No	Viljampole market
2016-10-18	Thigh	219	82	10	No	Viljampole market
2016-10-18	Light smoked breast	221	83	11	No	Viljampole market
2016-10-18	Light smoked breast	191	84	12	No	Viljampole market
2016-10-18	Light smoked breast	222	85	13	No	Viljampole market
2016-10-28	Wing	320	86	1	No	Viljampole market
2016-10-28	Wing	342	87	2	No	Viljampole market
2016-10-28	Wing	323	88	3	No	Viljampole market
2016-10-28	Wing	280	89	4	No	Viljampole market
2016-10-28	Wing	313	90	5	No	Viljampole market
2016-10-28	Thigh	191	91	6	No	Viljampole market
2016-10-28	Thigh	273	92	7	No	Viljampole market
2016-10-28	Thigh	202	93	8	No	Viljampole market
2016-10-28	Thigh	186	94	9	No	Viljampole market
2016-10-28	Thigh	240	95	10	No	Viljampole market
2016-10-28	Light smoked breast	166	96	11	No	Viljampole market
2016-10-28	Light smoked breast	220	97	12	No	Viljampole market
2016-10-28	Light smoked breast	181	98	13	No	Viljampole market

Planting date	Product	Weight (g)	No.	Plate no.	Campylobacter isolated	Buying place
2016-10-28	Light smoked breast	187	99	14	No	Viljampole market
2016-10-28	Light smoked breast	162	100	15	No	Viljampole market
2016-11-16	Thigh	205	101	1	No	Viljampole market
2016-11-16	Thigh	210	102	2	No	Viljampole market
2016-11-16	Thigh	195	103	3	No	Viljampole market
2016-11-16	Thigh	220	104	4	No	Viljampole market
2016-11-16	Thigh	225	105	5	No	Viljampole market
2016-11-16	Wing	280	106	6	No	Viljampole market
2016-11-16	Wing	275	107	7	No	Viljampole market
2016-11-16	Wing	270	108	8	No	Viljampole market
2016-11-16	Wing	285	109	9	No	Viljampole market
2016-11-16	Wing	295	110	10	No	Viljampole market
2016-11-16	Light smoked breast	250	111	11	No	Viljampole market
2016-11-16	Light smoked breast	235	112	12	No	Viljampole market
2016-11-16	Light smoked breast	245	113	13	No	Viljampole market
2016-11-16	Light smoked breast	255	114	14	No	Viljampole market
2016-11-16	Light smoked breast	240	115	15	No	Viljampole market
2016-12-11	Thigh	205	116	1	No	Viljampole market
2016-12-11	Thigh	215	117	2	No	Viljampole market
2016-12-11	Thigh	220	118	3	No	Viljampole market

Planting date	Product	Weight (g)	No.	Plate no.	Campylobacter isolated	Buying place
2016-12-11	Thigh	235	119	4	No	Viljampole market
2016-12-11	Thigh	210	120	5	No	Viljampole market
2016-12-11	Wing	310	121	6	No	Viljampole market
2016-12-11	Wing	280	122	7	No	Viljampole market
2016-12-11	Wing	295	123	8	No	Viljampole market
2016-12-11	Wing	275	124	9	No	Viljampole market
2016-12-11	Wing	275	125	10	No	Viljampole market
2016-12-11	Light smoked breast	255	126	11	No	Viljampole market
2016-12-11	Light smoked breast	240	127	12	No	Viljampole market
2016-12-11	Light smoked breast	235	128	13	No	Viljampole market
2016-12-11	Light smoked breast	260	129	14	No	Viljampole market
2016-12-11	Light smoked breast	245	130	15	No	Viljampole market