UNIVERSITI PUTRA MALAYSIA

OCCURRENCE OF EXTENDED-SPECTRUM BETA-LACTAMASE *ESCHERICHIA COLI* AT UNIVERSITY VETERINARY HOSPITAL (UVH), UNIVERSITI PUTRA MALAYSIA

ONG KAR SOON

lp
FPV 2022 38

OCCURRENCE OF EXTENDED-SPECTRUM BETA-LACTAMASE *ESCHERICHIA COLI* AT UNIVERSITY VETERINARY HOSPITAL (UVH), UNIVERSITI PUTRA MALAYSIA

ONG KAR SOON

FACULTY OF VETERINARY MEDICINE
UNIVERSITI PUTRA MALAYSIA
SERDANG, SELANGOR

2022

@COPYRIGHT UPM

CCURRENCE OF EXTENDED-SPECTRUM BETA-LACTAMASE *ESCHERICHIA COLI* AT UNIVERSITY VETERINARY HOSPITAL (UVH), UNIVERSITI PUTRA MALAYSIA

ONG KAR SOON

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfilment of the requirement of the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
Universiti Putra Malaysia
Serdang, Selangor Darul Ehsan

DECEMBER 2022

@COPYRIGHT UPM

CERTIFICATION

It is hereby certified that I have read this project paper entitled "Occurrence of extended-spectrum beta-lactamase *Escherichia coli* at University Veterinary Hospital (UVH), Universiti Putra Malaysia", by Ong Kar Soon and in my opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Final Year Project.

Associate Professor Dr. Chen Hui Cheng
DVM (UPM), MVM (UPM), DVSc (Guelph),
Associate Professor,
Faculty of Veterinary Medicine,
Universiti Putra Malaysia
(Supervisor)

Dr. Nur Indah Ahmad
DVM (UPM) MVSc (Utrecht) PhD (Edinburgh),
Senior Lecturer (Veterinary Public Health),
Faculty of Veterinary Medicine,
Universiti Putra Malaysia
(Co-Supervisor)

@COPYRIGHT UPM

ACKNOWLEDGMENTS

With deepest appreciation and gratitude, I thank my supervisor, Associate Professor Dr Chen Hui Cheng and my co-supervisor Dr Nur Indah Ahmad for their enthusiasm and patient guidance throughout this study. I would not be able to complete this project and presentation without their help.

In addition, I would like to thank Dr Afshar, for his help, company and moral support along this period.

Without forgetting my beloved family members, thank you for preparing good meals and a comfortable environment for me to complete this project.

Lastly, a special thank you to my fellow classmates of DVM2023 for the reminders, wishes and support along this journey.

@COPYRIGHT UPM

CONTENTS

@COPYRIGHT UPM

@COPYRIGHT UPM

LIST OF TABLES

@COPYRIGHT UPM

LIST OF FIGURES

@COPYRIGHT UPM

LIST OF ABBREVIATIONS

CTX-M : Cefotaximase Munich (a group of beta-lactamases)

ESBL : Extended spectrum beta-lactamase

TEM : Temoneira (a group of beta-lactamases named after a patient)

SHV : Sulfhydryl variable (a group of beta-lactamases)

AMR : Antimicrobial Resistance

MDR : Multidrug Resistant

*E. **coli*** *: Escherichia coli*

HUS : Hemolytic uraemic syndrome

TTP : Thrombotic thrombocytopenic purpura

UTI : Urinary Tract Infection

MIC : Minimum inhibitory concentration

mPCR : Multiplex polymerase chain reaction

MCC : MacConkey

MCC-CTX : MacConkey with cefotaxime

CLSI : Clinical Laboratory Standard Institute

@COPYRIGHT UPM

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999- Projek Ilmiah Tahun Akhir.

KEJADIAN EXTENDED-SPECTRUM BETA-LACTAMASE *ESCHERICHIA COLI* DI HOSPITAL VETERINAR UNIVERSITI, UNIVERSITI PUTRA MALAYSIA

Oleh

ONG KAR SOON

2022

Penyelia: Profesor Madya Dr. Chen Hui Cheng
Penyelia bersama : Dr. Nur Indah Ahmad

Antibiotik adalah salah satu ubat yang paling berguna dalam perubatan veterinar dan manusia. Walau bagaimanapun, kerintangan antimicrobial (AMR) telah muncul sebagai salah satu kebimbangan kesihatan awam. Bakteria *Escherichia coli* yang menghasilkan beta-lactamase spektrum lanjutan (ESBL-*E. coli)* adalah sumber kerintanganan antimikrobial yang tersebar luas dalam kalangan haiwan dan manusia. ESBL-*E. coli* pada haiwan kesayangan pernah dilaporkan. Tujuan kajian ini adalah untuk mengesan kejadian ESBL-*E. coli* pada haiwan yang dimasukkan ke wad, persekitaran premis kesihatan, dan kakitangan yang bekerja di Hospital Veterinar Universiti. Swab rektum dikumpulkan dari 8 kes haiwan yang diwadkan, 7 haiwan residen, 30 kakitangan, dan 59 permukaan yang lazim disentuh di UVH. Sampel swab dieram dalam air pepton berpenimbal semalaman pada 37℃. Kultur kemudiannya dicalit pada agar MacConkey yang ditambah dengan cefotaxime (1ug/L), dan dieram semalaman pada 37. Sub-kultur dilakukan pada koloni

@COPYRIGHT UPM

yang tumbuh untuk mendapatkan kultur tulen. Ujian biokimia dijalankan untuk mengesahkan *E. coli*. Ujian "Double disk diffusion' untuk pengesahan ESBL-*E. coli* dijalankan dalam 2 langkah, saringan dan plat pengesahan, mengikut protokol Clinical and Laboratory Standard Institute. Sebanyak 20 asingan *E. coli* didapati daripada 104 sampel, iaitu, anjing yang diwadkan (n = 1/2), anjing residen (n = 4), kucing yang diwadkan (n = 1/6), kucing residen (n = 3), permukaan lazim disentuh (n = 3/59) dan kakitangan UVH (n = 15/30). Daripada 20 asingan *E. coli,* 11 disahkan menghasilkan ESBL. Data ini tidak menunjukkan penyebaran kerintangan yang ketara daripada haiwan peliharaan kepada manusia atau persekitaran atau sebaliknya. Ia menunjukkan potensi ancaman kepada kesihatan awam dan keperluan langkah pencegahan dan kawalan jangkitan di klinik veterinar untuk mencegah penyebaran AMR ke komuniti.

***Kata kunci**: kerintangan antimikrobial,* ESBL-*E. coli, manusia, haiwan, persekitaran*

@COPYRIGHT UPM

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999- Final Year Project

OCCURRENCE OF EXTENDED-SPECTRUM BETA-LACTAMASE *ESCHERICHIA COLI* AT UNIVERSITY VETERINARY HOSPITAL (UVH), UNIVERSITI PUTRA MALAYSIA

by

ONG KAR SOON

2022

Supervisor: Associate Professor Dr. Chen Hui Cheng

Co-supervisor: Dr. Nur Indah Ahmad

Antibiotics were one of the most useful drugs in veterinary and human medicine. However, antimicrobial resistance (AMR) had became one of the emerging public health concerns. Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* is a widely distributed source of antimicrobial resistance for animals and humans. ESBL-producing

@COPYRIGHT UPM

E. coli has been reported in companion animals. The aim of this study was to detect and determine the occurrence of extended-spectrum beta-lactamase *E. coli* in warded animals, health premise environment, and personnel working at University Veterinary Hospital (UVH). Rectal swabs were collected from 8 warded animals and 7 resident animals, 30 staff, and 59 highly touchable surface in UVH. Swab samples were enriched in buffered peptone water and incubated overnight at 37°C. Enriched cultures were streaked on MacConkey agar supplemented with cefotaxime (1 ug/L) and incubated overnight at 37°C. Presumptive ESBL-*E. coli* were sub-cultured to obtain pure culture. A series of biochemical tests were conducted to confirm *E. coli*. The double disk diffusion (DDD) test was conducted to confirm the identification of ESBL-*E. coli* in a 2-step method, screening and confirmatory plates, based on the protocol by the Clinical and Laboratory Standard Institute (CLSI). A total of 20 presumptive ESBL-*E. coli* growth were obtained from the 104 samples, of which 11 were confirmed ESBL-*E. coli*. This data does not indicate significant wide-spread of resistance from pets to human or environment or inversely. This shows potential threat to public health, and highlights the need to foster IPC standards in veterinary health premises to prevent the spread of AMR into community.

Keywords*: antimicrobial resistance,* ESBL-*E. coli, humans, animals, environment*

@COPYRIGHT UPM

CHAPTER 1: INTRODUCTION

1.1 Background

Antibiotics were one of the most useful drugs in veterinary and human medicine. However, antimicrobial resistance had become one of the emerging public health concerns. Extended-spectrum beta-lactamase (ESBL) producing *E. coli* was a widely distributed source of antimicrobial resistance for animals and humans. ESBL producing *E. coli* was commonly reported among companion animals (Salgado-Caxito et al., 2021).

Extended-spectrum beta-lactamase can confer resistance to the third-generation cephalosporin (Rumi et al., 2019). In small animal practice, use of broad-spectrum cephalosporins were frequently linked to an increase in ESBL-producing *E. coli* in companion animals. Today, over 150 different ESBLs have been described (Bradford, 2001). The most common ESBL *E. coli* genotype detected are CTX-M, TEM, and SHV in both human and small animals (Salgado-Caxito et al., 2021).

A recent study reported significant shedding of ESBL producers in dogs and cats on admission and during hospitalization (Shnaiderman-Torban et al., 2020). In Malaysia, the occurrence of ESBL producing *E. coli* in companion animals is not well known. This information is essential to know the extent of similar problems in the local setting. By studying the occurrence of ESBL *E. coli* in the animals, the health premise environment,

@COPYRIGHT UPM

and the personnel working in the environment, the pattern of ESBL transmission may be identified.

1.2 Objectives and justifications

This study was conducted to detect and determine the occurrence of extended-spectrum beta-lactamase *E. coli* in warded animals, health premise environment, and personnel working at UVH.

The worldwide proliferation of extended-spectrum beta-lactamase had become an urgent global concern. Acquisition of antimicrobial resistance in human commensal bacteria such as *E. coli* had became a general threat to public health and increasing report of ESBL in companion animals and humans. Other than that, antimicrobial resistance gives rise to economic losses, reducing countries 'Growth Domestic product'. (Anderson et al., 2019) However, there is no published report on ESBL *E. coli* in small animals in Malaysia. There was concern that companion animals may play a role in the transmission of ESBL -producing Enterobacteriaceae since they interact closely with humans (Santaniello et al., 2021).

1.3 Hypotheses

The hypotheses that were tested in this study are:

@COPYRIGHT UPM

H_0: No ESBL-*E. coli* detected in the warded animal, health premise environment, and personnel in UVH

H_A: Presence of ESBL-*E. coli* in the warded animal, health premise environment, and personnel working in the environment in UVH

@COPYRIGHT UPM

CHAPTER 2: LITERATURE REVIEW

2.1 Enterobacteriaceae

Enterobacteriaceae basically were found in environment and intestine in human and animals. Enterobacteriaceae were like rod shaped, gram negative, non-sporulating, facultative aerobic. The gram-negative (-ve) rod-shaped bacteria *Escherichia. coli* belongs to the Enterobactericae family. The size is 1-3 x 0.4-0.7 m in size and 0.6 to 0.7 m in volume when viewed under a microscope. They can be arranged singly or in pairs and are motile because to peritrichous flagella, though certain strains are not. Some strains, such as type 1 (hemagglutinating and mannose-sensitive), which is present in both motile and non-motile strains, may be fimbriated. Public health was concerned about 3 forms of pathogenic *Escherichia coli*: enterotoxigenic *E. coli* (ETEC), diarrheagenic *E. coli*, and Shiga toxin-producing *E. coli* (STEC). E. coli with six different diarrheagenic pathotypes can affect both humans and animals.

2.2 Antimicrobial resistance

Antimicrobial resistance was a global issue that is getting worse. It lowers the efficacy of antibiotics used to treat infectious infections in people and animals, increasing morbidity and mortality as well as costs. The significant correlation between the use of antimicrobial drugs and the development of resistance is well known. The development of resistant pathogenic organisms or the horizontal transfer of resistance genes from one type of organism to another are two ways that antimicrobial resistance might spread.

@COPYRIGHT UPM

2.3 Beta lactam antibiotic

The largest and most used class of antimicrobial medicines both in Sweden and globally were beta-lactam antibiotics. The beta-lactam ring, a molecular structure, is what sets the group apart. Depending on the ring structure fused to the beta-lactam ring, they can be categorised into four separate categories based on their chemical makeup, although they are frequently divided into the following groups: penicillins, cephalosporins, carbapenems, monobactam, and beta-lactamase inhibitors.

Through inhibition of penicillin-binding proteins, beta-lactam antibiotics prevent the transpeptidation of the peptidoglycan component of cell walls; however, it is unknown how exactly this results in cell death. (Hongbaek et al., 2015) The majority of the substances used in beta-lactam antibiotics are semi-synthetic and derived from environmental bacteria and fungus. (Rodriguez-Herrera et al., 2019) There are more than 80 distinct beta-lactams in therapeutic usage. While some beta-lactams target only Gram-positive bacteria, others target both Gram-positive and Gram-negative bacteria and have a far broader antibacterial spectrum.

One of the earliest antibiotics to enter commercial production was penicillin G in the 1940s. They can be administered either orally or parenterally and are split into subgroups based on their antibacterial spectrum and stability against penicillinases. Because penicillins are frequently eliminated by the kidneys unmetabolized, their concentration in urine can be very high. (Martin et al., 2020)

@COPYRIGHT UPM

Ceftazidime was effective against a wide range of Gram-negative bacteria, including Enterobacteriaceae, particularly *E. coli, Klebsiella spp., Proteus spp., and Pseudomonas aeruginosa*. It is administered parenterally and primarily used to treat nosocomial pneumonia in patients with cystic fibrosis and cystic fibrosis, as well as probable septicaemia in individuals who are neutropenic.

Cefotaxime was a parenterally administered broad spectrum cephalosporin used to treat meningitis and other severe infections of the skin, soft tissues, and internal organs. The Enterobacteriaceae, particularly *E. coli, Klebsiella spp., Proteus spp., Salmonella spp., and Shigella spp*., as well as a several skin and respiratory tract infections, are covered by the antimicrobial spectrum. (Padda et al., 2022)

Beta-lactamases, including several ESBLs, are highly well tolerated by carbapenems. This broad spectrum of activity, which includes Gram-positive and Gram-negative aerobic and anaerobic bacteria, is the result of their special mechanism of outer membrane permeability. All carbapenems are administered parenterally. (Rawat et al., 2010)

The antibacterial combination of amoxicillin and clavulanic acid was effective against both Gram-positive and Gram-negative, aerobes and anaerobes, which can be found on the skin, respiratory tract, and saliva. It also had an impact on some members of the Enterobacteriaceae family. The medication was used to treat simple pneumonia, upper respiratory tract infections, UTIs, and bite wounds. (Evans et al., 2021)

@COPYRIGHT UPM

2.4 Extended-spectrum beta-lactamases

Extended spectrum beta-lactamases (ESBLs) were characterised as enzymes capable of hydrolyzing extended spectrum cephalosporin and are produced by specific bacteria. As a result, they were effective in combating beta-lactam drugs including ceftazidime, ceftriaxone, cefotaxime, and oxyiminomonobactam (Bradford, 2001; Paterson and Bonomo, 2005)

The most significant and often found beta-lactamase is TEM-1. It was expected that the presence of TEM-1 is responsible for more than 90% of *E. coli* ampicillin resistance (Livermore, 1995). Penicillin and first-generation cephalosporins can be hydrolyzed by TEM-1.

2.4.1 Classification of Extended-spectrum beta-lactamases-

Beta lactamase can be classified based on molecular structure and different schemes According to the Bush, Jacoby and Medeiros scheme, beta-lactamases were divided into four groups The first, and most extensively used, approach was developed by Ambler (Ambler et al., 1991).

Group I (Ambler Class C) beta-lactamases. This group, which was primarily present on chromosomes, was resistant to beta-lactamase inhibitors like clavolunate (Minami et al., 1980). The enzyme belongs to this class and is induced. Therefore, any beta-lactame antibiotic exposure to bacteria results in an increase in the production of

@COPYRIGHT UPM

enzymes. Penicillins, cephamycins, first-, second-, and third-generation cephalosporins, as well as beta lactam/beta lactamase inhibitor combos are all ineffective against Group I producer beta-lactamases. Cefepime and carbapenems are ineffective against them (Sanders et al., 1996).

Group 2 (Ambler Class A) enzymes. Since group 2 enzymes were carried via plasmid, they could quickly spread into different bacterial cells, leading to rapid resistance to such enzymes. The original group 2 enzymes were inhibited by beta-lactamase inhibitors such clavulanic acid, sulbactam, and tazobactam. The two primary enzymes in group 2 are TEM and SHV. Group 2 enzymes, often known as extended spectrum betalactamases or ESBLs, were resistant to beta lactamase and may hydrolyze ampicillin, first-, second-, and third-generation cephalosporins, as well as monobactams (Livermore, 1995).

Group 3 (Ambler Class B) enzymes. These metallo-enzymes can eliminate carbapenems (Burn-Buisson et al., 1987). *P. aeruginosa, Bacteroides fragilis*, and *Stenotrophomonas maltophilia* are frequently discovered to contain these enzymes.

Group 4 Beta lactamase. Group 4 beta-lactamases contains those unusual penicillinases not inhibited by clavulanic acid. Four of these enzymes exhibit high rates of hydrolysis with carbenicillin and/or cloxacillin.

2.4.2 Types of Extended-spectrum beta-lactamases

@COPYRIGHT UPM

Most ESBLs are derivatives of TEM or SHV enzymes. There were now more than 90 TEM-type beta-lactamases and more than 25 SHV-type enzymes. (Bradford, 2001) The most typical -lactamase seen in gram-negative bacteria is TEM-1. The creation of TEM-1 by *E. coli* is the cause of up to 90% of the organism's ampicillin resistance. This enzyme was also to responsible for the rising prevalence of ampicillin and penicillin resistance in N. gonorrhoeae and H. influenzae. Penicillins and early cephalosporins like cephalothin and cephaloridine can be hydrolyzed by TEM-1. The first -lactamase derivative, TEM-2, differed just in one amino acid from the original. (Barthelemy et al., 1985) The substrate profifile remained unchanged, however the isoelectric point changed from 5.4 to 5.6. There were only a few sites in the TEM enzyme where amino acid changes take place. Combinations of these amino acid modifications cause ESBL phenotypes to change subtly in a number of ways, such as their capacity to hydrolyze particular oxyimino-cephalosporins like ceftazidime and cefotaxime, or a shift in their isoelectric points, which can change from a pI of 5.2 to 6.5.

The SHV-1 beta-lactamase was most commonly seen in *K. pneumoniae* and accounts for up to 20% of the species' plasmid-mediated ampicillin resistance (Tzouvelekis et al., 1999)). Currently, the ESBL phenotype was found in the majority of SHV-type derivatives. SHV-10, on the other hand, has been shown to have an inhibitor-resistant phenotype. This enzyme appears to be derived from SHV-5, with the addition of one amino acid substitution of glycine for serine 130. The bulk of SHV-type ESBLs are discovered in *K. pneumoniae* strains. These enzymes, however, have also been discovered in *Citrobacter diversus, E. coli,* and *P. aeruginosa*. (Bradford, 1994)

@COPYRIGHT UPM

Recent years have seen the emergence of the CTX-M family of plasmid-mediated ESBLs, which preferentially hydrolyze cefotaxime. Although they had also been reported in other Enterobacteriaceae species, they have primarily been identified in *Salmonella enterica serovar Typhimurium* and *E. coli* strains. These enzymes only share about 40% of their DNA with the two most often isolated beta-lactamases, TEM or SHV, indicating that they were not very closely related to those enzymes (Tzouvelekis et al., 2000)). According to kinetic studies, the CTX-M-type beta-lactamases preferentially hydrolyze cefotaxime over ceftazidime and hydrolyze cephalothin or cephaloridine better than benzylpenicillin. Despite being identified from numerous locations around the world, strains containing CTX-M-type beta-lactamases have most frequently been linked to focused epidemics in eastern Europe,South America, and Japan. However, 23 *Salmonella* and *E. coli* isolates from Spain that were recently discovered to display the CTXM-9 beta-lactamase raises the possibility that this enzyme may also be prevalent throughout western Europe (Sabate et al., 2000)

2.4.3 Epedemiology of Extended-spectrum beta-lactamases

ESBLs are now a worldwide issue for hospitalised patients. Most likely because expanded-spectrum -lactam antibiotics were originally utilised there in clinical settings, the ESBL phenomenon first emerged in western Europe. But it didn't take long until ESBLs had been found in Asia and the United States. Depending on the institution, the incidence of ESBL generation in Enterobacteriaceae in the United States ranges from 0 to 25%, with the national average being approximately 3%. In Europe, there are significant

@COPYRIGHT UPM

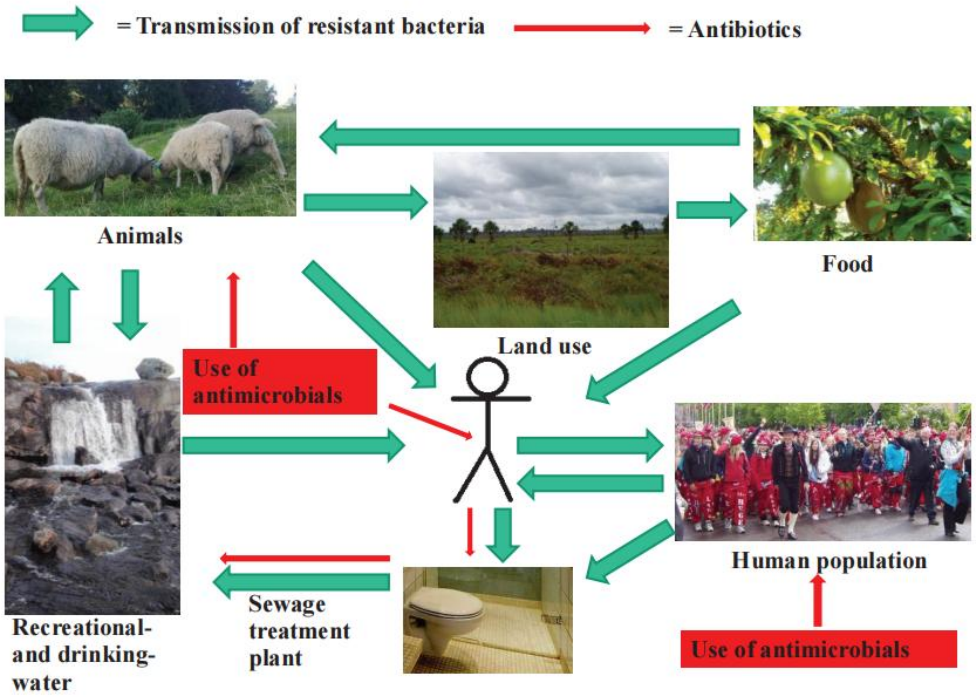
regional differences in the proportion of Enterobacteriaceae isolates that produce ESBLs. A study of 11 hospital laboratories in the Netherlands found that 1% of the strains of *E. coli* and *K. pneumoniae* had an ESBL (Stobberingh et al., 1999). The percentage of -lactam resistance resulting from ESBL synthesis in *E. coli* and *K pneumoniae* in Japan continues to be extremely low. An ESBL was found in 0.1% of *E. coli* and 0.3% of *K. pneumoniae* strains in a recent investigation of 196 institutions nationwide (Yagi et al., 2000). In other parts of Asia, *E. coli* and *K. pneumoniae* produce ESBLs to varying degrees, ranging from 4.8% in Korea to 8.5% in Taiwan and up to 12% in Hong Kong.

2.4.4 Mode of transmission

There are probable cycles of transmission of bacteria, plasmids, and other mobile genetic components containing CTX-M between different hosts, populations of humans and animals, and the environment, before returning to the human population. Selection and horizontal gene transfer that take place in the many compartments enhance these cycles. Each of these divisions' research findings will be presented separately.

Figure 2.1 - Dissemination of ESBL between the patient, the population and the environment

@COPYRIGHT UPM



Intestinal carriage. Through horizontal gene transfer in the gut, even transient bacteria carrying ESBL plasmids may give their resistance genes to a host. Faecal transport is a major factor in the ESBL transmission cycle because it is the cause of faecal-oral transmission as well as environmental pollution.

The potential spread of ESBL from food to humans is a significant topic of research. A genetic link between ESBL-genes present in food, animals, and humans has been demonstrated by at least one study. Antimicrobials used in food production may make it more likely for ESBL-producing bacteria to proliferate among farm animals.

Sewage treatment facilities are sources of ESBL environmental pollution since ESBL-*E. coli* has been found in their effluent and affluent. Even ESBL-genes that are still present from these plants are a source of pollution because bacteria may acquire them

through spontaneous transformation or other forms of horizontal gene transfer. Humans may contract bacteria that have been discharged into the environment or altered bacteria either directly through exposure to ambient fluids or dust or indirectly through the food chain or other animals. Therefore, choosing the best water treatment technique may limit the growth of bacterial resistance. These artificial aquatic systems may be a factor in the current spread of resistance.

2.4.5 Mechanism of Extended-spectrum beta-lactamases

Beta-lactam resistance is primarily caused by bacterial development of enzymes that cleave the beta-lactam ring or by a structural alteration of the penicillin binding proteins (resulting in a decrease in the drug's affinity). Reduced permeability or active transportation via efflux pumps are further mechanisms.

2.4.6 Detection method of Extended-spectrum beta-lactamases

Double-disk diffusion test. A swab of the organism was placed on a Muller - Hinton agar plate. An antibiotic disc containing one of the oxyimino-lactam antibiotics was positioned 30mm (centre to centre) away from the amoxicillin-clavulanic acid disc. A positive result was the enhancement of the oxyimino -lactam's zone of inhibition brought on by the synergy of the clavulanate found in Amoxy-clav disc. The test is more trustworthy now. By bringing the space between the discs closer together (20 mm), the

@COPYRIGHT UPM

sensitivity is increased. When clavulanate (4 g/ml) is added, the MHA's sensitivity is increased. First, inoculate the pure culture into the Muller Hinton agar plate by swiping the swab over the entire agar surface three times in a back-and-forth motion, rotating the plate by about 60 degrees each time.insert the antimicrobial-impregnated discs (clavulanic acid and cefotaxime) on top of the agar, cover with the lid, invert the plate and incubate the plates at 35°C for 16 to 18 hours. After 24 hours, read the results. Zones of inhibition have a smaller diameter as a result of *E. coli* resistance that produces a wider range of beta lactamases. (Rawat et al., 2010)

Three dimensional test. Advantage of simultaneous determination of antibiotics susceptibility and beta lactamase substract profile, 2 types of inoculam are prepared. Which is inoculam 1 contain 10^9 - 10^10 CFU/ml of active ESBL producer. Inoculam 2 contains 0.5 Mc Farland std (150 millions organism per ml)

The minimum inhibitory concentration (MIC) value is the lowest drug concentration at which the growth of a bacterial isolate is inhibited. The MIC values can be converted into an ordinal scale, where S stands for sensitive, I for intermediate, and R for resistant. Committees and organisations determine the thresholds at which a certain bacterial species is classified as S, I, or R, with the goal of determining whether the bacterium is treatable or not. (Kowalska-Krochmal et al., 2021)

PCR. DNA will be extracted from the samples using the boiling procedure in order to locate the genes TEM, SHV, and CTX-M for the genotype identification for ESBL E. coli. (Naas et al., 2007) The PCR is a thermal cycler that generates genomic

@COPYRIGHT UPM

sequences by annealing and denaturing at temperatures of 95°C for 30 seconds each, followed by an elongation step that lasts 1 minute and 5 minutes at 72°C. The National Center of Biotechnology Information's Basic Local Alignment Search Tool (BLAST) is used to retrieve and analyse the genomic sequences (NCBI). (Lorenz, 2012)

Multiplex polymerase chain reaction (mPCR). Real-time amplification is performed in 25 µL reactions with one negative control strain (N1) is used with a set of five positive control strains (P1–5). For a maximum of polymerase activity, a preliminary heating start at 95°C for 15 min. Then 30 cycles of 95°C for 15 sec; 50°C for 15 sec and 70°C for 20 sec. The fluorescence signals are detected in four different channels.

2.4.7 Treatment of Extended-spectrum beta-lactamases

The Trans 6-hydroxyethyl group in carbapenems makes them extremely resistant to the hydrolytic action of all ESBLs, making them the most reliable and effective antimicrobial agents. The most active drug, Meropenem, has a MIC lower than Imipenem's. There are only a few effective lactam, 7-methoxy cephalosporins, like Cefoxitin, Cefotetan, and Latamoxcef. Combination of a beta lactam and a beta lactamase inhibitor, such as piperacillin or amoxicillin clavulanate Tazobactam may be yet another choice to take into account. Although effective in vitro, cephamycins like Cefoxitin and Cefotetan are not advised for treating such infections due to how easily these strains can reduce the production of outer membrane proteins, making them resistant. Combination

@COPYRIGHT UPM

therapy with clavulanic acid is an option for urinary tract infections. (Chaudhary et al., 2004)

2.4.8 Prevention of Extended-spectrum beta-lactamases

Stewardship of antibiotics is crucial for reducing the rate at which bacteria acquire resistance and for preventing coselection in veterinary and human treatment. Since we found cefpodoxime-resistant strains in 14% of the studied pets, there is a need to raise veterinarians' and doctors' knowledge of the problems caused by antibiotic resistance. Transmission of cefpodoxime-resistant strains from pets to humans or vice versa cannot be ruled out. It is important to establish joint antibiotic monitoring programmes, like the Canadian Integrated Program for Antimicrobial Resistance Surveillance, that share databases of antibiotics used in both human and animal medicine. Recent studies revealed the possibility of synergistic interactions between the veterinary and human health sectors.

@COPYRIGHT UPM

Chapter 3: METHODOLOGY

3.1 Sample collection

A total of 104 fecal samples derived from warded dogs and cats, resident dogs and cats, University Veterinary Hospital staff, and highly touchable surface in UVH. For 15 warded dogs and cats were swabbed (rectal swab) at University Veterinary Hospital. Samples were collected upon the approval of the owners. Basic background information on dogs regardings species, types of antibiotics, route administration of antibiotics, duration use of antibiotics were obtained through case file. For human sampling, 30 fecal samples were collected from UVH staff. Basic background information on staff regarding on outdoor activities, 3 month prior prescribed antibiotics, hand hygiene during working hours, nutrition was obtained through an initial questionnaire. For 59 environment sample from highly touchable surface from front clinic, large animals ward, cat and dogs ward, operating theater (25 cm^2). Those targeted sites are chair, desk, floor surface, door knob, thermometer, computer, examination table, cage, trolley, muzzle and fridge. Types of inanimate objects and sampling site of each object are summarized in Table 3.1.

Table 3.1: List of inanimate objects and the area where swabs samples were taken

Types of inanimate objects	*Numbers of objects sampled*	*Sampling site*
Animal cage	*5*	*Whole surface*

@COPYRIGHT UPM

Floor surface	*4*	*25cm^2 surface*
Water tap	*4*	*Whole surface*
Door knob	*3*	*Whole surface*
chair	*4*	*25cm^2 surface*
desk	*3*	*25cm^2 surface*
computer	*4*	*Whole surface*
table	*4*	*25cm^2 surface*
Light switch	*2*	*Whole surface*

By using sterile swabs pre-moistened with normal saline, two swabs samples were taken simultaneously on each surface

3.2 Bacteria isolates

Fecal samples were enriched in buffered peptone water, and then cultured on MacConkey (MCC) agar containing 1mg/L of cefotaxime (MCC-CTX). Streak plate technique was used. All cultured media were incubated at 37℃ for 18-24 h aerobically in an incubator. When growth occurred on MCC, typical pink colonies suspected to be *E. coli* were selected for sub-cultured on Nutrient agar and incubated overnight at 37℃ for 18-24 h.

@COPYRIGHT UPM

3.3 Biochemical test

Suspected of *E. coli* typified by biochemical tests (Indole test, oxidase test, Triple sugar iron agar, sulfur indole motility test, citrate test, urease test). *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as quality control. Indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. Oxidase is a test that performed to determine which microorganisms possess the cytochrome oxidase enzyme (important in the electron transport chain). Oxidase negative Enterobacteriaceae and oxidase positive Pseudomadaceae are frequently distinguished using this method. Triple sugar iron agar (TSI) is a differential medium that contains lactose, sucrose, a small amount of glucose (dextrose), ferrous sulphate, and the pH indicator phenol red. It is used to differentiate the enterics based on the ability to reduce sulphur and ferment carbohydrates. SIM is a differential medium. It tests the ability of an organism to do several things: reduce sulphur, produce indole and swim through the agar (be motile). SIM is commonly used to differentiate members of Enterobacteriaceae. A specified medium called citrate agar is used to test an organism's ability to use citrate as its only source of carbon. It is frequently used to distinguish between Enterobacteriaceae family members. Urea test is used to identify bacteria capable of hydrolyzing urea using the enzyme urease.

3.4 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was determined by disk diffusion method on Mueller Hilton agar plates (Oxoid) according to Clinical Laboratory Standard Institute

@COPYRIGHT UPM

(CLSI) recommendations. Based on the observed diameters of the inhibition zones compared to the accepted standards interpretative zone diameter, isolates were categorised as susceptible, intermediate, or resistant. The antibiotics tested were chloramphenicol (30 µg), imipenem (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), doxycycline (30 µg), amoxicillin-clavunate acid (10 µg), cefazolin (30 µg), gentamicin (10 µg), enrofloxacin (5 µg), nitrofurantoin (30 µg), and sulfamethoxazole (25 µg) according to Clinical and Laboratory Standards Institute, CLSI. (CLSI, 2015a).

Table 3.2: Antibiotic Susceptibility Interpretative Criteria as described by CLSI VET01-S2 guideline (2013)

Antimicrobial agent	Disk content	Zone diameter (mm)		
		S	I	R
Chloramphenicol	30 µg	≧18	13-17	≦12
imipenam	10 µg	≧23	20-22	≦19
cefotaxime	30 µg	≧26	23-25	≦22
ceftazidime	30 µg	≧21	18-20	≦17
doxycycline	30 µg	≧14	11-13	≦10
amoxicillin-	10µg	≧18	14-17	≦13

@COPYRIGHT UPM

clavunate acid				
cefazolin	30µg	≧23	20-22	≦19
gentamicin	10µg	≧16	13-15	≦12
nitrofurantoin	30µg	≧17	15-16	≦14
enrofloxacin	5µg	≧23	17-22	≦17
sulfamethoxazole	25µg	≧16	11-15	≦10

3.5 ESBL identification

First ESBL disc screening test was evaluated using antibiotic discs of cefotaxime alone, ceftazidime alone, cefotaxime with clavulanic acid and ceftazidime with clavulanic acid on Mueller Hinton agar. (CLSI, 2015). For the ESBL confirmation, the Double-Disc Synergy Test was applied according to CLSI 2013; discs containing ceftazidime and cefotaxime were put next to a disc with amoxicillin plus clavulanic acid (20 mm centre to centre). The positive result is indicated when a ≧ 5-mm increase in a zone diameter for either antimicrobial agent tested in combination with amoxicillin-clavulanate acid. *E. coli* ATCC 25922 was used for quality control.

@COPYRIGHT UPM

3.6 Statistical analysis

Statistical analyses were conducted by using SPSS. Descriptive statistics will be used to report the occurrence, and antimicrobial susceptibility of ESBL *E. coli*. Potential risk factors will be checked using the Chi-square or Fisher's exact test. Significance will be set at $p < 0.05$ for all tests. The Chi-square test or Fisher's exact was used to investigate significant differences in the ESBL presence in UVH staff according to gender, outdoor activities, prior prescribed antibiotics. For univariable such as last time taken antibiotics, hand hygiene during working hours and nutrition, logistic regression test was used to investigate significant difference in potential risk factors.

@COPYRIGHT UPM

Chapter 4: RESULTS

4.1 Identification of *Escherichia coli* isolate

104 bacterial isolate were collected from ward dogs (n = 2), sick dog (n = 4), warded cats (n = 6), healthy cats (n = 3), highly touch surface (n = 59) and staff UVH (n = 30). Those strains that had grown on agar with cefotaxime were selected to carry out biochemical test.

Table 4.1: Isolates obtained from various specimens (n=104)

Total	Culture positives	Culture negatives
104	88	16

Out of the 104 specimens obtained, 88 were found to be culture positive and 16 were culture negative

4.2 Biochemical Test for *E. coli*

Biochemical identification revealed *E. coli* on MCC-CTX media. Out of the 104 specimens obtained, 88 were found to be culture positive and 16 were culture negative (88/104). Out of 88 isolates 20 (19.23%) were *Escherichia. coli*. For biochemical test, *Escherichia. coli* should be negative oxidase test, positive indole test, present of gas and acidic condition in Triple sugar iron agar, negative citrase test, negative urease test, absent of sulfur and motility in SIM agar.

@COPYRIGHT UPM

Table 4.2: Percentage of Gram-negative bacteria

Organism	Total	Percentage
Escherichia. coli	20	22.7
Klebsiella pneumonia	11	12.5
Klebsiella oxytoca	1	1.1
Proteus species	1	1.1

Out of 88 bacteria isolates 20 (22.7%) were *E. coli*, 11 (12.5%) were *Klebsiella pneumonia*, 1 (1.1%) were *Klebsiella oxytoca*, 1 (1.1%) were *Proteus. Species* and other organisms.

Table 4.3: Resistant Strain among E. coli

Number of Isolates	Resistant to 3rd generation cephalosporins
88	10

Out of 88 isolates, 11 (12.5%) were found to be ESBL-*E. coli* producers by Double disk diffusion method, 10 out of 11 were resistant to cefotaxime

@COPYRIGHT UPM

Table 4.4: Antimicrobial resistance patterns of Escherichia coli isolated

Antibiotics	S		I		R	
	N	(%)	N	(%)	N	(%)
Chloramphenicol	5	45	0	0	6	54
Imipenem	10	90	0	0	1	9
Cefotaxime	1	9	0	0	10	90
Doxycycline	4	36	1	9	6	54
Amoxicillin-clavulanic acid	7	63	1	9	3	27
Cefazolin	0	0	0	0	11	100
Enrofloxacin	0	0	9	81	2	18
Gentamicin	4	36	1	9	6	54
Nitrofurantoin	9	81	1	9	1	9
Sulfamethaxazole	3	27	0	0	8	72

N: Number; %: Percentage; S: susceptible; I: intemediate; R: resistant

©COPYRIGHT UPM

4.3 Antimicrobial resistance rate of ESBL to antibiotics

Antimicrobial resistance rate of ESBL for chloramphenicol (54.5%), imipenem (9%), cefotaxime (91%), doxycycline (54.5%), amoxicillin-clavulanic acid (27.3%), cefazolin (100%), enrofloxacin (18.2%), gentamicin (54.5%), nitrofurantoin (9.1%), sulfamethoxazole (73%).

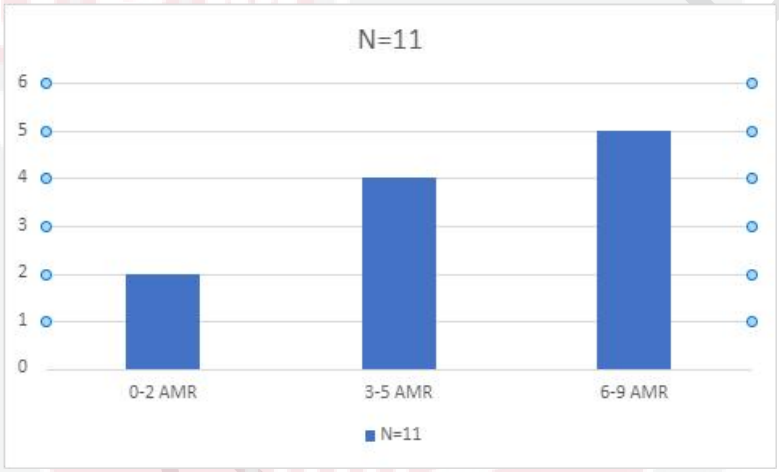


Figure 4.1: Multidrug resistance of 11 isolates

4.4 Risk factor associated with ESBL-*E. coli* contamination

Fisher's Exact test showed that prior antibiotics-use was not statistically significant to occurrence of ESBL-*E. coli.* (p= 0.174).

@COPYRIGHT UPM

Table 4.5: Association of prior antibiotics-use with ESBL-E. coli occurrence

	Value	*df*	*Asymptotic Significance (2-sided)*	*Exact Sig. (2-sided)*	*Exact Sig. (1-sided)*
Pearson Chi-square	*4.966*	*1*	*.026*		
Continuity Correction	*.774*	*1*	*.379*		
Likelihood ratio	*3.728*	*1*	*.054*		
Fisher's Exact Test				*.174*	*.174*
Linear-by-linear Association	*4.750*	*1*	*.029*		
N of valid case	*23*				

Univariable logistic regression model indicated only one of these factor that had the likelihood of causing ESBL-*E. coli* contamination, at p-value less than 0.05, which

@COPYRIGHT UPM

included is wash hand with alcohol (hand hygiene) while the others such as duration antibiotics used, hand hygiene during working hour, nutrition are not significant.

However, based on the multivariable logistic regression model, none of these factors significantly associated with ESBL-*E. coli* isolation. (Table 4.6) In this study, the possible low detection of *E. coli* in health-care worker maybe because the use of glove have reduced Health-care worker contamination rates by decreasing the acquisition of bacteria over time when compared to bare hands.

Table 4.6: Univariable and mutivariable factors associated with ESBL E. coli

Variables	P-value	Univariable analysis		Multivariable analysis	
		OR	95% CI	OR	95% CI
Duration of antibiotic used	0.951	1.022	0.509-2.054		
Wash hand with water	0.296	0.564	0.192-1.652		
Wash hand with alcohol	0.198	2.496	0.619-10.057		
Wash hand with soap	0.620	1.437	0.343-6.014		

@COPYRIGHT UPM

Wear disposable glove	0.361	0.683	0.301-1.549		

@COPYRIGHT UPM

Chapter 5: DISCUSSION

5.1 Antibiotic Stewardship

Identifying the risk factor associated with presence of ESBL-*E. coli* can provide a scientific basis for designing effective way to reduce the risk of ESBL-*E. coli*. Antibiotic Stewardship is crucial for reducing the rate at which bacteria acquire resistance and for preventing co-selection in veterinary and human treatment. Therefore, there is a need to raise veterinarians' and doctors' knowledge of the problems caused by antibiotic resistance (Anderrson, 2010). IPC standards, hand hygiene supplies, and hand hygiene compliance were largely insufficient in the clinic; therefore, improvement in these areas might help to contain the spread of the AMR.

5.2 Detection of ESBL method

Rectal swabs were used to collect *E. coli* from animal because the gastrointestinal system is the most important reservoir for nosocomial Gram-negative organisms such as MDR *E. coli* and *Enterobacter spp*. (Dyakova et al., 2017) In addition, clinical extraintestinal infection frequently precedes intestinal carriage in humans. (Urban et al., 2020)) Environmental samplings are usually performed by using moistened sterile swabs, and the area to swab should be fixed, for example 25cm^2(Aklilu et al., 2012).

In this study, MacConkey with cefotaxime was used to selectively isolate ESBL *E. coli* and other enterobacteriaceae. MacConkey can inhibit the growth of gram-positive

@COPYRIGHT UPM

bacteria without affecting the growth of coliform bacteria and *E. coli*. Furthermore, *E. coli* appear as pinkish colonies in the MacConkey agar. However, growth of Gram-negative bacteria other than *E. coli* were observed in plates. It can be confusing to differentiate *E. coli*. Therefore, biochemical test such as indole test, oxidase test, TSI, citrase test, urease test, SIM test must be performed to confirm for *E. coli*.

.

5.3 Antimicrobial resistance rate

High levels of AMR, particularly against beta-lactams, were found in the current investigation, along with a correlation with beta-lactamase inhibitors. The highest AMR rates were seen for amoxicillin (75%) and amoxicillin-clavulanic acid (77%), among the tested isolates.(Bertenolli et al., 2020). While compare to this study, the antimicrobial ressitance rate of ESBL *E. coli* for amoxicillin-clavulanic acid was low (27.3%). This could be due to the lack of sample collected.

Our study reported high level of resistance to first and third generation cephalosporins (cefazolin, 100% and cefotaxime, 90%). Antibiotic treatment is known to influence the acquisition of resistance in microorganism. Resistence to thhird-generation cephalosporin would leaveus with few options for treating patients with gram-negative bacteremia, and may require the use of carbapenem. (Han et al., 2014). Resistance to cephalosporins was of particular concern because these compounds, particularly C3G and

@COPYRIGHT UPM

C4G, are crucial for human medicine. They are rarely used in veterinary medicine and should not be used as the first line therapy.

5.4 Limitations

Limitation of this study includes the small sample size due to lack of time, where only 5 weeks were available for sampling and laboratory work.

@COPYRIGHT UPM

Chapter 6: CONCLUSION

In this study, the null hypothesis was rejected. ESBL-*E. coli* were detected in warded animals, heath premise environment, and personnel working in UVH. Based on the study, none of these factors (prior-antibiotics used, hand hygiene) was statistically significant to occurrence of ESBL-*E. coli.* Imipenam is still effective against ESBL-*E. coli* while the resistance rate of ESBL-*E. coli* to the first and third generation of cephalosporin (cefazolin and cefotaxime) were very high.

It is recommended to extend the period of study to obtainlarger sample size, as the sample size of this study is not representative. Furthure wide-scale studies amongst veterinary personnel, pet animals and their owners in Malaysia will better document the public health risk of ESBL *E. coli.* Next, the use of molecular technique can improve sensitivity of detection of the bacteria of interest.

@COPYRIGHT UPM

REFERENCES

Aliyu, A. B., Saleha, A. A., Jalila, A., & Zunita, Z. (2016). Risk factors and spatial distribution of extended spectrum β-lactamase-producing- Escherichia coli at retail poultry meat markets in Malaysia: a cross-sectional study. BMC Public Health, 16(1).

Abbott, S.L. Klebsiella, Enterobacter, Citrobacter, Serratia, Plesiomonas, and other Enterobacteriaceae. In Manual of clinical microbiology. Murray, P., Baron, E.J., Jorgensen, J.H., Landry, M.L. & Pfaller, MA., editors. 2007. Washington DC. ASM Presss. p. 698-715

Baede, V. O., Wagenaar, J. A., Broens, E. M., Duim, B., Dohmen, W., Nijsse, R., Timmerman, A. J., & Hordijk, J. (2015). Longitudinal Study of Extended-Spectrum-β-Lactamase- and AmpC-Producing Enterobacteriaceae in Household Dogs. Antimicrobial Agents and Chemotherapy, 59(6), 3117–3124.

Bauernfeind, A., Schweighart, S., & Grimm, H. (1990). A new plasmidic cefotaximase in a clinical isolate of Escherichia coli. Infection, 18(5), 294-298.

Bradford, P. A. (2001). Extended-Spectrum β-Lactamases in the 21st Century: Characterization, Epidemiology, and Detection of This Important Resistance Threat. Clinical Microbiology Reviews, 14(4), 933–951.

@COPYRIGHT UPM

Bennett, P. M. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. Br J Pharmacol 153, S347-357, doi:10.1038/sj.bjp.0707607 (2008)

Bidet, P., Burghoffer, B., Gautier, V., Brahimi, N., Mariani-Kurkdjian, P., El-Ghoneimi, A., ... & Arlet, G. (2005). In vivo transfer of plasmid-encoded ACC-1 AmpC from Klebsiella pneumoniae to Escherichia coli in an infant and selection of impermeability to imipenem in K. pneumoniae. Antimicrobial agents and chemotherapy, 49(8), 3562-3565.

Bonnet, R. (2004). Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrobial agents and chemotherapy, 48(1), 1-14.

Carvalho, I., Cunha, R., Martins, C., Martínez-Álvarez, S., Safia Chenouf, N., Pimenta, P., Pereira, A. R., Ramos, S., Sadi, M., Martins, N., Façanha, J., Rabbi, F., Capita, R., Alonso-Calleja, C., de Lurdes Nunes Enes Dapkevicius, M., Igrejas, G., Torres, C., & Poeta, P. (2021). Antimicrobial Resistance Genes and Diversity of Clones among Faecal ESBL-Producing Escherichia coli Isolated from Healthy and Sick Dogs Living in Portugal. Antibiotics, 10(8), 1013.

Chen, Y., Liu, Z., Zhang, Y., Zhang, Z., Lei, L., & Xia, Z. (2019). Increasing Prevalence of ESBL-Producing Multidrug Resistance Escherichia coli From Diseased Pets in Beijing, China From 2012 to 2017. Frontiers in Microbiology, 10.

@COPYRIGHT UPM

Coudran, P. E. (1997). Occurrence and Detection of ESBL in member of the Family Enterobacteriaceae at a Veterans Medical Center Seek and You May Find. JCM, 2593, 2597.

Cavaco, L. M., Abatih, E., Aarestrup, F. M., & Guardabassi, L. (2008). Selection and persistence of CTX-M-producing Escherichia coli in the intestinal flora of pigs treated with amoxicillin, ceftiofur, or cefquinome. Antimicrobial agents and chemotherapy, 52(10), 3612-3616.

Donnenberg, M.S. Enterobacteriaceae. In Principles and practice of infectious diseases. Mandell, G.L., Bennett, J.E. & Dolin, R., editors. 2005. London. Elsevier, Churchill, Livingstone. p. 2567-2586.

Donnenberg, M.S. Enterobacteriaceae. In Principles and practice of infectious diseases. Mandell, G.L., Bennett, J.E. & Dolin, R., editors. 2005. London. Elsevier, Churchill, Livingstone. p. 2567-2586.

Dolejska, M., Villa, L., Poirel, L., Nordmann, P. & Carattoli, A. Complete sequencing of an IncHI1 plasmid encoding the carbapenemase NDM-1, the ArmA 16S RNA methylase and a resistance-nodulation-cell division/multidrug efflux pump. The Journal of antimicrobial chemotherapy 68, 34-39 (2013).

@COPYRIGHT UPM

Ewers, C., Bethe, A., Semmler, T., Guenther, S., & Wieler, L. (2012). Extended-spectrum β-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: a global perspective. Clinical Microbiology and Infection, 18(7), 646–655.

Espinosa-Gongora, C., Shah, S. Q. A., Jessen, L. R., Bortolaia, V., Langebæk, R., Bjørnvad, C. R., & Guardabassi, L. (2015). Quantitative assessment of faecal shedding of β-lactam-resistant Escherichia coli and enterococci in dogs. Veterinary Microbiology, 181(3–4), 298–302.

Farmer III, J., Boatwright, K.D. & Janda, J.M. Enterobacteriaceae: introduction and identification. In Manual of clinical microbiology. Murray, P., Baron, E.J., Jorgensen, J.H., Landry, M.L. & Pfaller, M.A., editors. 2007. Washington DC. ASM Press. p. 649-669.

Fisher, J. F., Meroueh, S. O., & Mobashery, S. (2005). Bacterial resistance to β-lactam antibiotics: compelling opportunism, compelling opportunity. Chemical reviews, 105(2), 395-424.

Ghafourian, S., Sadeghifard, N., Soheili, S., & Sekawi, Z. (2015). Extended spectrum beta-lactamases: definition, classification and epidemiology. Current Issues in Molecular Biology, 17(1), 11-22.

©COPYRIGHT UPM

Gagliotti, C., Balode, A., Baquero, F., Degener, J., Grundmann, H., Gür, D., ... & Heuer, O. (2011). Escherichia coli and Staphylococcus aureus: bad news and good news from the European Antimicrobial Resistance Surveillance Network (EARS-Net, formerly EARSS), 2002 to 2009. Eurosurveillance, 16(11), 19819.

Gandolfi-Decristophoris, P., Petrini, O., Ruggeri-Bernardi, N., & Schelling, E. (2013). Extended-spectrum β-lactamase-producing Enterobacteriaceae in healthy companion animals living in nursing homes and in the community. American Journal of Infection Control, 41(9), 831–835.

Hong, J. S., Song, W., Park, H. M., Oh, J. Y., Chae, J. C., Jeong, S., & Jeong, S. H. (2020). Molecular Characterization of Fecal Extended-Spectrum β-Lactamase- and AmpC β-Lactamase-Producing Escherichia coli From Healthy Companion Animals and Cohabiting Humans in South Korea. Frontiers in Microbiology, 11.

Huber, H., Zweifel, C., Wittenbrink, M. M., & Stephan, R. (2013). ESBL-producing uropathogenic Escherichia coli isolated from dogs and cats in Switzerland. Veterinary Microbiology, 162(2–4), 992–996.

Holmes, A. H., Moore, L. S., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., ... & Piddock, L. J. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. The Lancet, 387(10014), 176-187.

Liu, J., Du, S. X., Zhang, J. N., Liu, S. H., Zhou, Y. Y., & Wang, X. R. (2019). Spreading of extended-spectrum β-lactamase-producing Escherichia coli ST131 and Klebsiella

@COPYRIGHT UPM

pneumoniae ST11 in patients with pneumonia. Chinese Medical Journal, 132(16), 1894–1902.

Ljungquist, O., Ljungquist, D., Myrenås, M., Rydén, C., Finn, M., & Bengtsson, B. (2016). Evidence of household transfer of ESBL-/pAmpC-producing Enterobacteriaceae between humans and dogs – a pilot study. Infection Ecology &Amp; Epidemiology, 6(1), 31514

Mittal, A. K., Bhardwaj, R., Mishra, P., & Rajput, S. K. (2020). Antimicrobials Misuse/Overuse: Adverse Effect, Mechanism, Challenges and Strategies to Combat Resistance. The Open Biotechnology Journal, 14(1).

Matthew, M., Hedges, R. W., & Smith, J. T. (1979). Types of beta-lactamase determined by plasmids in gram-negative bacteria. Journal of Bacteriology, 138(3), 657-662.

Marchetti, L., Buldain, D., Gortari Castillo, L., Buchamer, A., Chirino-Trejo, M., & Mestorino, N. (2021). Pet and Stray Dogs as Reservoirs of Antimicrobial-Resistant Escherichia coli. International Journal of Microbiology, 2021, 1–8.

Nataro, J., Bopp, C.A., Fields, P.I., Kaper, J.B. & Stockbine, N.A. Escherichia, Shigella, and Salmonella, In Manual of clinical microbiology. Murray, P., Baron, EJ., Jorgensen, J.H., Landry, M.L. & Pfaller, M.A., editors. 2007. Washington DC. ASM Press. p. 670-687.

©COPYRIGHT UPM

Neftel, K. A., & Hübscher, U. (1987). Effects of beta-lactam antibiotics on proliferating eucaryotic cells. Antimicrobial agents and chemotherapy, 31(11), 1657-1661.

Olson, A. B., Silverman, M., Boyd, D. A., McGeer, A., Willey, B. M., Pong-Porter, V., Daneman, N., & Mulvey, M. R. (2005b). Identification of a Progenitor of the CTX-M-9 Group of Extended-Spectrum β-Lactamases from Kluyvera georgiana Isolated in Guyana. Antimicrobial Agents and Chemotherapy, 49(5), 2112–2115.

Pitout, J. D. D., Reisbig, M. D., Venter, E. C., Church, D. L., & Hanson, N. D. (2003). Modification of the double-disk test for detection of Enterobacteriaceae producing extended-spectrum and AmpC β-lactamases. Journal of Clinical Microbiology, 41(8), 3933-3935.

Subramanya, S. H., Bairy, I., Metok, Y., Baral, B. P., Gautam, D., & Nayak, N. (2021). Detection and characterization of ESBL-producing Enterobacteriaceae from the gut of subsistence farmers, their livestock, and the surrounding environment in rural Nepal. Scientific Reports, 11(1).

Schmitt, K., Biggel, M., Stephan, R., & Willi, B. (2022). Massive Spread of OXA-48 Carbapenemase-Producing Enterobacteriaceae in the Environment of a Swiss Companion Animal Clinic. Antibiotics, 11(2), 213.

Schmieder, R. & Edwards, R. Insights into antibiotic resistance through metagenomic approaches. Future Microbiology 7, 73-89

@COPYRIGHT UPM

Simonsen, G. S., & Urdahl, A. M. (2017). NORM/NORM-VET 2016-Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway.

Simpson, I. N., Harper, P. B., & O'Callaghan, C. H. (1980). Principal beta-lactamases responsible for resistance to beta-lactam antibiotics in urinary tract infections. Antimicrobial Agents and Chemotherapy, 17(6), 929-936.

Schjørring, S., & Krogfelt, K. A. (2011). Assessment of bacterial antibiotic resistance transfer in the gut. International journal of microbiology, 2011.

Subha, A., & Ananthan, S. (2002). Extended spectrum beta lactamase (ESBL) mediated resistance to third generation cephalosporins among Klebsiella pneumoniae in Chennai. Indian journal of medical microbiology, 20(2), 92-95.

Sanders, C. C., Barry, A. L., Washington, J. A., Shubert, C., Moland, E. S., Traczewski, M. M., ... & Mulder, R. (1996). Detection of extended-spectrum-beta-lactamase-producing members of the family Enterobacteriaceae with Vitek ESBL test. Journal of clinical microbiology, 34(12), 2997-3001.

Schwaber, M. J., Raney, P. M., Rasheed, J. K., Biddle, J. W., Williams, P., McGowan Jr, J. E., & Tenover, F. C. (2004). Utility of NCCLS guidelines for identifying extended-spectrum β-lactamases in non-Escherichia coli and non-Klebsiella spp. of Enterobacteriaceae. Journal of Clinical Microbiology, 42(1), 294-298.

@COPYRIGHT UPM

Titelman, E. (2013). Extended-spectrum beta-lactamase-producing enterobacteriaceae: epidemiology and dynamics of fecal carriage. Karolinska Institutet (Sweden).

@COPYRIGHT UPM