Antibiotic Resistance of *Enterobacteriaceae strains* Isolated from Different Animals Gastrointestinal Tracts

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Abstract

In this study we monitored antibiotic resistance in *Enterobacteriaceae* strains isolated from different animals gastrointestinal tracts (GIT). We isolated *Enterobacteriaceae* from chicken, ducks, lambs, pigs, sheeps, cows and rabbits collected from slovakian farms. *Enterobacteriaceae* strains were cultivated on MacConkey agar at 35° ± 2°C at 24 hours. Pure cultures of *Enterobacteriaceae* strains were obtained by four-way streak method on Chromogenic coliform agar. Identification of purified *Enterobacteriaceae* strains was done by Enterotest 24 and MALDI TOF MS. For susceptibility testing disk diffusion method was used according by EUCAST. We determined the most resistance in *Enterobacteriaceae* strains against streptomycin, tetracycline, ampicillin, piperecillin, levofloxacine, chloramphenicol and smaller level of resistance against amikacin, ceftriaxone and ofloxacine. Equally we detected resistance to more antibiotics in one strain. The most resistance was *Salmonella enterica* ser. Typhimurium. Also *E. coli* was resistance against four antibiotics and *Raoultella ornithinolytica* too. Antibiotic resistance was found in other isolated strains too.

Keywords:*Enterobacteriaceae*, antibiotics resistance, GIT, animals

1. Introduction

Antibiotic resistance is significant health, social and economic problem at this time. Antibiotic resistance of bacteria is biological risk, which increases morbidity and mortality of animal and human [1]. Keyser *et al.* [2] note that in recent years, accumulating problems with bacteria, which are resistant to antibiotics, leading to predictions that we return to the time before the discovery of antibiotics. Resistant bacteria from the intestines of food animals may be transferred to retail meat products resulting from fecal contamination during various stages of the slaughter process (e.g., evisceration) and subsequent handling of animal tissue [3]. Endogenous bacterial flora may play an important role as acceptor and donor of transmissible drug resistance genes [4, 5]. The *Enterobacteriaceae* family is commonly used as an indicator of fecal contamination during food microbiological analyses, and includes important zoonotic bacteria such as *Salmonella* spp., *Yersinia* spp. and *Escherichia coli*. *Enterobacteriaceae* are the significant causes of serious infection, and many of the most important members of this family are becoming increasingly resistant to currently available antimicrobials [6]. Recently, antimicrobial resistance has been reported in bacteria isolated from organic dairy products [7, 8], and in poultry products related to *Salmonella* and *Campylobacter* [9, 10]. However, little information relative to commensal bacteria isolated from poultry meat and milk products is currently available. Consequently, the main goal of the present study was to investigate the prevalence of antimicrobial

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susceptibility found in *Enterobacteriaceae* isolates derived from chicken meat and milk products.

2. Materials and methods

Collection of samples

Samples were collected from different farms of Slovakia from 2009 to 2013. We collected a 280 samples from different animals GIT. We isolated strains from GIT of chickens, sheeps, pigs, cows, lambs ducks and rabbits. Samples were collected by rectal swabs kit containing (Copan Inovation, Italy) and transported to the laboratory. Not all isolates were tested against the same collection of antibiotics and therefore not all antibiotics has the same numbers of tested strains.

Cultivation of *Enterobacteriaceae* strains

All samples were spread on the surface of agar by rectal swabs directly. Bacteria were cultivated on MacConkey agar (Biomark Pune, India) at 35±2°C for 24 hours in aerobic condition. Growed bacterial collonies were purified by four-ways streak plate method on Chromogenic coliform agar (Oxoid, UK) in the same condition. For recultivation of not clear collonies the same procedure were used in the same condition. Puryfied collonies were picked-up from the agar and suspended into the physiological solution adjusted to equal 0.5 McF° for the antibiotic susceptibility testing.

Antibiotic susceptibility testing

Prepared physiological solutions with bacteria adjusted to equal 0.5 McF° were spread by sterile L-rods on the surface of Mueller-Hinton agar (Oxoid, UK) in 100µl volume. Antibiotics discs (Oxoid, UK) wich we used in this experiment were follow: ampicillin (AMP) 10 µg/disc, chloramphenicol (CHL) 30 µg/disc, amikacin (AMI) 30 µg/disc, gentamicin (GEN) 10 µg/disc, piperacillin (PIP) 30 µg/disc, cefotaxime (CTX) 5 µg/disc, ceftriaxone (CRO) 30 µg/disc, doripenem (DOR) 10 µg/disc, meropenem (MEM) 10 µg/disc, levofloxacin (LVX) 5 µg/disc, ofloxacin (OFX) 5 µg/disc. Incubation of bacterial strains on Mueller-Hinton agar were done at 35±2°C for 16-20 hours according by EUCAST [11]. Interpretation of inhibition zones around the discs were done by EUCAST [12] (Breakpoint tables for interpretation of MICs and zone diameters, version 5.0 valid from 2015-01-01.

Identification of *Enterobacteriaceae* strains

The basic identification of *Enterobacteriaceae* strains were done on Chromogenic coliform agar (Oxoid, UK). Equally we identified bacteria by ENTEROtest 24 (Erba Lachema, CZ). Procedure for ENTEROtest 24 is described into the manufacturer manual. Evaluation of biochemical results were evaluated by TNW Lite 7.0 software (Erba Lachema, CZ). For the better identification of isolated strains matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS Biotyper) (Bruker Daltonics GmBH, Germany) was used. Method for preparing of samples was described previous by authors Kmeť and Drugdová [13].

3. Results and discussion

In this study we researched antibiotic resistance of *Enterobacteriaceae* strains isolated from gastrointestinal tracts of animals. We collected maximal 280 samples from GIT of animals from slovakian farms. We tested 280 *Enterobacteriaceae* strains against chloramphenicol and we detected resistance in the level of 5.71 %. The most resistance showed bacteria against ampicillin (22.54 %), in this case we tested 244 strains of *Enterobacteriaceae*. Equally we found resistance (19.64 %) against piperacillin where we tested 56 isolates. Also 92 isolates were tested against amikacin and we detected that 4.35 % isolates were resistant only. Resistance against ceftriaxone (2.35 %) from 85 isolates, levofloxacin (19.3 %) from 57 isolates and ofloxacin (1.32 %) from 76 isolates were detected. Resistance to other antibiotics we didnt detected in this research. All isolates were sensitive to gentamicin, cefotaxime, doripenem and meropenem. Exactly results are described into the table 1.

Table 1. Antibiotic resistance in *Enterobacteriaceae* strains isolates from GIT of animals

Antibiotic	Number of tested isolates	Perecentage of resistance
Ampicillin (AMP)	244	22.54
Piperacillin (PIP)	56	19.64
Amikacin (AMI)	92	4.35
Gentamicin (GEN)	62	0
Chloramphenicol (CHL)	280	5.71
Cefotaxime (CTX)	18	0
Ceftriaxone (CRO)	85	2.35
Doripenem (DOR)	27	0
Meropenem (MEM)	76	0
Levofloxacin (LVX)	57	19.3
Ofloxacin (OFX)	76	1.32

After the identifications of *Enterobacteriaceae*strains we separated each identified strains and we detected resistance in this isolates. We found strains as *E. coli*, *Serratia* spp., *S. odorifera* bv. 1, *S. fonticola*, *Klebsiella* spp., *K. oxytoca*, *K. pneumoniae*, *Citrobacter farmeri*,

C. freundii, *C. gillenii*, *Enterobacter* spp., *E. aerogenes*, *E. cloacae*, *Yersinia* spp., *Y. enterocolitica*, *Raoultella ornithinolytica*, *Proteus mirabilis*, *P. vulgaris*, *Shigella flexneri* and *Salmonella enterica* ser. Typhimurium. The most resistant strain which we found in this experiment was *Salmonella enterica* ser. Typhimurium which was resistant against six antibiotics (ampicillin, piperacillin, ceftriaxone, levoflxacin, ofloxacin and chloramphenicol). We isolated it from chicken intestinal tracts. The second most resistant bacteria, *Escherichia coli* was isolated from intestinal tracts of chicken, lambs, pigs, sheeps and cows and it was resistant against levofloxacin, piperacillin, ampicillin and ofloxacin. Other isolated and identified bacteria were resistant to less those two antibiotics and some bacteria is naturally resistant to antibiotics. Equally we determined that the most spread resistance in slovakian farms is against ampicillin, penicilins antibiotics respectivelly.

Table 2. Identified Enterobacteriaceae strains, their origin and resistance profile

Bactrial strain	Source of isolate	Resistance
Escherichia coli	Chickens, lambs, pigs, sheeps, Cows	LVX, PIP, AMP, OFL
Serratia spp.	Chickens	AMP
Serratia odorifera bv. 1	Lambs	ND
Serratia fonticola	Chickens	AMP
Klebsiella spp.	Ducks, lambs, pigs	AMP^{IR}, CHL, LVX
Klebsiella oxytoca	Chickens	AMP^{IR}
Klebsiella pneumoniae	Ducks	ND
Citrobacter farmeri	Lambs	AMP
Citrobacter freundii	Chickens	AMP^{IR}
Citrobacter gillenii	Rabbits	AMI, CTX
Enterobacter spp.	Ducks	AMP
Enterobacter aerogenes	Ducks, sheeps, pigs	AMP^{IR}
Enterobacter cloacae	Chickens	ND
Yersinia spp.	Chickens	AMP
Yersinia enterocolitica	Ducks	AMP^{IR}
Raoultella ornithinolytica	Cows, chickens	AMP, CHL
Proteus mirabilis	Chickens	ND
Proteus vulgaris	Chickens	AMP^{IR}
Shigella flexneri	Chickens	CHL
Salmonella enterica ser. Typhimurium	Chickens	AMP, PIP, CRO, LVX, OFL, CHL

Legend: AMP – ampicillin, LVX – levoflocaxin, PIP – piperacillin, OFL – ofloxacin, CHL – chloramphenicol, AMI – amikacin, CRO – ceftriaxone, ND – not detected, ^{IR} – intrisically resistance[1].

[1] – intrinsically resistance described by EUCAST [14] (Expert rules in antimicrobial susceptibility testing, version 1, April 2008)

Antibiotic resistance of bacteria isolated from intestinal tracts of animal studied authors as Lei *et al.* [15] too. They determined resistance against ampicillin, gentamicin, cholarmphenicol, tetracycline, nalixid acid and levofloxacin in pigs, chickens and ducks intestinal tracts. Also Unno *et al.*, [16] tested *E. coli* isolated from intestinal tracts of different animals and they determined resistance against ampicillin from 9.3 to 72.9 %, against gentamicin from 0 to 29.2 %, against streptomycin from 18.5 to 72.9 %, against piperacillin from 7.4 to 61.7 %, against chloramphanicol from 1.9 to 46.8 % and resistance against tetracycline from 9.3 to 91.7 %. Many authors meets in the opinion that resistance of bacteria is differ from study to study [17-20].

4. Conclusion

These results showed that the most spread resistance in bacteria, *Enterobacteriaceae* respectivelly, is antibiotic resistant against ampicillin, penicillins antibiotics respectivelly in Slovakia. Equaly resistant against levofloxacin was determined in the greater extent. Many identified bacteria from *Enterobacteriaceae* showed resistance against ampicillin the most often. Also multi-resistant bacteria as *E. coli* and *S. enterica* ser. Typhimurium were determined. Therefore is very necessary to monitor and find resistance in bacteria from gastrointestinal tracts of animals, because GITs are considered as reservoirs of antibiotic resistance.

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