

Phenotypic characterization of ESBL- and AmpC- type beta-lactamases in *Enterobacteriaceae* from chicken meat and dairy products

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Summary: The excess and off-label use of antibiotics results in development of antibiotic resistance among microorganisms. Although microbiological criteria have been appropriately considered in the Food Codex, an inspection for antibiotic-resistant bacteria has not come into force yet. Beta-lactamase producing *Enterobacteriaceae* adversely affects the human health by leading to therapeutic failures against infections. The objective of this study was to characterize ESBL- and/or AmpC- type beta-lactamases in *Enterobacteriaceae* isolated from chicken meat, raw milk and unpacked-fresh cheese samples phenotypically. In this study, a total of 327 samples (109 chicken meat, 135 raw milk and 83 unpacked fresh cheese) was examined microbiologically by performing pre-enrichment, enrichment on selective media, and oxidase test according to the Criteria by ISO/DIS21528-2. Overall, 80 ESBL- and/or AmpC positive isolates were identified by mass spectrometer. The most prevalent strain was *Escherichia coli* (68.8%), followed by *Klebsiella pneumoniae* (8.8%), *Enterobacter cloacae* (7.5%), *Citrobacter* spp. (6.2%), *Hafnia alvei* (6.2%), and *Klebsiella oxytoca* (2.5%). The beta-lactamases were screened by disc diffusion, disc diffusion confirmation, and MIC determination according to the Guidelines of Clinical and Laboratory Standards Institute. The most common beta-lactamase type was found as ESBL in 75 isolates, followed by a combination of ESBL & AmpC in 10 isolates, and AmpC in five isolates, respectively. In conclusion, our study showed that ESBL- and/or AmpC-type beta-lactamases were the most common enzymes in *Enterobacteriaceae* in the analyzed foods.

Keywords: AmpC, antibiotic resistance, dairy product, *Enterobacteriaceae*, ESBL.

Tavuk eti ve süt ürünleri kaynaklı *Enterobacteriaceae* suşlarında ESBL- ve AmpC- tipi beta laktamazların fenotipik karakterizasyonu

Özet: Aşırı ve bilinçsiz antibiyotik kullanımı mikroorganizmalarda antibiyotik direnci gelişimi ile sonuçlanmaktadır. Gıda kodeksinde mikrobiyolojik kriterler olmasına rağmen, antibiyotik dirençliliği için düzenleme henüz yapılmamıştır. Beta-laktamaz üreten *Enterobacteriaceae* suşları enfeksiyonlara karşı tedaviyi başarısız kılarak, insan sağlığını olumsuz etkilemektedir. Bu çalışmada tavuk eti, çiğ süt ve açık taze peynir örneklerinden izole edilen *Enterobacteriaceae* suşlarında GSBL- ve/veya AmpC- tipi beta-laktamazların varlıklarının incelenmesi amaçlanmıştır. Araştırmada, 109 adet tavuk eti, 135 adet çiğ süt ve 83 adet açık taze peynir olmak üzere toplam 327 adet gıda örneğinde ISO/DIS21528-2 talimatı uyarınca ön ve selektif zenginleştirme ile oksidaz testi uygulanarak mikrobiyolojik inceleme yapılmıştır. Toplam 80 adet GSBL- ve/veya AmpC- pozitif izolat kütle spektrometresi ile tiplendirilmiştir. İzolatlarda *Enterobacteriaceae* dağılımının %68,8 *Escherichia coli*, %8,8 *Klebsiella pneumoniae*, %7,5 *Enterobacter cloacae*, %6,2 *Citrobacter* spp., %6,2 *Hafnia alvei* ve %2,5 *Klebsiella oxytoca* olduğu saptanmıştır. Tiplendirilmiş izolatlarda beta-laktamazların karakterizasyonu Klinik ve Laboratuvar Standartları Kurumu talimatlarına (CLSI 2013) göre disk difüzyon, disk difüzyon konfirmasyonu ve MİK değeri tespiti ile yapılmıştır. İnceleme sonucu 75 adet izolatta GSBL-, 10 adet izolatta GSBL- ve AmpC- kombinasyonu ve beş adet izolatta AmpC- tipi enzimler karakterize edilmiştir. Sonuç olarak, analiz edilen gıda maddelerinde GSBL- ve/veya Amp- tipi beta-laktamazların baskın enzimler oldukları saptanmıştır.

Anahtar sözcükler: AmpC, antibiyotik direnci, *Enterobacteriaceae*, GSBL, hayvansal ürünler.

Introduction

The consumption of antibiotics in the food-animals for growth promotion and disease prevention is twice as used for humans in the world (35). The use of antibiotics cannot be controlled effectively due to economic concerns of the animal farming sector largely ignoring risks

associated with human and animal health (33, 40). Therefore, some foods of animal origin are under question for transmission of antibiotic resistant bacteria, which might be responsible for colonization and infection of the humans (33).

Beta-lactamases are the most prevalent mechanism of antibiotic resistance that inactivate beta-lactam antibiotics, including penicillins, cephalosporins, and monobactams (9, 28). These enzymes are encoded by an extrachromosomal DNA fragment called plasmid. A plasmid can be transferred genetically between the same and/or different bacteria (20). The beta-lactamases currently receiving the most attention are documented as extended spectrum beta-lactamases (ESBL) and aminopenicillin-deactivating cephalosporinase (AmpC) (4).

The resistance to beta-lactam antibiotics has been well-documented in the family of *Enterobacteriaceae*, including *Klebsiella* spp., *Escherichia (E.) coli*, *Proteus* spp., *Enterobacter* spp., *Citrobacter* spp., and *Salmonella* spp. (26, 42, 43). But, the patterns of resistance vary among the species (19). The recent studies have indicated that *E. coli* has gained beta-lactam resistance, and increasingly observed in some foods of animal origin (15). However, their impact on human health still remains incomplete across the World, including Turkey (2, 6).

In this study, ESBL- and AmpC- type beta-lactamases have been characterized phenotypically in *Enterobacteriaceae* isolated from chicken meat, raw milk and unpacked-fresh cheese.

Materials and Methods

Reference cultures: An ESBL positive strain *K. pneumoniae* ATCC 700603 and an ESBL negative strain *E. coli* ATCC 25922 were used for control testing, respectively.

Food samples: During the year 2014, a total of 327 food samples (109 chicken meat, 135 raw milk and 83 unpacked cheese) were collected randomly from chicken farms, bulk tanks in dairy farms, public bazaars and food chain markets located in Sakarya, Kocaeli, and İstanbul. All samples were put into sterile sampling bags, and taken to the laboratory in a sample carry case (JPB, UK) at 4°C. The microbiological evaluation was started in the same day.

Microbiological evaluation: Twenty-five grams of chicken meat and cheese in 225 mL of *Enterobacteriaceae* Enrichment Broth (LABM, UK), and 10 mL of raw milk in 90 mL of the same broth was homogenized in a sterile bag (Interscience, France). The suspension was then incubated at 37°C for 18-24 h under aerobic condition. After that, 10 µL of the suspension was directly streaked onto a Chromatic™ ESBL+AmpC agar (Liofilchem, Turkey) allowing the growth of both ESBL and AmpC producers. The plate was again incubated at 37°C for 18-24 h under aerobic condition. The Pink-reddish-mauve, green-blue and brown colonies were selected according to the manufacturer's instructions. The suspicious colonies were then sub-cultured onto Tryptic Soy Agar (Merck,

Turkey), and allowed for incubation at 37°C for 18-48 h. Their oxidase activity was tested by Bactident Oxidase Testing Kit (Merck, Turkey). Finally, the isolates were identified by mass spectrometer (Vitek® MS bioMérieux, France).

Disc screening and confirmation of ESBL suspicious isolates: After identification, the isolates were suspended in a sterile salt solution (0.85% NaCl) to 0.5 McFarland density, and transferred onto Mueller–Hinton agar (Liofilchem, Turkey) using sterile swabs. Cefpodoxime (CPD 10 µg), cefotaxime (CTX 30 µg), and ceftazidime (CAZ 30 µg) containing antibiotic discs (MAST CPD10, UK) were placed on the plate. Disc diffusion confirmation was performed by combinations of CPD, CTX, and CAZ±Clavulanate (CLA 10 µg) (MAST D67C). The disc-inserted plates were then incubated at 37 °C for 18-24 h. The breakpoints with zone diameters and zones of inhibition were evaluated according to the criteria as described by CLSI (2013) (5).

Antimicrobial susceptibility based on minimal inhibitory concentration (MIC): MIC determination was performed for ESBL- and AmpC-type beta-lactamases according to the manufacturer's instructions on Micronaut-S Beta-Lactamase VII plate (Merlin Diagnostika, Germany). A 50 µL aliquot of 0.5 McFarland-standardized suspension of the isolate was vortexed in 10 mL of Mueller Hinton Broth (Merck, Germany). After that, 100 µL of this suspension was pipetted into each well of the 96-well plate, followed by incubation at 37 °C overnight. The readings were then taken by Thermo Scientific™ Multiskan FC spectrometer, and automatically analyzed by MCN6 Software (Sifin, Germany).

Results

Microbiological results: A total of 327 samples (109 chicken meat, 135 raw milk and 83 unpacked-fresh cheese) were examined microbiologically according to the Criteria by ISO/DIS21528-2. Overall, 80 isolates were positive as ESBL- and/or AmpC-producing *Enterobacteriaceae*. The most prevalent strain was *E. coli* (68.8%), followed by *K. pneumoniae* (8.8%), *E. cloacae* (7.5%), *Citrobacter* spp. (6.2%), *H. alvei* (6.2%), and *K. oxytoca* (2.5%) (Figure 1).

Beta-lactamase-types: The types of beta-lactamases were determined by disc diffusion, disc diffusion confirmation, and MIC determination according to the Criteria by CLSI (2013) (5). The most common type was found as ESBL in 75 isolates (55 *E. coli*, seven *K. pneumoniae*, five *E. cloacae*, five *Citrobacter* spp., one *H. alvei*, and two *K. oxytoca*), followed by ESBL & AmpC in 10 isolates (seven *E. coli*, one *E. cloacae*, one *Citrobacter* spp., and one *H. alvei*), and AmpC in five isolates (four *H. alvei* and one *E. cloacae*), respectively. All the results were presented in Table 1 and Figures 1, 2, 3.

Table 1. Sample- and species-based distribution of ESBL- and/or AmpC.
Tablo 1. GSBL- ve/veya AmpC- beta-laktamazların örnek ve tür bazlı dağılımı.

Type of bacteria	Type of food	ESBL	ESBL&AmpC	AmpC	Total
<i>E. coli</i>	Chicken meat	28	2	-	28
	Raw milk	23	3	-	23
	Unpacked fresh cheese	4	2	-	4
	Subtotal	55	7	-	55
<i>K. pneumoniae</i>	Chicken meat	2	-	-	2
	Raw milk	-	-	-	-
	Unpacked fresh cheese	5	-	-	5
	Subtotal	7	-	-	7
<i>E. cloacae</i>	Chicken meat	2	-	-	2
	Raw milk	3	1	-	3
	Unpacked fresh cheese	-	-	1	1
	Subtotal	5	1	1	6
<i>Citrobacter spp.</i>	Chicken meat	1	-	-	1
	Raw milk	3	-	-	3
	Unpacked fresh cheese	1	1	-	1
	Subtotal	5	1	-	5
<i>H. alvei</i>	Chicken meat	-	-	-	-
	Raw milk	-	-	-	-
	Unpacked fresh cheese	1	1	4	5
	Subtotal	1	1	4	5
<i>K. oxytoca</i>	Chicken meat	-	-	-	-
	Raw milk	-	-	-	-
	Unpacked fresh cheese	2	-	-	2
	Subtotal	2	-	-	2
Total		75	10	5	80

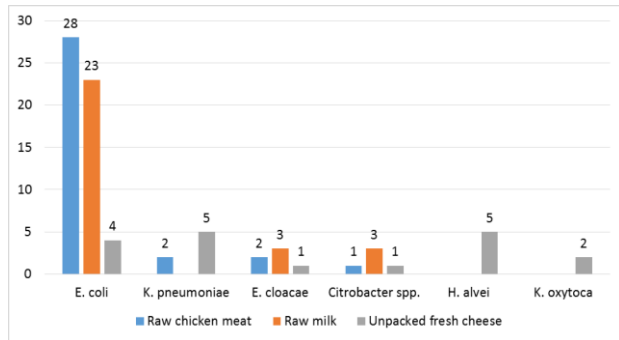


Figure 1. Sample-based distribution of ESBL- and/or AmpC-positive isolates.
Şekil 1. GSBL- ve/veya AmpC- pozitif izolatların örnek bazlı dağılımı.

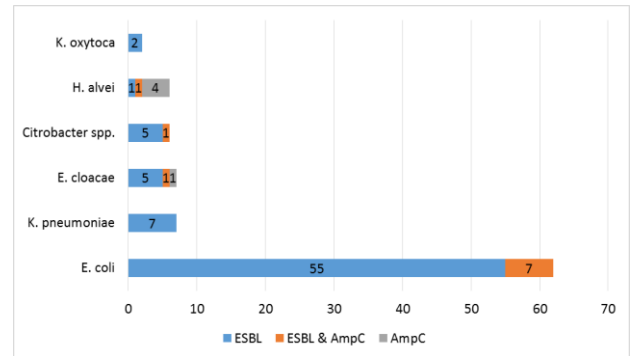


Figure 3. Species-based distribution of beta-lactamases.
Şekil 3. Beta-laktamazların tür bazlı dağılımı.

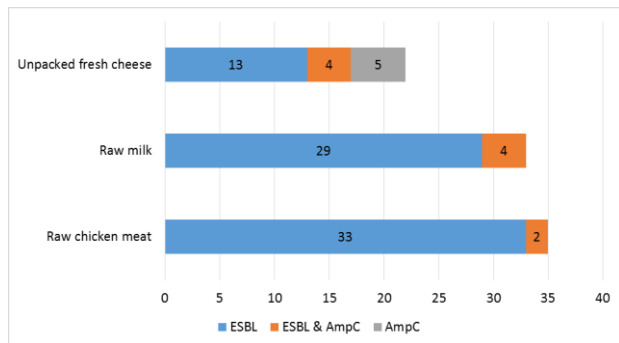


Figure 2. Sample-based distribution of beta-lactamases.
Şekil 2. Beta-laktamazların örnek bazlı dağılımı.

The average zone differences of CAZ±CVA, CTX±CVA and CPD±CVA were 9.2±4.4 mm, 12.9±6.3 mm and 14.3±6.3 mm in ESBL positive isolates, respectively, and 9.5±4.4 mm, 14.5±6.5 mm and 16.7±6.4 mm in ESBL & AmpC positive isolates, respectively, while 15.5±1.9 mm for CPD±CVA in AmpC positive isolates. 24 were resistant to CTX ($\geq 16 \mu\text{g/mL}$), 20 to CAZ (MIC $\geq 16 \mu\text{g/mL}$), 10 to COX ($\leq 4 \mu\text{g/mL}$), 18 to CEP ($=64 \mu\text{g/mL}$), and 3 to CMC ($=1/4 \mu\text{g/mL}$) out of 75 ESBL positive isolates. Among ESBL & AmpC positive isolates, nine were resistant to CTX ($=128 \mu\text{g/mL}$), 10 to

CAZ (=64 µg/mL), 2 to MER (=64 µg/mL), 4 to COX (≤ 4 µg/mL), 2 to ERT (>1 µg/mL), 8 to CEP (≥ 128 µg/mL), and 3 to CMC ($\leq 0.25/4$ µg/mL), respectively. The AmpC positive isolates showed resistance to CTX (=8 µg/mL) in 3, to CAZ (≥ 16 µg/mL) in 1, COX (≥ 16 µg/mL) in 4, and ERT (>1 µg/mL), respectively.

Discussion and Conclusion

In this study, a total of 327 samples (109 chicken meat, 135 raw milk and 83 unpacked-fresh cheese) were examined microbiologically. Overall, 80 isolates were positive as ESBL- and/or AmpC-producing *Enterobacteriaceae*. The most prevalent strain was *E. coli* (68.8%), followed by *K. pneumoniae* (8.8%), *E. cloacae* (7.5%), *Citrobacter* spp. (6.2%), *H. alvei* (6.2%), and *K. oxytoca* (2.5%). The phenotypic determination of beta-lactamases revealed that the most common beta-lactamase type was ESBL in 75 isolates (55 *E. coli*, seven *K. pneumoniae*, five *E. cloacae*, five *Citrobacter* spp., one *H. alvei*, and two *K. oxytoca*), followed by ESBL & AmpC in 10 (seven *E. coli*, one *E. cloacae*, one *Citrobacter* spp., and one *H. alvei*), and AmpC in five (four *H. alvei* and one *E. cloacae*), respectively.

Many antibiotics that were formerly effective against bacterial infections are no longer effective because of resistance development (38). The off-label and over use of antibiotics has fueled the spread of resistant bacteria and their resistance-encoding genetic elements among humans, animals, food, water and the environment (8, 21, 39). Despite of these facts, the average consumption rate of antibiotics per kilogram for food animal produced annually will globally increase nearly double by 2030 (3, 35). By 2050, the infections associated with antibiotic resistant bacteria could cause the death of 10 million people a year all over the World with a burden of \$100 trillion: more than the size of the current World economy (22). However, there is not actual data about the use of antibiotics in food animals in Turkey (39). The related studies from Turkey in this area are quite limited (3, 13, 14). Our study, therefore, contributed to an underestimation of the ESBL- and/or Amp-type enzymes in *Enterobacteriaceae* from some foods of animal origin.

Foods from animal origin could easily be contaminated by *Enterobacteriaceae* (1). The unhygienic consumption of these foods could be food safety and public health issue (10). Therefore, they should be free from *Enterobacteriaceae*, including the resistant ones (30, 31). The beta-lactamase producing *Enterobacteriaceae* are considered as major agents of some infections for conferring to penicillins, 1st, 2nd and 3rd-generation cephalosporins, and aztreonam (23, 36). These strains may colonize in the intestinal tract, and exchange their resistance-encoding genetic material with commensal bacteria of the human (37). The recent studies indicated

that beta-lactamases characterized in both human and foods were the same to each other (16). In this study, we detected ESBL- and/or AmpC-type beta-lactamases in *E. coli*, *K. pneumoniae*, *E. cloacae*, and *Citrobacter* spp., *H. alvei*, and *K. oxytoca*. Our findings related to the types of beta-lactamase positive enterobacteria in chicken meat, raw milk and unpacked-fresh cheese were similar to the results obtained in Belgium (29), Germany (27), China (41), Holland (230), Poland (17), and Denmark (11), respectively.

According to the Ministry of Health of Turkey (www.uhes.saglik.gov.tr), the antibiotic resistance patterns from clinical isolates have significantly spread, particularly in *E. coli* (33.2% in 2008 up to 48.83% in 2013) and *K. pneumoniae* (40% in 2008 up to 49.69% in 2013). But, the role of the foods on this increasing rate of beta-lactamase positive enterobacteria has not been seriously addressed in Turkey so far (18). Therefore, our study is important for providing the presence of antibiotic-resistant enterobacteria in the analyzed foods.

AmpC-type beta-lactamase is associated with multiple antibiotic resistances (32). In our study, we detected AmpC- production in *E. coli*, *E. cloacae*, *Citrobacter* spp., and *H. alvei*. For instance, if raw milk flavor is required, the best culture to add is *H. alvei*. However, we determined that *H. alvei* isolates could even be resistant to the beta-lactam antibiotics. The co-existence of ESBL- and AmpC- is a growing concern all over the world, leaving limited therapeutic options (8). Therefore, a failure to detect these multi-resistance patterns contributes to their uncontrolled spread (33).

The antimicrobial susceptibility based on MIC values revealed that ESBL positive isolates were resistant to CTX, CAZ, COX, CEP and CMC, while ESBL & AmpC positive ones were resistant to CTX, CAZ, COX, CEP, CMC, MER and ERT. As seen in the used antibiotic agents, a co-existing pattern of ESBL with AmpC suggested two different agents, including meropenem (MER) and ertapenem (ERT). For alone AmpC producers, the antibiotic agents were CTX, CAZ, COX and ERT. All of these beta-lactam agents is of importance in veterinary medicine (12, 29).

The detection of ESBL's co-presence with AmpC in an isolate with ERT susceptibility could be considered as one of the indicators of *K. pneumoniae* carbapenemase (KPC) activity. But, MIC test could not detect it. This means that carbapenem non-susceptible ESBL isolate is a potent problem in the future if it is not precisely detected.

Although microbiological criteria have been considered appropriate to the Food Codex, an inspection for antibiotic-resistant enterobacteria has not come into force yet (7, 25). The ESBLs are mainly encoded by plasmids and mobile genetic elements such as integron, insertion sequence, transposon and plasmid. Multiple

studies have reported that these genetic elements can easily be transferred to the commensal microflora of the humans through unhygienic foods (33, 34). Thus, each transmission may be important in mediating the spread of resistance-coding genes to the same and/or different microorganisms (14).

In conclusion, our study revealed that ESBL- and AmpC- were the most common beta-lactamases in *Enterobacteriaceae* from the analyzed foods. Accordingly, excessive and/or unconscious use of antibiotics in the farm animals should be considered, but there also is a need for advanced molecular studies to understand their epidemiology and dissemination ways.

Conflicts of Interest

The authors declare that there is no conflict of interest.

References

1. **Alvarez-Fernandez E, Cancelo A, Diaz-Vega C, et al.** (2013): *Antimicrobial resistance in E. coli isolates from conventionally and organically reared poultry: a comparison of agar disc diffusion and Sensi Test Gram-negative methods.* Food Control, **30**, 227-234.
2. **Apata DF** (2009): *Antibiotic resistance in poultry.* Int J Poult Sci, **8**, 404-408.
3. **Ata Z, Dinç G, Yılbar A, et al.** (2015): *Extended spectrum beta-lactamase activity and multidrug resistance of Salmonella serovars isolated from chicken carcasses from different regions of Turkey.* Vet J Ankara Univ, **62**, 119-123.
4. **Babic M, Hujer AM, Bonomo RA** (2006): *What's new in antibiotic resistance? Focus on beta-lactamases.* Drug Resist Updat, **9**, 142-156.
5. **CLSI Clinical and Laboratory Standards Institute** (2013): *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement, CLSI Document M100-S23, CLSI, Wayne PA.*
6. **Dai L, Lu LM, Wu CM, et al.** (2008): *Characterization of antimicrobial resistance among Escherichia coli isolates from chickens in China between 2001 and 2006.* FEMS Microbiol Lett, **286**, 178-183.
7. **Demirtürk N, Demirdal T** (2004): *The problem of the antimicrobial drug resistance.* KTD, **5**, 17-21.
8. **EFSA** (2011): *Scientific opinion on the public health risks of bacterial strains producing extended-spectrum beta-lactamases and/or AmpC beta-lactamases in food and food-producing animals.* EFSA Journal, **9**, 2322-2417.
9. **Falagas ME, Karageorgopoulos DE** (2009): *Extended-spectrum beta-lactamase-producing organisms.* J Hosp Infect, **73**, 345-354.
10. **FAO** (2014): *Food balance sheets.* (Available at <http://faostat.fao.org/site/610> (Accessed 26 May 2014).
11. **García-Tello A, Gimbernat H, Redondo C, et al.** (2014): *Extended-spectrum beta-lactamases in urinary tract infections caused by Enterobacteria: Understanding and guidelines for action.* Actas Urol Esp, **38**, 678-684.
12. **Ghafourian S, Sadeghifard N, Soheili S, et al.** (2014): *Extended spectrum beta-lactamases: Definition, classification and epidemiology.* Curr Issues Mol Biol, **17**, 11-22.
13. **Gündogan N, Avci E** (2013): *Prevalence and antibiotic resistance of extended-spectrum beta-lactamase (ESBL) producing Escherichia coli and Klebsiella species isolated from foods of animal origin in Turkey.* Afr J Microbiol Res, **7**, 4059-4064.
14. **Kaya S, Çetin E, Arıkan S, et al.** (2007): *Tavuklardan izole edilen E coli, Klebsiella ve enterokoklarda antibiyotik duyarlılık durumları.* SDÜ Tıp Fak Dergisi, **14**, 24-27.
15. **Lavilla S, Gonzalez-Lopez JJ, Miro E, et al.** (2008): *Dissemination of extended-spectrum beta-lactamase-producing bacteria: The food-borne outbreak lesson.* J Antimicrobial Chem, **61**, 1244-1251.
16. **Lopez-Cerrero L, Egea P, Torres E, et al.** (2012): *Increased raw poultry meat colonization by extended spectrum beta-lactamase-producing Escherichia coli in the South of Spain.* Int J Food Microbiol, **159**, 69-73.
17. **Makaa L, Maćkiwa E, Ścieżyńska H, et al.** (2014): *Antimicrobial susceptibility of Salmonella strains isolated from retail meat products in Poland between 2008 and 2012.* Food Control, **38**, 199-204.
18. **Manges AR, Smith SP, Lau BJ, et al.** (2007): *Retail meat consumption and the acquisition of antimicrobial resistant Escherichia coli causing urinary tract infections: A case-control study.* Foodborne Pathog Dis, **4**, 419-431.
19. **McEwen SA, Fedorka-Cray PJ** (2002): *Antimicrobial use and resistance in animals.* Clin Infect Dis, **34** (Suppl 3), 93-106.
20. **Morosini MI, García-Castillo M, Coque TM, et al.** (2006): *Antibiotic coresistance in extended-spectrum-beta-lactamase-producing Enterobacteriaceae and in vitro activity of tigecycline.* Antimicrob Agents Chemother, **50**, 2695-2699.
21. **Neu HC** (1992): *The crisis in antibiotic resistance.* Science, **257**, 1064-1073.
22. **O'Neill J** (2014): *Antimicrobial resistance: Tackling a crisis for the health and wealth of nations. Review on antimicrobial Resistance.* (Available at <http://amr-review.org>). (Accessed December 2014).
23. **Overdeest I, Willemsen I, Rijnsburger M, et al.** (2011): *Extended-spectrum beta-lactamase genes of Escherichia coli in chicken meat and humans, the Netherlands.* Emerg Infect Dis, **17**, 1216-1222.
24. **Özpinar H, Turan B, Tekiner İH, et al.** (2013): *Evaluation of pathogenic Escherichia coli occurrence in vegetable samples from district bazaars of İstanbul using real time PCR.* Lett Appl Microbiol, **57**, 362-367.
25. **Phillips I, Casewell M, Cox T, et al.** (2004): *Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data.* J Antimicrobial Chem, **53**, 28-52.
26. **Samaha-Kfoury JN, Araj GF** (2003): *Recent developments in beta lactamases and extended spectrum beta lactamases.* BMJ, **327**, 1209-1213.
27. **Schwaiger K, Huther S, Hölzel C, et al.** (2012): *Prevalence of antibiotic-resistant enterobacteriaceae isolated from chicken and pork meat purchased at the*

- slaughterhouse and at retail in Bavaria, Germany. *Int J Food Microbiol*, **154**, 206-211.
28. **Shashwati N, Kiran T, Dhanvijay AG** (2014): *Study of extended spectrum β -lactamase producing Enterobacteriaceae and antibiotic coresistance in a tertiary care teaching hospital*. *J Nat Sci Biol Med*, **5**, 30-35.
 29. **Smet A, Martel A, Persoons D, et al.** (2009): *Broad-spectrum β -lactamases among Enterobacteriaceae of animal origin: Molecular aspects, mobility and impact on public health*. *FEMS Microbiology Reviews*, **34**, 295-316.
 30. **Stuart JC, van den Munckhof T, Voets G, et al.** (2012): *Comparison of ESBL contamination in organic and conventional retail chicken meat*. *Int J Food Microbiol*, **154**, 212-214.
 31. **Sudarwanto M, Akineden Ö, Odenthal S, et al.** (2015): *Extended-spectrum β -lactamase (ESBL)-producing Klebsiella pneumoniae in bulk tank milk from dairy farms in Indonesia*. *Foodborne Pathog Dis*, **12**, 585-590.
 32. **Taneja N, Rao P, Arora J, et al.** (2008): *Occurrence of ESBL & Amp-C β -lactamases & susceptibility to newer antimicrobial agents in complicated UTI*. *Indian J Med Res*, **127**, 85-88.
 33. **Tekiner IH, Özpınar H** (2016): *Occurrence and characteristics of ESBL-producing Enterobacteriaceae from foods of animal origin*. *Braz J Microbiol*, **47**, 444-451.
 34. **Thorsteinsdottir TR, Haraldsson G, Fridriksdottir V, et al.** (2010): *Prevalence and genetic relatedness of antimicrobial resistant Escherichia coli isolated from animals, foods and humans in Iceland*. *Zoonoses Public Hlth*, **57**, 189-196.
 35. **Van Boeckel TP, Brower C, Gilbert M, et al.** (2015): *Global trends in antimicrobial use in food animals*. *Proc Natl Acad Sci USA*, **112**, 5649-5654.
 36. **Van den Bogaard AE, Stobberingh EE** (2000): *Epidemiology of resistance to antibiotics. Links between animals and humans*. *Int J Antimicrob Agents*, **14**, 327-335.
 37. **Van den Bogaard AE** (2001): *Human health aspects of antibiotic use in food animals: A review*. *Tijdschr Diergeneeskd*, **126**, 590-595.
 38. **Webb GF, D'Agata EMC, Magal P, et al.** (2002): *A model of antibiotic-resistant bacterial epidemics in hospitals*. *Proc Natl Acad Sci*, **99**, 2293-2298.
 39. **WHO** (2015): *Global Action plan on antimicrobial resistance*. ISBN 9789241509763. Avenue Appia 20, 1211 Geneva, Switzerland.
 40. **Yıbar A, Soyutemiz E** (2013): *Antibiotics use in food-producing animals and possible residual risk*. *Atatürk Üniv Vet Bil Derg*, **8**, 97-104.
 41. **Zheng H, Zeng Z, Chen S, et al.** (2012): *Prevalence and characterisation of CTX-M β -lactamases amongst Escherichia coli isolates from healthy food animals in China*. *Int J Antimicrob Agents*, **39**, 305-310.
 42. **Zurfluh K, Hächler H, Nüesch-Inderbinen M, et al.** (2013): *Characteristics of extended-spectrum β -lactamase- and carbapenemase-producing Enterobacteriaceae Isolates from rivers and lakes in Switzerland*. *Appl Environ Microbiol*, **79**, 3021-3026.
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