Molecular Typing of Extended-spectrum beta-lactamase producing *E. coli* (EPEC) isolates from different sources by PFGE

A. Mushtaq[1*], T. S. Rai[1], A. K. Arora[1], R. Singh[2], A. Arun Prince Milton[3]

[1]Department of Veterinary Microbiology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, 141004, India
[2]Centre of One Health, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, 141004, India
[3]Division of Animal and Fisheries Sciences, ICAR Complex for NorthEastern Hill Region, Umiam, Meghalaya, 793103, India.
*Corresponding author: Asiya Mushtaq Email: drasiya.mushtaq29@gmail.com

Journal of Livestock Science (ISSN online 2277-6214) 16: 721-727
Received on 5/2/2025 Accepted on 15/12/25; Published on 20/12/25
doi. 10.33259/JLivestSci.2025.721-727

Abstract

Antimicrobial resistance in humans and animals develops because of antibiotic overuse, inappropriate drug dosages, and inadequate treatment regimens. In the past few decades, a global increase in antimicrobial resistance to β-lactamases has augmented the worldwide occurrence of ESBL-producing Enterobacterales. The spread of resistant isolates and antibiotic-resistance genes between humans and animals takes place via food chain. The bacterial genotyping methods can be employed to identify the predominant strain of bacteria, study clonal distribution, and conduct epidemiological surveillance. A total of 12 ESBL- producing *E. coli* (EPEC) isolates from sheep, goats and pigs and from an animal handler were subjected to genotypic analysis using the Pulsed- field gel electrophoresis (PFGE) technique. The PFGE results were examined using BioNumerics software. Based on the clustering of isolates, a dendrogram was generated and their genetic relatedness was analyzed. PFGE revealed similarity among various animal species taken from different farms suggesting clonal dissemination between same and different species of animals.

Keywords: ESBL, *E. coli*, Sheep, Goats, Pigs, Genotyping, PFGE

Introduction

Antimicrobial resistance in humans and animals develops because of antibiotic overuse, inappropriate drug dosages, and inadequate treatment regimens (Chauhan et al., 2016; Ayukekbong et al., 2017; Rahman et al., 2020). On the contrary, bacteria have adapted various defense mechanisms to evade the effects of several antibiotics by producing hydrolyzing enzymes (Mahamat et al., 2021). The best illustrated are the extended-spectrum beta-lactamase (ESBL) group that is capable of hydrolyzing third generation cephalosporins and aztreonam (Al-Hasso & Mohialdeen, 2023). While clavulanic acid shows high efficacy in identifying ESBLs in detection tests (Rawat & Nair, 2010), commercial amoxicillin-clavulanate and ticarcillin-clavulanate combinations have limited activity against most ESBL producers (Toussaint & Gallagher, 2015). The most commonly used system for β-lactamases nowadays is the molecular classification, that divides them into four groups (A, B, C and D) based on amino acid sequences and the functional classification, which takes into account substrate and inhibitor profiles (Bush & Jacoby, 2010).

A global increase in antimicrobial resistance due to β-lactamases has increased drastically over the past few decades, escalating the infection rates caused by ESBL- producing Enterobacteriaceae (Eltai et al., 2018). Infections associated with Gram-negative bacteria producing ESBL enzymes are increasingly causing community-acquired infections, the most common being the urinary tract infections (Meier et al., 2011). An earlier published report on the transfer of ESBL-producing *E. coli* (EPEC) across the food chain suggests the spread of EPEC isolates (Zhang et al., 2016; Barilli et al., 2019) and their antibiotic-resistance genes from food animals to humans (Geser et al., 2012). The increased cross-spread of ESBL producing strains represents a major public health concern (Adler et al., 2016). The plasmids and clonal expansion of certain lineages are the driving forces responsible for the spread of EPEC in clinical and extra-clinical settings (Davies & Davies, 2010).

The bacterial genotyping methods can be employed to identify the predominant strain of bacteria, study clonal distribution (Wolska & Szweda, 2012), virulence and resistance factors and conduct epidemiological surveillance (Ochoa-Díaz et al., 2018). These methods facilitate the differentiation of bacterial strains based on their genetic makeup and generate a specific genetic profile for each strain (Yildirim et al., 2011). Among various genotyping methods, pulsed-field gel electrophoresis (PFGE) stands out for being the most successful and commonly used genotyping technique for a range of commonly occurring pathogens (Neoh et al., 2019). In PFGE, the separation of large segments of DNA occurs under the influence of an alternating electric current. The movement of DNA through the gel takes place in a zig-zag pattern, giving it more time and field to separate as bands. In this way, species with similar genetic patterns show a similar DNA separation profile than the dissimilar or less similar ones (Parizad et al., 2016).

The interspecies transfer of resistant mobile elements is largely attributed to the shared environment between humans and animals (Cao et al., 2022; Nadimpalli et al., 2023). Keeping this in mind the present study was planned to analyze the clonal relationship among EPEC isolates from different sources by molecular typing using the PFGE technique. The molecular typing of EPEC isolates revealed clonal dissemination between same and different species of animals from different farms.

Material and Methods

Animal Ethics

The permissions for collecting faecal samples and hand swabs from different farms in various districts in Punjab were taken from the Institutional Biosafety Committee and Institutional Animal Ethics Committee, GADVASU, Ludhiana, India (IBSC/2021/1765-66, Dated: 13/10/2021, GADVASU/2021/IAEC/61/23, Dated: 19/10/2021) and the Institutional Ethics Committee of Dayanand Medical College & Hospital, Ludhiana, India, DMCH/R&D/2021/104, Dated: 10/09/2021), respectively.

Place of Study

The study was carried out at Department of Veterinary Microbiology, College of Veterinary, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, India.

ESBL isolates

ESBL producing *E. coli* strains isolated from rectal swabs of sheep, goats, pigs, and hand swabs of animal handlers which were earlier examined for antibiotic sensitivity test were used for this study. The ESBL production from *E. coli* isolates was initially screened using HiCrome ESBL agar (Himedia, India). The phenotypic confirmation of ESBL production was done using the double disc-synergy test (DDST) where the positive isolates were subjected to DDST.

Overall, 12 EPEC isolates (4 samples each from sheep and goats, 3 from pigs and 1 hand swab of a handler) were randomly selected from a bulk of samples for this study.

PFGE

The PFGE analysis of EPEC isolates was done using CHEF-DR III System (BIO-RAD Laboratories, Hercules, CA) as per the procedure described in CDC guidelines (CDC 2013). The bacterial colonies were added to cell suspension buffer (prepared as per the protocol) and then adjusted to 1.0 McFarland scale. From the adjusted cell suspensions, 200 µl was transferred in a 1.5 ml microcentrifuge tube and equal volume (200 µl) of 1% SeaKem Gold agarose and 10 µl volume of proteinase K (20mg/mL) was added and mixed by gentle pipetting. This mixture (100 µl) was then immediately poured in plug molds avoiding bubble formation and left undisturbed for 15 minutes till they solidified. The cell lysis buffer was prepared as per the protocol and the plugs were subjected to lysis in this buffer for 3 hours at 54 ºC. This was followed by washing the plugs twice with distilled water and five times with a TE buffer (pH 8.0) for 15 minutes each at 54 ºC.

The plugs were subjected to digestion with 50U of restriction enzyme, *Xba*I at 37 ºC for 3 hours, after which the digested DNA was subjected to electrophoresis. The conditions for electrophoresis were voltage: 6V, an initial switch time: 6.76 s, a final switch time: 35.38 s, included angle of 120˚ followed by a run time of 20 h. In the next step, ethidium bromide solution was employed to stain the PFGE gel in a closed container for 20-30 min. After destaining the gel, an image was captured using a gel documentation system for analysis.

The PFGE results were analyzed using BioNumerics software (version-7.6) program. The genetic similarity or relatedness of the ESBL isolates was determined by observing the number of DNA bands produced. Subsequently, the unweighted pair group method with arithmetic mean algorithm (UPGMA) was used for dendrogram analysis that builds a dendrogram based on the clustering method. This described the degree of relationship (percentage of similarity) among various PFGE profiles, and if the similarity index was ≥ 90, the isolates were considered similar and belonging to the same PFGE cluster.

Results

In this study, the PFGE results revealed two major clusters at 89% similarity, cluster I included 4 isolates where isolate 6 and 10 were exhibiting 93% similarity, while isolate 8 was 91.5% similar to these and isolate 7 was 91% similar to isolates 6, 10 and 8. In cluster II, six isolates clustered together on the basis of 90% similarity. Isolate 12 was different from rest of the isolates by 10%. Isolates 1 and 4 exhibited the maximum (94%) similarity while isolates 5 and 9 showed 92% similarity. Isolates (2 and 11) were found to be un-typable by PFGE. The isolates 1 and 4 with maximum similarity were taken from same species (goats) but from different farms. Isolates 6, 10, 5 and 9 were taken from different species (sheep and pigs) and farms indicating clonal dissemination between different farms. Since isolate 12 was taken from an animal handler, it was genetically distinct from the animal strains. PFGE banding pattern of ESBL producing *E. coli* isolates and dendrogram showing clonal relationship among various isolates is shown in figures 1 and 2.

Discussion

The molecular and epidemiological studies of various microbes have been done using PFGE technique. This method is efficient in investigating the source of infection and its spread. Therefore, it is considered as a gold standard for analysis of epidemiology of many microbes including *E. coli* (Pal, 2015; Neoh et al., 2019). However, whole genome sequencing (WGS) has recently emerged as an alternative to PFGE due to its superior resolution for bacterial strain typing in molecular epidemiology. Many studies have demonstrated higher resolution and discriminatory power of WGS compared to PFGE (Pendleton et al., 2013; Salipante et al., 2015) as it can detect even small genetic differences that may be missed by PFGE (Moore et al., 2015). While WGS helps in accurate identification and provides more detailed genomic information, it may not always exactly conclude relatedness between isolates (Yang et al., 2016). In clinical settings, the application of WGS remains a challenge because of high-cost, time and the need for sophisticated bioinformatics tools (Pendleton et al., 2013).

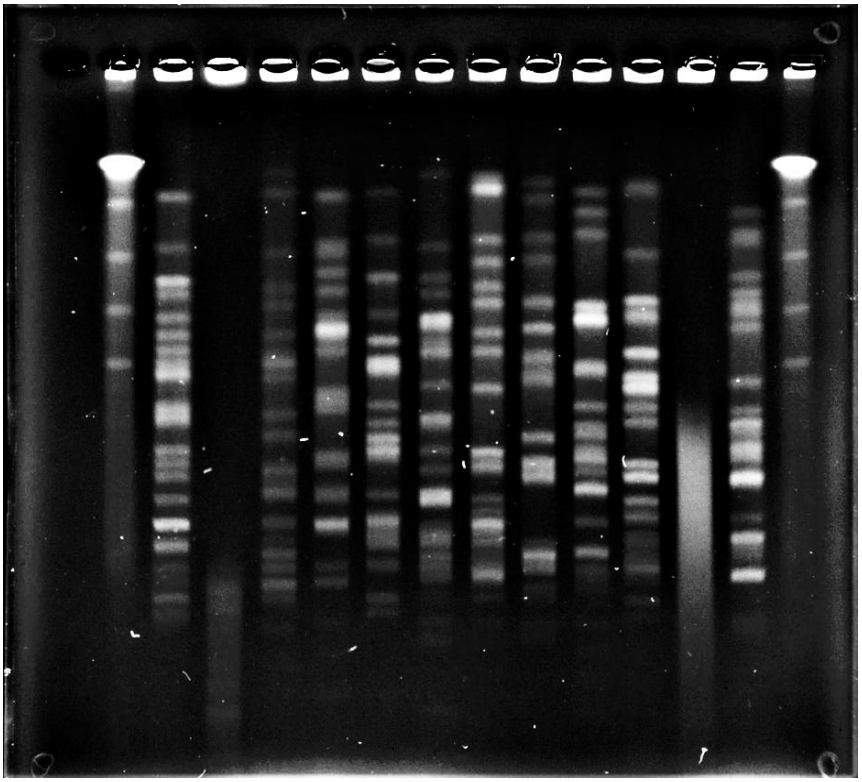

Fig. 1: PFGE banding pattern of ESBL producing *E. coli* isolates

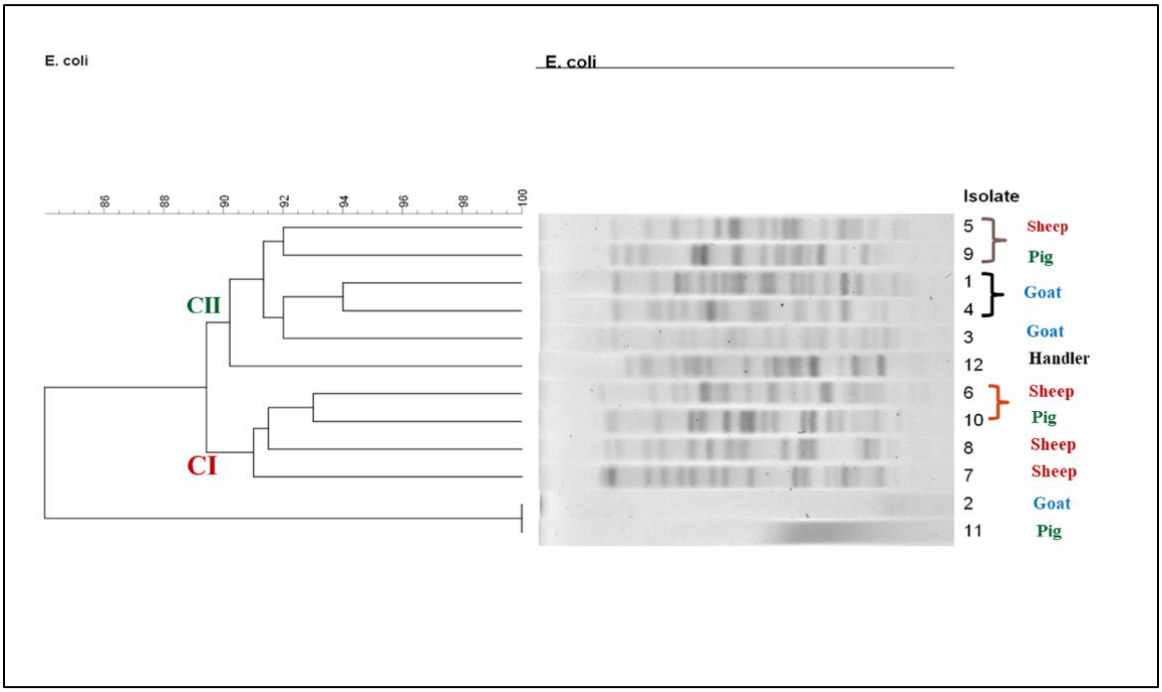

Fig. 2: Dendrogram and PFGE profiles of ESBL producing *E. coli* isolates

Discussion

The molecular and epidemiological studies of various microbes have been done using PFGE technique. This method is efficient in investigating the source of infection and its spread. Therefore, it is considered as a gold standard for analysis of epidemiology of many microbes including *E. coli* (Pal, 2015; Neoh et al., 2019). However, whole genome sequencing (WGS) has recently emerged as an alternative to PFGE due to its superior resolution for bacterial strain typing in molecular epidemiology. Many studies have demonstrated higher resolution and discriminatory power of WGS compared to PFGE (Pendleton et al., 2013; Salipante et al., 2015) as it can detect even small genetic differences that may be missed by PFGE (Moore et al., 2015). While WGS helps in accurate identification and provides more detailed genomic information, it may not always exactly conclude relatedness between isolates (Yang et al., 2016). In clinical settings, the application of WGS remains a challenge because of high-cost, time and the need for sophisticated bioinformatics tools (Pendleton et al., 2013).

PFGE technique continues to be a valuable tool for molecular subtyping and investigating local outbreaks of bacterial pathogens due to its usefulness and established protocols (Besser, 2015). In our study, EPEC isolates from different animals were genotyped using PFGE and clustering of isolates revealed clonal dissemination among different species of animals. The findings this paper is in line with findings of an earlier study in which PFGE profiles of multidrug-resistant *E. coli* strains isolated from food and pet animals were analyzed. It showed clonal dissemination of bacterial strains among different animals (Lei et al., 2010). Similar results showing clonal diffusion of *E. coli* isolates between different animals were reported in another study (Zhu et al., 2023). A report from food animals revealed that all the CMY-2 positive *E. coli* isolates which were subjected to PFGE analysis displayed both a common phylogenetic and geographic origin (Guo et al., 2014). Reports of previous study conducted to examine clonal relationship among *E. coli* isolates from food-producing animals showed unrelated pulsotypes (Ben Sallem et al., 2012).

Conclusion

PFGE profile revealed clonal dissemination between same and different species of animals from different farms highlighting the possibility of interspecies transfer of mobile genetic elements.

Acknowledgement

The authors wish to thank the Dean and Director of Research, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) to approve the research.

Conflict of Interest

The author(s) declare no conflict of interest.

References

1) Al-Hasso, M.Z. and Mohialdeen, Z.K., 2023. A Review of Extended Spectrum β-Lactamases: Definition and Types. Kirkuk University Journal for Scientific Studies, 18 **(1)**, 43-61.
2) Adler, A., Katz, D.E. and Marchaim, D., 2016. The continuing plague of extended-spectrum β-lactamase–producing Enterobacteriaceae infections. Infectious Disease Clinics, 30 **(2)**, 347-375.
3) Ayukekbong, J.A., Ntemgwa, M. and Atabe, A.N., 2017. The threat of antimicrobial resistance in developing countries: causes and control strategies. Antimicrobial Resistance and Infection Control, 6,1-8.
4) Barilli, E., Vismarra, A., Villa, Z., Bonilauri, P. and Bacci, C.E.S.L.E., 2019. ESβL *E. coli* isolated in pig's chain: Genetic analysis associated to the phenotype and biofilm synthesis evaluation. International Journal of Food Microbiology, 289, 162-167.
5) Ben Sallem, R., Ben Slama, K., Sáenz, Y., Rojo-Bezares, B., Estepa, V., Jouini, A., Gharsa, H., Klibi, N., Boudabous, A. and Torres, C., 2012. Prevalence and characterization of extended-spectrum beta-lactamase (ESBL)–and CMY-2–producing *Escherichia coli* isolates from healthy food-producing animals in Tunisia. Foodborne Pathogens and Disease, 9 **(12)**, 1137-1142.
6) Besser, J., 2015. Pulsed-field gel electrophoresis for disease monitoring and control. Methods in Molecular Biology,1301, 3-7.
7) Bush, K. and Jacoby, G.A., 2010. Updated functional classification of β-lactamases. Antimicrobial Agents and Chemotherapy, 54 **(3)**, 969-976.
8) Cao, H., Bougouffa, S., Park, T.J., Lau, A., Tong, M.K., Chow, K.H. and Ho, P.L., 2022. Sharing of antimicrobial resistance genes between humans and food animals. MSystems, 7 **(6)**, e00775-22.

9) Centers for Diseases Control and Prevention (CDC). Standard operating procedure for PulseNet PFGE of *Escherichia coli* O, 157;2013. Available from: http://www.pulsenetinternational.org/assets/PulseNet/uploads/pfge/PNL05_Ec-Sal-ShigPFGEprotocol.pdf

10) Chauhan, P.M., Thumar, H.K., Bhagat, A., Sharma, V.K., Chauhan H.C.; Patel, M.R. 2016. Comparative efficacy of antibiotic sensitivity tests for management of acute clinical Escherichia Coli mastitis in crossbred cow. Journal of Livestock Science 7: 41-45

11) Davies, J. and Davies, D., 2010. Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews, 74 **(3)**, 417-433.

12) Eltai, N.O., Al Thani, A.A., Al-Ansari, K., Deshmukh, A.S., Wehedy, E., Al-Hadidi, S.H. and Yassine, H.M., 2018. Molecular characterization of extended spectrum β-lactamases Enterobacteriaceae causing lower urinary tract infection among pediatric population. Antimicrobial Resistance and Infection Control, 7 **(90)**, 1-9.

13) Geser, N., Stephan, R. and Hächler, H., 2012. Occurrence and characteristics of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. BMC Veterinary Research, 8 **(21)**, 1-9.

14) Guo, Y.F., Zhang, W.H., Ren, S.Q., Yang, L., Lü, D.H., Zeng, Z.L., Liu, Y.H. and Jiang, H.X., 2014. IncA/C plasmid-mediated spread of CMY-2 in multidrug-resistant *Escherichia coli* from food animals in China. PLoS One, 9 **(5)**, e96738.

15) Lei, T., Tian, W., He, L., Huang, X.H., Sun, Y.X., Deng, Y.T., Sun, Y., Lv, D.H., Wu, C.M., Huang, L.Z. and Shen, J.Z., 2010. Antimicrobial resistance in *Escherichia coli* isolates from food animals, animal food products and companion animals in China. Veterinary Microbiology, 146 **(1-2)**, 85-89.

16) Mahamat, O.O., Kempf, M., Lounnas, M., Tidjani, A., Hide, M., Benavides, J.A., Carrière, C., Bañuls, A.L., Jean-Pierre, H., Ouedraogo, A.S. and Dumont, Y., 2021. Epidemiology and prevalence of extended-spectrum β-lactamase-and carbapenemase-producing Enterobacteriaceae in humans, animals and the environment in West and Central Africa. International Journal of Antimicrobial Agents, 57 **(1)**, 106203.

17) Meier, S., Weber, R., Zbinden, R., Ruef, C. and Hasse, B., 2011. Extended-spectrum β-lactamase-producing Gram-negative pathogens in community-acquired urinary tract infections: an increasing challenge for antimicrobial therapy. Infection, 39, 333-340.

18) Moore, G., Cookson, B., Gordon, N.C., Jackson, R., Kearns, A., Singleton, J., Smyth, D. and Wilson, A.P.R., 2015. Whole-genome sequencing in hierarchy with pulsed-field gel electrophoresis: the utility of this approach to establish possible sources of MRSA cross-transmission. Journal of Hospital Infection, 90 **(1)**, 38-45.

19) Nadimpalli, M.L., Stegger, M., Viau, R., Yith, V., de Lauzanne, A., Sem, N., Borand, L., Huynh, B.T., Brisse, S., Passet, V. and Overballe-Petersen, S., 2023. Plugging the leaks: antibiotic resistance at human–animal interfaces in low-resource settings. Frontiers in Ecology and the Environment, 21 **(9)**, 428-434.

20) Neoh, H.M., Tan, X.E., Sapri, H.F. and Tan, T.L., 2019. Pulsed-field gel electrophoresis (PFGE): A review of the "gold standard" for bacteria typing and current alternatives. Infection, Genetics and Evolution, 74, 103935.

21) Ochoa-Díaz, M.M., Daza-Giovannetty, S. and Gómez-Camargo, D., 2018. Bacterial genotyping methods: from the basics to modern. Methods in Molecular Biology, 1734,13-20.

22) Pal, P., 2015. Pulsed-Field Gel Electrophoresis as a molecular tool for characterizing genomes of certain food-borne bacterial isolates-A Review. International Letters of Natural Sciences, 2, 13-23.

23) Parizad, E.G., Parizad, E.G. and Valizadeh, A., 2016. The application of pulsed field gel electrophoresis in clinical studies. Journal of Clinical and Diagnostic Research, 10 **(1)**, DE01-04.

24) Pendleton, S., Hanning, I., Biswas, D. and Ricke, S.C., 2013. Evaluation of whole-genome sequencing as a genotyping tool for *Campylobacter jejuni* in comparison with pulsed-field gel electrophoresis and flaA typing. Poultry Science, 92 **(2)**, 573-580.

25) Rahman, M.M., Husna, A., Elshabrawy, H.A., Alam, J., Runa, N.Y., Badruzzaman, A.T.M., Banu, N.A., Al Mamun, M., Paul, B., Das, S. and Rahman, M.M., 2020. Isolation and molecular characterization of multidrug-resistant *Escherichia coli* from chicken meat. Scientific Reports, 10 **(1)**, 21999.

26) Rawat, D. and Nair, D., 2010. Extended-spectrum β-lactamases in Gram Negative Bacteria. Journal of Global Infectious Diseases, 2 **(3)**, 263-274.

27) Salipante, S.J., SenGupta, D.J., Cummings, L.A., Land, T.A., Hoogestraat, D.R. and Cookson, B.T., 2015. Application of whole-genome sequencing for bacterial strain typing in molecular epidemiology. Journal of Clinical Microbiology, 53 **(4)**, 1072-1079.

28) Toussaint, K.A. and Gallagher, J.C., 2015. β-lactam/β-lactamase inhibitor combinations: from then to now. Annals of Pharmacotherapy, 49 **(1)**, 86-98.

29) Wolska, K. and Szweda, P., 2012. Genotyping techniques for determining the diversity of microorganisms. Genetic diversity in microorganisms, 53.
30) Yang, S., Hemarajata, P., Hindler, J., Ward, K., Adisetiyo, H., Li, F., Aldrovandi, G.M., Green, N.M., Russell, D., Rubin, Z. and Humphries, R.M., 2016. Investigation of a suspected nosocomial transmission of blaKPC3-mediated carbapenem-resistant Klebsiella pneumoniae by whole genome sequencing. Diagnostic Microbiology and Infectious Disease, 84 **(4)**, 337-342.
31) Yildirim, İ.H., Yildirim, S.C. and Koçak, N., 2011. Molecular methods for bacterial genotyping and analyzed gene regions. Journal of Microbiology and Infectious Diseases, 1 **(1)**, 42-46.
32) Zhang, H., Zhai, Z., Li, Q., Liu, L., Guo, S., Li, Q., Yang, L., Ye, C., Chang, W. and Zhai, J., 2016. Characterization of extended-spectrum β-lactamase–producing *Escherichia coli* isolates from pigs and farm workers. Journal of Food Protection, 79 **(9)**, 1630-1634.
33) Zhu, D.M., Ding, Q., Li, P.H., Wang, Y.L., Li, Y.Z., Yu Li, X., Li, G.M., Ma, H.X. and Kong, L.C., 2023. Antimicrobial resistance in *E. coli* of animal origin and discovery of a novel ICE mobile element in Northeast China. BMC Veterinary Research, 19 **(1)**, 255.