Original Research Article

Phenotypic and genotypic detection of extended-spectrum β-lactamase producing and fluoroquinolone resistant *Escherichia coli* isolated from Pigs and its antibiogram pattern

ABSTRACT

Antimicrobial resistance (AMR) in food animals is a global issue. Particularly, the dissemination of Extended Spectrum Beta-Lactamase (ESBL) and fluoroquinolone (FQ) resistant *Escherichia coli* in food-producing animals posed a public health threat to the efficacy of various antibiotics. Therefore, this study aimed to detect the presence of multidrug-resistant *Escherichia coli* from fecal samples of pigs in Puducherry. In total, 16 faecal samples from apparently healthy pigs of different age groups were chosen for this study and subjected to detection of ESBL production and FQ resistance*. E. coli* was isolated from all the faecal samples (100%) and confirmed by biochemical tests and Polymerase Chain Reaction. All the *E. coli* isolates were found to be positive for ESBL production and fluoroquinolone resistance by phenotypic method. Out of 16 *E. coli* isolates, 11 (68.75%) isolates were positive for the presence of ESBL-producing genes, in which, 2 harbored *bla* TEM and 9 harboured *bla* SHV genes. In total 13/16 (81.2%) *E. coli* isolates harbored PMQR genes, in which 11 isolates harbored the *qnr*S gene and 11 isolates harbored the *qnr*B gene. The results showed that 9 isolates harbored both *qnr*S and *qnr*B genes in combination. Antimicrobial susceptibility test showed that the majority of the isolates exhibited a high level of resistance against Amoxycillin/clavulanic acid (100%), Cefpodoxime (100%), Cefotaxime (80%), Aztreonam (70%), Ceftazidime (70%), Enrofloxacin (70%) and Ceftriaxone (40%). The present study showed the presence of ESBL-producing and FQ-resistant *E. coli* in swine in the Puducherry region and their antibiogram pattern.

1. INTRODUCTION

Food animals, including pigs, are the important source for the development of antimicrobial-resistant (AMR) bacteria through the usage of antibiotics at a sub-therapeutic concentration in the form of feed additives and growth promoters in the farms (1). This leads to the rapid emergence of one of the most important resistance mechanisms extended spectrum β-lactamases (ESBLs), which has a serious impact on the treatment or therapeutic strategy of various infections mainly caused by the enteric bacteria, namely *Escherichia coli, Salmonella* spp., and *Klebsiella pneumoniae* (2). The close association between animals and humans and the interaction between commensal and pathogenic bacteria present in the animal gut possibly trigger such infection in human patients (3). Among the enteric bacteria, *Escherichia coli* is a commensal bacterium in the gut of animals and humans. The pathogenic strains can cause both intestinal and extra-intestinal infections in animals and humans (4). The commensal *E. coli* present in food animals can also acquire antibiotic resistance genes through

mobile genetic elements either from the animal itself, where they are maintained under various antibiotic selection pressures, or from the environment (5). *E. coli* is transmitted to humans through the food chain during slaughter, improper cooking, and unhygienic handling of food (6). The rapid emergence of extended-spectrum β-lactamases (ESBLs) in livestock and companion animals has been recorded and published worldwide (7). The primary cause of resistance to β-lactam antibiotics is the production of β-lactamases. Extended-spectrum β-lactamases (ESBLs) are a group of β-lactamase enzymes produced by Gram-negative bacteria, which can hydrolyze all cephalosporins and aztreonam but their activity is inhibited by clavulanic acid (8). Another group of important drugs were quinolones are broad-spectrum antibiotics and widely used in clinical practice. It plays an important role in the treatment of coliform infections (9) and is also used in the treatment and prevention of diseases in pigs worldwide (10). Due to overuse, resistance to quinolone antibiotics is increasingly common and spreading in some developing countries (11).

In food animal production, pig farming is a significant component of the livestock sector worldwide and pork is considered a major traded commodity globally. As per 20th livestock, the total pig population in the country is 9.06 million which accounts for 1.7% of the total livestock population. There is a growing market demand for pork products in India, driven by changing dietary preferences, urbanization, and a rising middle-class income. Although there are sporadic reports on AMR enteric bacteria of animal origin from different parts of India available, so far, very little is known about the AMR bacteria in pigs of India (12). There are not many reports on antimicrobial-resistant *E. coli* in pigs that have been carried out in India. Therefore, this study aimed to determine the antimicrobial resistance pattern of ESBL-producing and fluoroquinolone resistance *E. coli* isolated from fecal samples of pigs in Puducherry.

2. MATERIALS AND METHOD

2.1 Collection and Processing of Samples

Pigs of different age groups were reared under swill feeding to minimize the input costs were chosen for this study and a total of 16 fecal samples from pigs were collected, irrespective of their sex, age, or breed from two unorganized pig farms in Puducherry. The collected samples were processed at the Department of Veterinary Microbiology, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry.

2.2 Isolation and Identification of *E. coli*

Each fecal sample was individually inoculated into sterile test tubes containing Luria broth and then incubated for 18 hours at 37°C. A loopful of culture was streaked onto MacConkey's agar plate and incubated at 37°C for 24 hours. The pink lactose-fermenting colonies were chosen for Gram staining. The gram-negative bacteria were identified as *E. coli* based on their morphology, cultural characteristics on MacConkey's agar and EMB agar, and biochemical reactions as per Bergey's Manual of Systematic Bacteriology (13). The isolates biochemically characterized as *E. coli* were further confirmed by PCR, followed by detection of antimicrobial resistance.

2.3 Detection of *E. coli* using Polymerase Chain Reaction

Preparation of template DNA from *E. coli* strains was carried out as described by Kim *et al.,* (14) and Zhang *et al.,* (15) with minor modifications. PCR was performed for genotypic confirmation of *E. coli* by utilizing primers targeting the *alr* gene (16). PCR amplification was carried out in an automated thermal cycler (Eppendorf Mastercycler, Germany), as per PCR conditions described by Yokoigawa et al. (16). The resulting PCR products were analysed through 1.5% agarose gel electrophoresis under a UV transilluminator.

2.4 Phenotypic Identification of ESBL Production and FQ Resistance

All the *E. coli* isolates were screened for resistance against five indicator antimicrobial agents: Cefotaxime (30µg), ceftazidime (30µg), cefpodoxime (10µg), ceftriaxone (30µg), and aztreonam (30µg). Resistance to at least one indicator antibiotic was considered a "positive" screening test for ESBL production. The isolates positive by phenotypic screening were further confirmed by the combination method by using cefotaxime (30µg), cefotaxime plus clavulanic acid (30/10µg) discs (Hi-Media) on Mueller–Hinton agar plates by Kirby-Bauer disc diffusion method following CLSI guidelines (17). The isolate was confirmed as an ESBL producer when the inhibition zone diameter around the combination disc was ≥ 5 mm when compared to a disc containing cephalosporin alone (17). Similarly, phenotypic confirmation of fluoroquinolone resistance was carried out. The samples were streaked onto Mac Conkey agar supplemented with ciprofloxacin antibiotic (1mg/L) as per Bhardwaj *et al.* (18).

2.4 PCR for antimicrobial resistance genes

The isolates were further subjected to PCR for the detection of both ESBLs and fluoroquinolone-resistant genes and analysed.

Table 1: Primers details used for PCR in this study and their amplicon size

Target	Primer sequence (5'-3')	Annealing Temperature	Size (bp)	Reference
alr gene	CTGGAAGAGGCTAGCCTGGACGAG AAAATCGGCACCGGTGGAGCGATC	72°C	366	(16)
bla TEM	ATGAGTATTCAACATTTCCG CTGACAGTTACCAATGCTTA	56°C	867	(19)
bla SHV	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	56°C	393	(20)
bla CTX-M	CAATGTGCAGCACCAAGTAA CGCGATATCGTTGGTGGTG	59°C	540	(21)
*qnr*S	GCAAGTTCATTGAACAGGGT TCTAAACCGTCGAGTTCGGCG	52°C	428	(22)
*qnr*B	GGMATHGAAATTCGCCACTG TTTGCYGYYCGCCAGTCGAA	50°C	264	(22)

2.5 Antibiotic susceptibility testing

Antimicrobial susceptibility test was done on the Mueller-Hinton agar (HiMedia, Mumbai) plate as per the recommendation of the Clinical Laboratory Standard Institute (2019) using the commercially available antimicrobial discs (HiMedia) includes Amoxycillin/clavulanic acid (30µg), Cefpodoxime (10µg), Cefotaxime (30µg), Aztreonam (30µg), Ceftazidime (30µg), Enrofloxacin (30µg), and Ceftriaxone (30µg).

3. RESULTS AND DISCUSSION

In this present study, *E. coli* was isolated from all 16 fecal samples of swine (100%) and identified phenotypically using cultural and biochemical characterization followed by genotypic confirmation by PCR using the primer targeting the *alr* gene specific for *Escherichia coli* (366 bp) (Fig.3). All the *E. coli* isolates showed characteristic pink colored colonies in MacConkey agar (*Fig*.1), metallic sheen in EMB agar (*Fig.*2), gram-negative bacilli on microscopic examination, and biochemically they were catalase positive, oxidase negative, Indole test positive, Methyl red test positive, Voges Proskauer test negative, Citrate utilization test negative and urease test negative. The study on *E. coli* isolation from healthy pigs of organized farms showed higher occurrence (97%) in the Netherlands (23) and varying prevalence rate (49%) in Portugal (24) and 37% in Germany (25). In north-eastern India, 100% occurrence of *E. coli* in healthy and diarrhoeic pigs were reported (26). A lower occurrence (2 - 3%) was observed in rectal swabs of healthy pigs in Hong Kong (27)and Japan (28).

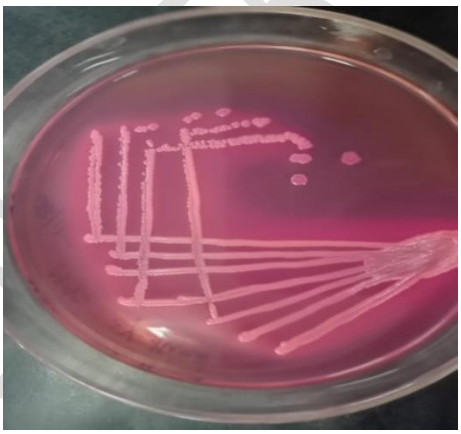

Fig 1. The lactose fermenting colonies of *E. coli* on MacConkey agar

Fig 2. The characteristic metallic sheen appearance of *E. coli* on EMB agar

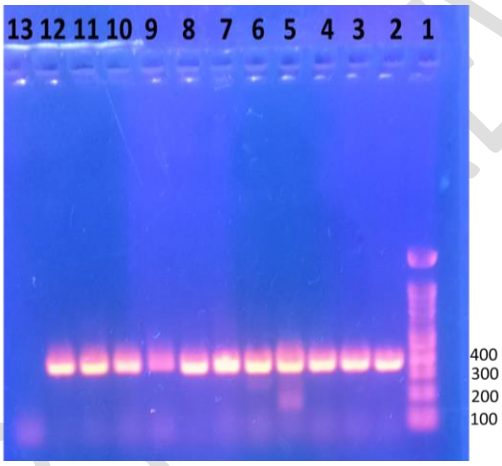

Fig 3. Agarose gel electrophoresis showing the results of polymerase chain reaction amplified product of 366 bp for the *alr* gene in *E. coli* isolates. Lane 1: 100 bp ladder; Lane 2 to 11: Field isolates positive for *alr* gene in *E. coli*; Lane 12: Positive control; Lane 13: Negative control

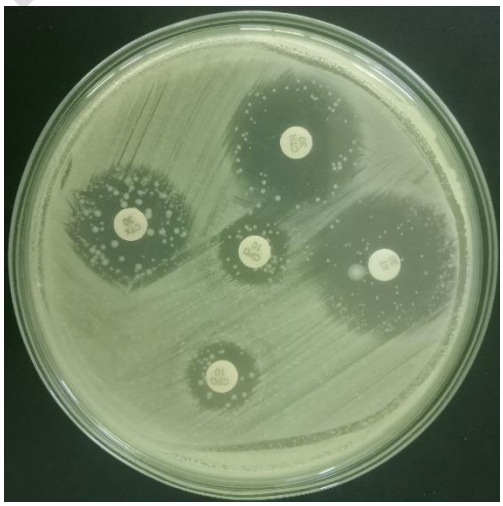

Fig 4. Phenotypic detection of *E. coli* isolates by indicator antibiotic discs

In this study, all the *E. coli* isolates were detected to be ESBL producers by the indicator discs method (Fig.4) and further confirmed by the combination disc method. Previous studies have reported the varying occurrence rate of ESBL-producing *E. coli* (6 to 70%) in India (29,30,31,32).

In the present study, out of 16 isolates, 11 (68.75%) isolates were found positive for ESBL-producing genes by PCR, in which 2 (18.18%) isolates harbored *bla* TEM (Fig. 6) and 9 (81.81%) isolates harbored *bla* SHV gene (Fig. 5). Two isolates harbored both *bla* TEM and *bla* SHV. None of the isolates were positive for CTX-M gene in this study. However, previous reports from other parts of the world indicated a comparatively higher prevalence of CTX-M among *E. coli* isolates from healthy pigs (15%) in Switzerland (33), 2-10% in China (10) and 21% from South Korea (34). Previous studies reported that the *bla* SHV gene (6.25%) alone was detected in *E. coli* from healthy pigs (35) and a lower prevalence (5.7%) of *bla* SHV was observed in organized pigs earlier (24).

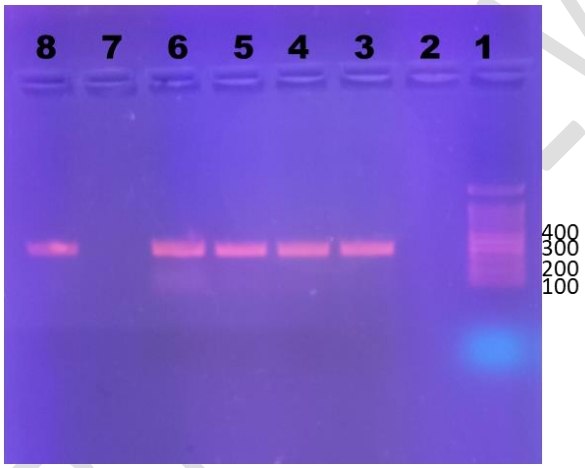

Fig 5. Agarose gel electrophoresis showing the results of polymerase chain reaction amplified product of 393 bp for the SHV gene in *E. coli* isolates. Lane 1: 100 bp ladder; Lane 2: Negative control; Lane 3: Positive control; Lane 4,5,6,7,8,9: Field isolates for SHV gene in *E. coli*;

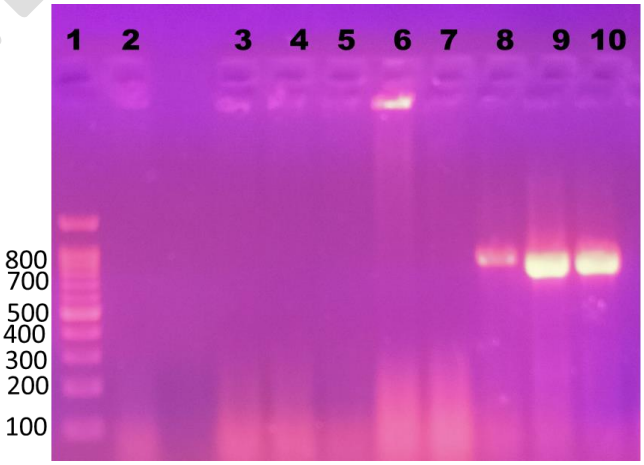

Fig 6. Agarose gel electrophoresis showing the results of polymerase chain reaction amplified product of 867 bp for the TEM gene in *E. coli* isolates. Lane 1: 100 bp ladder; Lane 3,4,5,6: Field isolates for TEM gene in *E. coli*; Lane 8: Positive control; Lane 2: Negative control

In this study about 13 (81.2%) samples were phenotypically positive for fluoroquinolone resistance. In total 13/16 (81.2%) *E. coli* isolates harbored FQ-resistant genes, in which 11 isolates harbored the *qnr*B gene (Fig. 7) and 11 isolates harbored the *qnr*S gene (Fig. 8). The results showed that 9 isolates harbored both *qnr*S and *qnr*B genes in combination. The resistance rate against fluoroquinolones was in the range of 74.4–82.6%, which is consistent with previous studies (36, 37,38). Differences in geographical location, sampling pattern, and other factors could have influenced the occurrence rate in the present study.

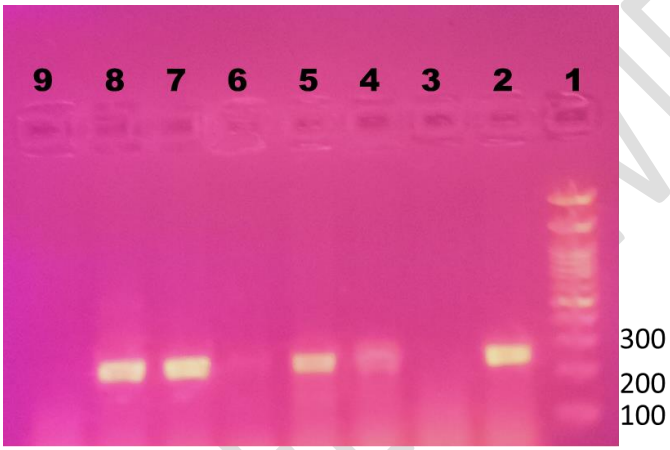

Fig 7. Agarose gel electrophoresis showing the results of polymerase chain reaction amplified product of 264 bp for the *qnr*B gene in *E. coli* isolates. Lane 1: 100 bp ladder; Lane 2&3,4,5: Field isolates for *qnr*B gene in *E. coli*; Lane 6: Positive control; Lane 7: Negative control

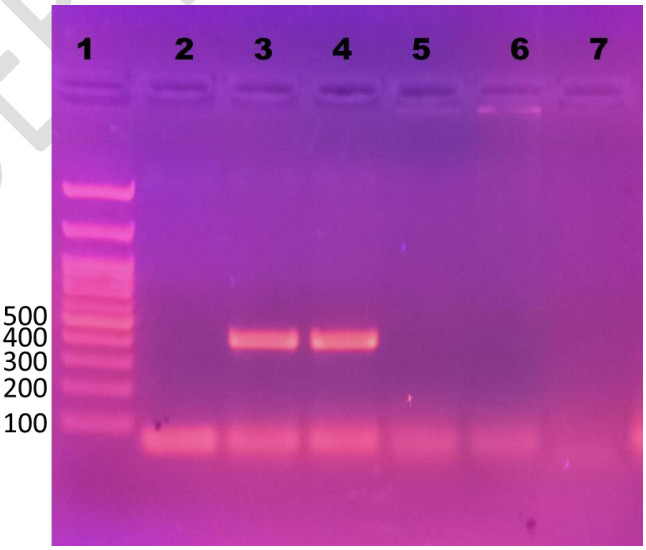

Fig 8. Agarose gel electrophoresis showing the results of polymerase chain reaction amplified product of 428 bp for the *qnr*S gene in *E. coli* isolates. Lane 1: 100 bp ladder; Lane 4,5,6,7: Field isolates for *qnr*S gene in *E. coli*; Lane 2: Negative control; Lane 3: Positive control

In this study, antimicrobial susceptibility test showed that the majority of the isolates exhibited a high level of resistance against Amoxycillin/clavulanic acid (100%), Cefpodoxime (100%), Cefotaxime (80%) followed by Aztreonam (70%), Ceftazidime (70%), Enrofloxacin (70%) and least resistant to Ceftriaxone (40%). In the Dutch samples, a high prevalence of resistance was observed in *E. coli* for three commonly used antibiotics in pig medicine, amoxycillin (70-94%), oxytetracycline (78-98%), and trimethoprim (62-96%) (39). Sasirekha (40) reported a similar resistance rate to Cefotaxime (84%), Ceftazidime (85%), and Ceftriaxone (75%) which is a higher resistant result when compared with the present study, among the *E. coli* isolates (Bangalore). All FQ-resistant *E. coli* isolates of suckling piglets showed MDR with high levels of resistance to aminoglycosides (100.0%), penicillins (100.0%), tetracyclines (97.7%), and phenicols (90.7%) (41). *E. coli* isolates from unorganized farms of pigs in Mizoram showed maximum resistance recorded against cefalexin (73.91%) and sulfafurazole/sulfisoxazole (73.91%), followed by ampicillin (72.46%), and trimethoprim (64.49%) (31).

4. CONCLUSION

The present study revealed the presence of *E. coli* carrying ESBL and fluoroquinolone-resistant genes in swine in the Puducherry region. The study indicates that the feces of swine can be a source of AMR and could spread in contact with humans and the external environment, resulting in a potential threat to public health and the environment.

REFERENCES

1. Aarestrup F M. Monitoring of antimicrobial resistance among food animals: Principles and limitations. Journal of Veterinary Medicine. Series B. 2004. 51; 380–388. https://doi.org/10.1111/j.1439-0450.2004.00775.x.
2. Hamilton WL and Wenlock R. Antimicrobial resistance: Amajor threat to public health. http://cambridgemedicine.org/hamilton2016/. Last accessed on 29-04-2018.
3. Venkatvasan R, Antony P, Mukhopadhyay H, Jayalakshmi V, Srinivas V. V, Thanislass J, Stephen S. Characterization of methicillin - Resistant Staphylococcus aureus from goats and their relationship to goat handlers using multi-locus sequence typing (MLST). Small Ruminant Research. 2020;186:106097. https://doi.org/10.1016/j.smallrumres.2020.106097.
4. Samanta I, Joardar SN, Das PK, Das P, Sar TK, Dutta TK, Bandyopadhyay S, Batabyal S, Isore D. Virulence Repertoire, Characterization, and Antibiotic Resistance Pattern Analysis ofEscherichia coliIsolated from Backyard Layers and Their Environment in India. Avian Diseases.2014. 58:39–45. https://doi.org/10.1637/10586-052913-reg.1.
5. González-Zorn B and Escudero JA. Ecology of antimicrobial resistance: humans, animals, food and environment. PubMed.2012; 15: 101–109. https://doi.org/10.2436/20.1501.01.163
6. Frye JG and Jackson CR. Genetic mechanisms of antimicrobial resistance identified in Salmonella enterica, Escherichia coli, and Enteroccocus spp. isolated from U.S. food animals. Frontiers in Microbiology.2013; 4. https://doi.org/10.3389/fmicb.2013.00135
7. Singaravelan T, Antony PX, Vasu J, Srinivas MV, Shanmugam VP, Shanmugam S, Mukhopadhyay HK. Isolation and Molecular Characterization of Extended Spectrum Beta-lactamase (ESBL) Producing Escherichia Coli from Dogs. Indian Journal of Veterinary Sciences and Biotechnology.2022. 18: 86-91.
8. Puii L, Dutta TK, Roychoudhury P, Kylla H, Chakraborty S, Mandakini R, Kawlni L, Samanta I, Bandopaddhay S, Singh S. Extended spectrum beta-lactamase producing Shiga-toxin producing-Escherichia coliin piglets, humans and water sources in North East region of India. Letters in Applied Microbiology. 2019.69: 373–378. https://doi.org/10.1111/lam.13216.

9. Michael, J.P., Jason, A.W., 2014. Bacterial topoisomerase inhibitors: quinolones and beyond. Antimicrobials, pp. 307–326
10. Tian G, Wang H, Zou L, Tang J, YingWang Z, Ye M, Tang J, Zhang Y, Zhang A, Yang X, Xu C, Fu Y. Detection of CTX-M-15, CTX-M-22, and SHV-2 Extended-Spectrum β-Lactamases (ESBLs) in *Escherichia coli* Fecal-Sample Isolates from Pig Farms in China. Foodborne Pathogens and Disease.2009; 6: 297–304. https://doi.org/10.1089/fpd.2008.0164.
11. Hart CA and Kariuki S. Antimicrobial resistance in developing countries. The BMJ.1998. 317:647–650. https://doi.org/10.1136/bmj.317.7159.647
12. Mandakini R, Dutta TK, Roychoudhury P, Subudhi P, Samanta I, Bandyopadhayay S. Isolation, identification and molecular characterization of multidrug resistant Escherichia coli recovered from pigs of Arunachal Pradesh, India. Indian Journal of Animal Health. 2019;58: 153. https://doi.org/10.36062/ijah.58.2spl.2019.153-168.
13. Bergey's Manual of Systematic Bacteriology, (1984). Vol 1 (edited by Kreig N.R.) & Vol II (edited by Sneath PHA) Williams & Wilkins.
14. Kim SH, Jeong HS, Kim YH, Song SA, Lee JY, Oh SH, Kim HR, Lee JN, Kho WG, Shin JH .Evaluation of DNA extraction methods and their clinical application for direct 122 detection of causative bacteria in continuous ambulatory peritoneal dialysis culture fluids from patients with peritonitis by using broad-range PCR. Ann Lab Med. 2012;32:119- 25.
15. Zhang J, Zheng B, Zhao L, Wei Z, Ji J, Li L, and Xiao Y. Nationwide high prevalence of CTX-M and an increase of CTX-M-55 in Escherichia coli isolated from patients with community-onset infections in Chinese county hospitals. BMC Infectious Diseases. 2014. https://doi.org/10.1186/s12879-014-0659-0
16. Yokoigawa K, Inoue K, Okubo Y and Kawai H. Primers for Amplifying an Alanine Racemase Gene Fragment to Detect E. coli Strains in Foods. Journal of Food Science. 1999; 64: 571–575. https://doi.org/10.1111/j.1365-2621.1999.tb15087.x
17. Clinical and Laboratory Standards institute. Performance Standards for Antimicrobial Susceptibility Testing: TwentyFourth Informational Supplement. M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA, USA. 2019.
18. Bhardwaj K, Shenoy S, Baliga S, Unnikrishnan B, Baliga B S and Shetty V K. Research Note: Characterization of antibiotic resistant phenotypes and linked genes of Escherichia coli and Klebsiella pneumoniae from healthy broiler chickens, Karnataka, India. Poultry Science.2021. 100; 101094.
19. Rasheed JK, Jay C, Metchock B, Berkowitz FE, Weigel LM, Crellin J, Steward CD, Hill BC, Medeiros AA, Tenover FC. Evolution of extended-spectrum beta-lactam resistance (SHV-8) in a strain of Escherichia coli during multiple episodes of bacteremia. Antimicrobial Agents and Chemotherapy.1997: 41:647–653. https://doi.org/10.1128/aac.41.3.647.
20. Colom K, Pérez J J, Alonso R, Fernández-Aranguiz A, Lariño E and Cisterna R. Simple and reliable multiplex PCR assay for detection of blaTEM, blaSHV and blaOXA-1 genes in Enterobacteriaceae. FEMS Microbiology Letters. 2003; 223: 147–151. https://doi.org/10.1016/s0378-1097(03)00306-9
21. Dutta TK, Warjri I, Roychoudhury P, Lalzampuia H, Samanta I, Joardar SN, Bandyopadhyay S and Chandra R. Extended-Spectrum-β-Lactamase-Producing Escherichia coli Isolate Possessing the Shiga Toxin Gene (stx 1) Belonging to the O64 Serogroup Associated with Human Disease in India. Journal of Clinical Microbiology. 2003; 51: 2008–2009. https://doi.org/10.1128/jcm.00575-13
22. Cattoir V, Poirel L, Rotimi V O, Soussy C J and Nordmann P. Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. The Journal of Antimicrobial Chemotherapy.2017; 60: 394–397. https://doi.org/10.1093/jac/dkm204
23. Nijsten R, London N, van den Bogaard A, Stobberingh E. Antibiotic resistance among Escherichia coli isolated from fecal samples of swine farmers and swine. J Antimicrob Chemother.1996;37:1131–1140.
24. Ramos S, Silva N, Jones-Dias D, Sousa M, Capelo-Martínez JL. Brito F, Caniça M, grejas G, Poeta P. Clonal Diversity of ESBL-Producing Escherichia coli in Pigs at Slaughter Level in Portugal. Foodborne Pathogens and Disease.2013;10:74–79. https://doi.org/10.1089/fpd.2012.1173

25. Von Salviati, Christina et al. “Extended-spectrum beta-lactamases (ESBL)/AmpC beta-lactamases-producing Escherichia coli in German fattening pig farms: a longitudinal study.” *Berliner und Munchener tierarztliche Wochenschrift* vol. 2014; 127: 412-9.
26. Rajkhowa, Swaraj, and Dilip Kumar Sarma. “Prevalence and antimicrobial resistance of porcine O157 and non-O157 Shiga toxin-producing Escherichia coli from India.” *Tropical animal health and production*.2014 ; 46: 931-7. doi:10.1007/s11250-014-0587-4
27. Duan RS, Sit TH, Wong SS, Wong RC, Chow KH, Mak GC and Ho PL. Escherichia coli producing CTX-M β-lactamases in food animals in Hong Kong. *Microbial drug resistance*.2006; *12*: 145-148.
28. Hiroi M, Yamazaki F, Harada T, Takahashi N, Iida N, Noda Y, Yagi M, Nishio T, Kanda T, Kawamori F, Sugiyama K Prevalence of extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae in food-producing animals. J Vet Med Sci.2012; 74:189–195.
29. Lalzampuia H, Dutta TK, Warjri I, Chandra R. PCR-Based Detection of Extended-Spectrum β-Lactamases (bla CTX-M-1 and bla TEM) in Escherichia coli, Salmonella spp. and Klebsiella pneumoniae Isolated from Pigs in North Eastern India (Mizoram). Indian Journal of Microbiology.2013;53:291–296. https://doi.org/10.1007/s12088-013-0378-z
30. Tamta S, Kumar OV, Pruthvishree B, Karthikeyan R, Rupner RN, Chethan G, Dubal ZB, Sinha DK, Singh BR. Faecal carriage of extended spectrum beta-lactamase (ESBL) and New Delhi metallo beta-lactamase(NDM) producing Escherichia coli between piglets and pig farmworkers. Comparative Immunology Microbiology and Infectious Diseases. 2020;73:101564. https://doi.org/10.1016/j.cimid.2020.101564.
31. Mandakini R, Roychoudhury P, Subudhi PK, Kylla H, Samanta I, Bandyopadhayay S, Dutta TK. Higher prevalence of multidrug-resistant extended-spectrum β-lactamases producing Escherichia coli in unorganized pig farms compared to organized pig farms in Mizoram, India. Veterinary World/Veterinary World.2020; 13:2752–2758. https://doi.org/10.14202/vetworld.2020.2752-2758.
32. Lalruatdiki, A., Dutta, T. K., Roychoudhury, P., & Subudhi, P. (2018). Extended-spectrum β-lactamases producing multidrug resistance Escherichia coli, Salmonella and Klebsiella pneumoniae in pig population of Assam and Meghalaya, India. Veterinary World, 11(6), 868–873. https://doi.org/10.14202/vetworld.2018.868-873.
33. Geser N, Stephan R and Hächler H. Occurrence and characteristics of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. BMC Veterinary Research.2012; 8(1). https://doi.org/10.1186/1746-6148-8-21.
34. Tamang MD, Nam H, Kim S, Chae MH, Jang G, Jung S, Lim S. Prevalence and Molecular Characterization of CTX-Mβ-Lactamase–ProducingEscherichia coliIsolated from Healthy Swine and Cattle. Foodborne Pathogens and Disease.2013; 10:13–20. https://doi.org/10.1089/fpd.2012.1245.
35. Bardoň J, Husičková V, Chromá M, Kolář M. Prevalence and Characteristics of Escherichia coli Strains Producing Extended-Spectrum β-Lactamases in Slaughtered Animals in the Czech Republic. Journal of Food Protection. 2013. 76; 1773–1777. https://doi.org/10.4315/0362-028x.jfp-13-114.
36. Gao L, Tan Y, Zhang X, Hu J, Miao Z, Wei L and Chai T. Emissions of Escherichia coli Carrying Extended-Spectrum β-Lactamase Resistance from Pig Farms to the Surrounding Environment. International Journal of Environmental Research and Public Health.2015; 12: 4203–4213. https://doi.org/10.3390/ijerph120404203.
37. Zhu S, Y Xing, T Hu and L. Lei. Detection and analysis of the fluoroquinolone resistance gene ssequence from 15 clinical isolates of antibiotic-resistant Escherichia coli. Chin. J. Vet. Sci. .2011; 31:875–880.
38. Xiao YH, Shen P, Wei Z, Chen Y.B, Kong HS, Yang Q. 2010 report of Mohnarin national bacterial resistant surveillance. Chin. J. Nosocomiology. 2011; 23:4896–4902.
39. Van Den Bogaard, A. E., London, N., & Stobberingh, E. E. (2000). Antimicrobial resistance in pig faecal samples from The Netherlands (five abattoirs) and Sweden. Journal of Antimicrobial Chemotherapy, 45(5), 663–671. https://doi.org/10.1093/jac/45.5.663
40. Sasirekha B and Shivakumar S. Occurrence of Plasmid-Mediated AmpC β-Lactamases Among Escherichia coli and Klebsiella pneumoniae Clinical Isolates in a Tertiary Care Hospital in

Bangalore. Indian Journal of Microbiology.2011; 52: 174–179. https://doi.org/10.1007/s12088-011-0214-2.
41. Seo K, Do KH, Lee WK. Molecular characteristics of fluoroquinolone-resistant Escherichia coli isolated from suckling piglets with colibacillosis. BMC microbiology.2022; 22:216. https://doi.org/10.1186/s12866-022-02632-9.