



Characterisation of *Escherichia coli* Recovered from Wild Animals Kept at Bikaner Zoo for Their Antibiotic Resistance

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Authors' contributions

This work was carried out in collaboration among all authors. Author SZ designed the study, wrote the protocol, conducted the experiments and wrote the first draft of the manuscript. Authors KG and Diwakar managed the analyses of the study and contributed in conducting experiments. Author FA also helped in conducting experiments. Author AKK supervised all the authors for conducting this research, guided in methodology and analysis of the study and also reviewed the original draft. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i330408

Editor(s):

(1) Dr. Vasudevan Mani, Qassim University, Saudi Arabia.

Reviewers:

(1) Iwu Chidozie Declan, University of Fort Hare, South Africa.

(2) Márió Gajdács, University of Szeged, Hungary.

(3) Teresita Sainz Espuñes, Universidad Autónoma Metropolitana Xochimilco Campus, Mexico.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/55265>

Original Research Article

Received 01 January 2020

Accepted 07 March 2020

Published 17 March 2020

ABSTRACT

In the present study, a total of 41 *E. coli* isolates obtained from *Boselaphus tragocamelus* (5), *Antelope cervicapra* (18) and *Gazella gazelle* (18) maintained at Bikaner (Rajasthan) zoo were subjected to antibiogram determination against 15 antibiotics belonging to four different classes and were also genotyped for detecting presence of *bla*_{TEM}, *sul-2*, *strA* and *aadA* genes. Antibiogram study revealed highest efficacy of ciprofloxacin (90.2%) followed by nalidixic acid (75.6%) and chloramphenicol (68.4%) and high resistance to β lactam, Sulfamethoxazole and Aminoglycoside antibiotics. The overall presence of *bla*_{TEM}, *sul-2*, *strA* and *aadA* genes was detected in 95.12%, 80.48%, 60.97% and 87.8% in isolates.

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Keywords: Antibiogram; *Escherichia coli*; *aadA*; *bla_{TEM}*; *strA* and *sul-2*.

1. INTRODUCTION

Antimicrobial resistance is a matter of great concern due to the reduced efficacy or failures of antibiotics to treat various bacterial infections [1]. There are many probable reasons for emergence of development of antimicrobial resistance of which one reason could be the secretion of antimicrobial agents by another bacterium such as *Streptomyces* mainly as a means of microbial competition for an ecological niche [2,3,4,5]. Another possible reason could be the excessive use of antibiotics which can exert a selection pressure on bacteria which results in accumulation of resistance genes in microbiota converting them into a pathogenic bacterium which are otherwise commensals [6,7,8,9]. Hence, the natural selection is one of the main causes which has supported the selection and evolution of multiresistant phenotypes of bacteria and their spread to different areas without direct use of antimicrobials [10]. Mutations, horizontal transfer of genes and transfer of already resistant bacteria found in the faecal matter, manure, sewage water, soil, clinical specimen of other animals and human could also be the other possible sources of emergence of resistant genes in bacteria [11,3].

Wild animals are rarely exposed to antimicrobial agents and there are very few reports available on the presence of antibiotic resistant bacteria from wild captive animals but sufficient data is available which depicts the presence of antibiotic resistant bacteria in the vicinity of captive wild animals [12,13].

Escherichia coli is a commensal bacterium commonly found in the intestinal gut of animals and humans [14,15] and is responsible for causing intestinal infections [16,17]. This organism is commonly used as a potential indicator of faecal contamination and is expected to be reservoir of antibiotic resistance genes [6] because of the regular use of antibiotics for treatment of infectious diseases. This is one of the major growing concerns in veterinary medicine as the resistant bacteria can enter into other ecological niches and can cause a potential risk to human health. Keeping this in view, study of antibiotic resistance situations in a particular area is prerequisite in order to access the possible risk of its transfer to other individuals. At present, there are few reports available on isolation and prevalence of antibiotic resistance

genes on *E. coli* found in wild captive animal species. To our knowledge, there are very few data available in India which can clearly depicts the situation.

Hence the present study was conducted to investigate phenotypic and genotypic properties of *E. coli* isolates obtained from wild captive animals at Bikaner zoo in regard to their antibiotic resistance. Our studies identified the presence of *bla_{TEM}*, *sul2*, *strA* and *aadA* resistance genes in *E. coli* from captive wild animal populations. *bla_{TEM}* provides resistance against various β -lactam antibiotics like penicillin and Ampicillin. *sul2* gene provides resistance against sulfamethoxazole antibiotics, *strA* work against streptomycin and *aadA* gene codes for aminoglycoside adenyl transferase enzyme which allows selection for resistance to aminoglycoside antibiotics such as spectinomycin and streptomycin.

2. METHODOLOGY

2.1 Sampling

For isolation of *E. coli*, the faecal samples from wild captive animals at Bikaner (Rajasthan) zoo were collected after permission from appropriate authorities. A total of 46 freshly voided faecal samples were collected from *Gazella bennettii*, (18) *Antelope cervicapra*, (20) *Boselaphus tragocamelus* (8) in June 2018. All the samples were kept at ambient temperature, transported to the laboratory and incubated overnight in the nutrient broth for 24 hours.

2.2 Isolation and Identification of *E. coli*

The isolation and identification of *E. coli* from faecal samples was carried out by conventional methods as described by Cowan and Steel [18] and Quinn et al. [19]. Each sample was inoculated on to the EMB agar by streaking and incubated aerobically at 37°C for 18 to 24 hours. Appearance of green metallic sheen on EMB agar revealed the presence of *E. coli*. Colonies with typical *E. coli* morphology were further sub-cultured until pure isolated colonies were obtained. The obtained pure *E. coli* colonies were gram stained and biochemical identification was done using Vitek 2 automated system (BioMerieux India private LTD). The colonies were also genotypically confirmed by PCR using 16 S rRNA as described by Khaled et al. [20].

The confirmed isolated colonies were streaked on agar slants and maintained for further use.

2.3 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was performed by the agar disc diffusion method as described by Bauer et al. [21] and as per clinical and laboratory standards institute [22]. A total of 15 antimicrobial agents belonging to different classes and generations were tested in this study (Table 1). Diameter of zone of inhibition was observed and results were interpreted as sensitive, intermediate and resistant isolates for different antimicrobial agents. The isolates with resistance to one or more antimicrobial agents were selected for detection of genes for antimicrobial resistance.

2.4 Detection of Genes for Antibiotic Resistance

Polymerase chain reaction was carried out with a Veriti 96 well thermocycler to detect presence of *bla*_{TEM}, *aadA*, *strA* and *sul-2* genes amplified through specific primers (Table 1) and using specific conditions (Table 2). For PCR 25 µl of PCR mixture was used, which included 7.5 µl of Nuclease free H₂O, .3µM of forward primer, .3µM of reverse primer, 12.5 µl of master mix and 50ng/ul of Template DNA. The amplified DNA was visualized by Gel electrophoresis in 1% agarose gel in 1X TBE buffer.

3. RESULTS

A total of 41 *E. coli* isolates from *G. bennettii*, (18), *A. cervicapra*, (18) and *B. tragocamelus* (5) were recovered after phenotypic and genotypic confirmation for analysis in this study. High prevalence of *E. coli* (82%) in faecal matter was also observed by Akond et al. [23]. The results of antibiogram study were interpreted as per the

literature supplied by the manufacturer. Accordingly, the response of organisms was characterized as sensitive, intermediate and resistant. The antibiotic susceptibility pattern of *E. coli* isolates from captive wild Animals faeces samples has been outlined in Table 3. The Antibiogram against all the isolates revealed that most effective antibiotic was Ciprofloxacin followed by Nalidixic acid, Chloramphenicol and Gentamicin (Table 3) and no resistance was recorded against Ciprofloxacin, Nalidixic acid and Gentamicin and little resistance was exhibited against Chloramphenicol.

Resistance spectrum of *E. coli* for 15 antibiotics tested in descending order was respectively polymyxin B, Cephalothin, Cefepime, Cefaclor, Piperacillin, Cefexime and Co- Trimoxazole, Ampicillin and Tetracycline, Colistin, Chloramphenicol, followed by no resistance in case of Ciprofloxacin, Gentamicin, and Nalidixic acid. It was shown that none of the isolates were found resistant against Ciprofloxacin, Gentamicin and Nalidixic acid and 80.5% isolates were found resistant against Polymyxin B (Table 3). The highest sensitivity was recorded for the antibiotic Ciprofloxacin followed by Nalidixic acid. Least Sensitivity was found against Co-Trimoxazole followed by Tetracycline.

The detection of genes for antibiotic resistance in the isolates by PCR revealed that of the total 41 isolates, *bla*_{TEM} gene was present in 39 (95.12%) isolates and one isolate each from *Antelope cervicapra* (5.5%) and *Boselaphus tragocamelus* (20%) was found not to possess this gene.

The *sul2* gene was present in 25 (60.97%) isolates. All the *Gazella bennettii* samples were found positive for this gene, whereas 13 (72.22%) isolates from *Antelope cervicapra* and three (60%) from *Boselaphus tragocamelus* samples did not have *sul2* gene.

Table 1. Primers and amplicon sizes for detection of genes for antibiotic resistance

Gene	Primer sequence	Amplicon size
<i>bla</i> _{TEM}	TTA ACT GGC GAA CTA CTT AC GTC TAT TTC GTT CAT CCA TA	227 bp
<i>Sul2</i>	CGG CAT CGT CAA CAT AAC CT TGT GCG GAT GAA GTC AGC TC	721 bp
<i>StrA</i>	ATG GTG GAC CCT AAA ACT CT CGT CTA GGA TCG AGA CAA AG	893 bp
<i>aadA</i>	GTG GAT GGC GGC CTG AAG CC AAT GCC CAG TCG GCA GCG	525 bp

Table 2. PCR conditions for detection of genes for antimicrobial resistance

Gene	Initial denaturation	Denaturation	Annealing temperature	Extension	Final extension
<i>bla_{TEM}</i>	94°C (4 min)	94°C (1 min)	50°C	72°C (2 min)	72°C (7 min)
<i>Sul2</i>	94°C (4 min)	94°C (1 min)	66°C	72°C (2 min)	72°C (7 min)
<i>strA</i>	94°C (4 min)	94°C (1 min)	56.5°C	72°C (2 min)	72°C (7 min)
<i>aadA</i>	94°C (4 min)	94°C (1 min)	63°C	72°C (2 min)	72°C (7 min)

Table 3. Antibiogram results of *E. coli* isolates from faeces of Chinkara, black buck and blue bull from Bikaner Zoo

S. no.	Antibiogram disc (Concentration/Disc)	Sensitive (%)	Intermediate (%)	Resistant (%)
1.	Polymyxin B (300 ug)	4.9	14.6	80.5
2.	Cephalothin (30 ug)	7.3	14.6	78.1
3.	Cefepime (30 ug)	7.4	14.6	78.0
4.	Cefaclor (30 ug)	21.9	9.8	68.3
5.	Piperacillin (100 ug)	7.4	26.8	65.8
6.	Cefixime (5 ug)	9.8	26.8	63.4
7.	Co-trimoxazole (CoT) (1.25/23.75 ug)	2.4	34.2	63.4
8.	Ampicillin (10 ug)	12.2	29.3	58.5
9.	Tetracycline (30 ug)	2.5	39.9	58.5
10.	Colistin (10 ug)	27.9	62.2	9.9
11.	Chloramphenicol (30 ug)	68.4	17.1	9.5
12.	Imipenem (10 ug)	53.6	41.7	4.7
13.	Ciprofloxacin (5 ug)	90.2	9.8	0
14.	Nalidixic acid (10 ug)	75.6	24.4	0
15.	Gentamicin (10 ug)	68.3	31.7	0

The *strA* gene was detected in 33 (80.48%) isolates. None of the isolates from *Gazella bennettii* was found negative for *strA* gene whereas six (33%) isolates from *Antelope cervicapra* and two from *Boselaphus tragocamelus* (40%) were found negative for this gene.

The gene *aadA* was present in 36 (87.80%) isolates. All the *Gazella bennettii* samples were found positive for this gene whereas 3 (16.66%) isolates from *Antelope cervicapra* and two (40%) from *Boselaphus tragocamelus* samples did not have *sul2* gene.

4. DISCUSSION

Increasing antimicrobial resistance in bacterial population is a global problem because it creates trouble in fighting infectious diseases. Improper and excessive use of antimicrobial agents along with poor environmental sanitation and low personnel hygiene have been attributed to such problems. There is paucity of data in the literature about the susceptibility to antibiotics in *E. coli* isolates of healthy wild captive animals. If data are available they are restricted to a low

number of animals. There are strong evidences available in literature supporting a direct relationship between the frequency of antibiotic resistance observed in *E. coli* isolates of captive wild animals and the degree of contact of these animals with humans which suggest the anthropogenic nature of Antibiotic resistance in *E. coli* isolates obtained from faecal matter of wild animals [24].

We investigated the occurrence of antibiotic resistance in commensal *E. coli* from wild captive animals in Bikaner district of Rajasthan state in India. It is an area with limited study on antibiotic resistance and use of antimicrobial agents. In our study, a high resistance against piperacillin (65.8) was recorded which were in accordance with Najam et al. [25], who also recorded almost similar results for piperacillin where they recorded very high resistance (95%) to Piperacillin in *E. coli* isolates.

Our findings also corroborated observation of Read et al. [26] who reported 0% resistance to ciprofloxacin and low resistance to Gentamicin in *E. coli*. In a previous study, Guo et al. [27] reported that 55% isolates of *E. coli* were

resistant to gentamicin but in our study no isolate was found resistant to gentamicin, these results were similar to the results obtained by Trivia et al. (2006) who also observed no isolate resistant to gentamicin. Similarly, in our study, 80.5% of the isolates were found resistant to polymyxin B, which were not consistent to results obtained by Lanz et al. [28] who observed no isolates resistant to this antibiotic. Chayani et al. [29], Pachori and Kulkarni [30] and Malakar [31] have also reported low sensitivity of *E. coli* isolates against Tetra cyclin as observed in the present investigation. Similar findings of resistance were reported by Cid et al. [32] and Khan et al. [33].

Kozak et al. [34] also observed high resistance to tetracycline among *E. coli* isolates obtained from small wild mammals. The high resistance against tetracycline among *E. coli* isolates is also in accordance with previous values obtained from similar reports [35,36,37,38]. Tetracycline is being used as first line antibiotic for treatment and growth promotion among food animals [39]. It is likely that resistance genes present on mobile genetic elements [40] got transferred from food animals to humans and then from humans to wild captive animals. Even tetracycline used in animal feed can create a selection pressure on bacteria harbouring resistant genes which give these bacteria an opportunity to grow in tetracycline susceptible environments.

The present findings also confirmed the earlier results of Andrasevic et al. [41] who found *E. coli* isolates resistant to ampicillin, co-trimoxazole, ciprofloxacin and gentamicin.

Similar to the observed pattern of phenotypic resistance, genes conferring resistance to β -lactamase, aminoglycoside adenosyl transferase, streptomycin/spectinomycin, and sulfamethoxazole were also found to be common among the isolates. Our results were almost similar to Adelowo et al. [42] who also observed high prevalence of *bla*_{TEM} (85%), *sul2* (67%), *aadA* (65%) and *strA* (70%) in poultry animals. However, it seemed that local and geographical factors and the factors other than antibiotic use may be contributing to the selection of resistance among the present isolates. All the genes seem to have a widespread occurrence within the study area. The widespread occurrence of all four antibiotic resistance genes observed in this study may be a result of their co selection and transfer with other resistance determinants on mobile genetic elements.

Escherichia coli isolates from humans have been found to be important reservoirs of quinolone resistance genes. In our study detection of quinolone resistance in *E. coli* isolates may be a result of plasmid mediated transfer of quinolone resistance genes from humans to captive animals. This is of concern as fluoroquinolones were listed by the World Health Organization as critically important antimicrobials for human health [43]. Therefore, it seems very important to pay more attention to decrease the spread of resistance in the captive areas where the interaction between human and animals are more common.

Though we tested for the resistance genes most commonly found in *E. coli*, but the absence of resistance genes in some of the isolates resistant to a particular antibiotic may be because of presence of some known mechanism not under this study or some unknown mechanism.

Though a definite conclusion on the association between drug use and occurrence of antimicrobial resistance cannot be drawn through this study as we did not include areas without antimicrobial use or the areas without any human interference but the results of this study highlights the need of routine screening for antibiotic resistance using both phenotypic and genotypic methods in commensal bacteria from wild captive animals.

5. CONCLUSION

This study suggests that close proximity to humans, unhygienic practices, poor maintenance of wild captive areas, are the reasons which increase the chances of antimicrobial resistance in *E. coli* isolates in captive wild animals of small areas like Bikaner zoo. The data of our study reveals that wild captive animals may act as a reservoir of antibiotic resistant genes which may be mobilized into other ecological populations. The data from the present study accounts for the critical need for regulation of antimicrobial drug usage in wild captive animals and highlights for continuous monitoring of antibiotic resistance.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

ACKNOWLEDGEMENT

We thank Forest department of Rajasthan, Jaipur, for permitting to collect samples from wild captive animals from Bikaner zoo. Thanks, are also due to Council of scientific and Industrial research, New Delhi for providing SRF to first author.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Authier S, Paquette D, Labrecque O, Messier S. Comparison of susceptibility to antimicrobials of bacterial isolates from companion animals in a veterinary diagnostic laboratory in Canada between 2-time points 10 years apart. *The Canadian Veterinary Journal*. 2006;47(8): 774.
2. Martinez JL. Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environ. Pollut*. 2009a;157: 2893–2902.
3. Martinez JL. The role of natural environments in the evolution of resistance traits in pathogenic bacteria. *Proc. Biol. Sci*. 2009b;276:2521–2530.
4. Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. Call of the wild: Antibiotic resistance genes in natural environments. *Nat. Rev. Microbiol*. 2010;8:251–259.
5. D'Costa VM, King CE, Kalan L, Morar M, Sung WW, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R, Golding GB, Poinar HN, Wright GD. Antibiotic resistance is ancient. *Nature*. 2011;477: 457–461.
6. Van den Bogaard AE, Stobberingh E. Epidemiology of resistance to antibiotics links between animals and humans. *Intern. J. Antimicrob. Agents*. 2000;14:327–335.
7. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. *J. Antimicrob. Chemother*. 2004;54:321–332.
8. Moyaert H, De Graef EM, Haesebrouck F, Decostere A. Acquired antimicrobial resistance in the intestinal microbiota of diverse cat populations. *Res. Vet. Sci*. 2006;81:1–7.
9. De Graef EM, Decostere A, Devriese LA, Haesebrouck F. Antibiotic resistance among fecal indicator bacteria from healthy individually owned and kennel dogs. *Microb. Drug determinants in pig isolates from three herds with different histories of antimicrobial agent exposure*. *Appl. Environ. Microbiol*. 2004;59:1467–147.
10. O'Brien TF. Emergence, spread, and environmental effect of antimicrobial resistance: How use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. *Clinical Infectious Diseases*. 2002; 34(Supplement_3):S78-S84.
11. Kummerer K, Henninger A. Promoting resistance by the emission of antibiotics from hospitals and households into effluent. *Clin. Microbiol. Infect*. 2003;9: 1203–1214.
12. Sato G, Oka C, Asagi M, Ishiguro N. Detection of conjugative R plasmids conferring chloramphenicol resistance in *Escherichia coli* isolated from domestic and feral pigeons and crows. *Zentralbl. Bakteriol. Orig. A*. 1978;241:407–417.
13. Kanai H, Hashimoto H, Mitsuhashi S. Drug resistance and conjugative R-plasmid in *Escherichia coli* strains isolated from wild birds (Japanese tree sparrows, green pheasants and bamboo partridges). *Jpn. Poult. Sci*. 1981;18:234–239.
14. Tannock GW. Normal microflora. In: *An Introduction to Microbes Inhabiting the Human Body*, Chapman and Hall, London; 1995.
15. Sørum H, Sunde M. Resistance to antibiotics in the normal flora of animals. *Vet. Res*. 2001;3(2):227–241.
16. Sa'enz Y, Zarazaga M, Brin'as L, Lantero M, Ruiz-Larrea F, Torres C. Antibiotic resistance in *Escherichia coli* isolates obtained from animals, foods and humans in Spain. *Int. J. Antimicrob. Agents*. 2001;18:353–358.
17. Rosas I, Salinas E, Marti'nez L, Calva E, Cravioto A, Eslava C, Ama'bile-Cuevas CF. Urban dust faecal pollution in Mexico City: Antibiotic resistance and virulence factors of *Escherichia coli*. *Int. J. Hyg. Environ. Health*. 2006;209:461–470.
18. Cowan ST, Steel KS. *Manual for the identification of medical bacteria*, 2nd Ed. Cambridge University Press, Cambridge; 1975.
19. Quinn PJ, Carter ME, Markey BK, Carter GR. *General procedures in microbiology*. Clinical Veterinary Microbiology. Wolfe Publishing, London. 1994;48.

20. Khaled A, Razik El, Abdel Rahman, Youssef F, Alaa M, Hazem A. Direct identification of major pathogen of bubaline subclinical mastitis in Egypt using PCR. *American Journal of Science*. 2010;6(10): 652-660
21. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*. 1966;45(4): 493.
22. CLSI. Performance standards for antimicrobial susceptibility testing; Seventeenth Informational Supplement. Clinical and Laboratory Standards Institute M100-S17: 1. Wayne, Pennsylvania. 2007;32-50.
23. Akond MA, Alam S, Hassan SMR, Shirin M. Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. *Internet Journal of Food Safety*. 2009;11:19-23.
24. Skurnik DR, Ruimy A, Andremont C, Amarin P, Rouquet B, Picard, Denamur E. Effect of human vicinity on antimicrobial resistance and integrons in animal faecal *Escherichia coli*. *J. Antimicrob. Chemother.* 2006;57:1215–1219.
25. Najam M, Koppad M, Halesh L, Siddesh K. Detection of carbapenem resistance in extended spectrum beta lactamase producing *Escherichia coli* isolates in a tertiary care hospital. *Indian J Microbiol Res*. 2015;2(3):138-41.
26. Read SC, Gyles CL, Clarke RC, Lior H, McEwen S. Prevalence of verocytotoxigenic *Escherichia coli* in ground beef, pork and chicken in southwestern Ontario. *Epidemiol. Infect.* 1990;105:11–20.
27. Guo S, Wakeham D, Brouwers HJ, Cobbold RN, Abraham S, Mollinger JL, Trott DJ. Human-associated fluoroquinolone-resistant *Escherichia coli* clonal lineages, including ST354, isolated from canine faeces and extraintestinal infections in Australia. *Microbes and Infection*. 2015;17(4):266-274.
28. Lanz R, Kuhnert P, Boerlin P. Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. *Veterinary Microbiology*. 2003;91(1):73-84.
29. Chayani N, Tiwari S, Sarangi G, Mallick B, Mohapatra A, Paty BP, Das P. Role of azithromycin against clinical isolates of family *Enterobacteriaceae*: A comparison of its minimum inhibitory concentration by three different methods. *Indian Journal of Medical Microbiology*. 2009;27(2):107.
30. Pachori RR, Kulkarni NS. Antibiogram of pathogens associated with Pelvic inflammatory disease (PID).
31. Malakar M. Antimicrobial susceptibility patterns of *Escherichia coli* isolated from urine and stool samples of infected patients in Lakhimpur district of Assam, India; 2014.
32. Cid D, Blanco M, Blanco JE, Santa Quiteira JR, de la Fuente R, Blanco J. Serogroups, toxins and antibiotic resistance of *Escherichia coli* strains isolated from diarrhoeic goat kids in Spain. *Veterinary Microbiology*. 1996;53(3-4): 349-354.
33. Khan A, Das SC, Ramamurthy T, Sikander A. Antibiotic resistance, virulence gene, and molecular profile of shiga toxin-producing *Escherichia coli* isolates from diverse source in Calcutta, India. *Journal of Clinical Microbiology*. 2002;40(6):2009-2015.
34. Kozak GK, Boerlin P, Janecko N, Reid-Smith RJ, Jardine C. Antimicrobial resistance in *Escherichia coli* isolates from swine and wild small mammals in the proximity of swine farms and in natural environments in Ontario, Canada. *Applied and Environmental Microbiology*. 2009; 75(3):559-566.
35. Boerlin PR, Travis CL, Gyles R, Reid-Smith N, Janecko H, Lim V, Nicholson SA, McEwen R, Friendship, Archambault M. Antimicrobial resistance and virulence genes of *Escherichia coli* isolates from swine in Ontario. *Appl. Environ. Microbiol.* 2005;71:6753–6761.
36. Lee C, Langlois BE, Dawson KA. Detection of tetracycline resistance determinants in pig isolates from three herds with different histories of antimicrobial agent exposure. *Appl. Environ. Microbiol.* 1993;59(5):1467-1472.
37. Maynard C, Bekal S, Sanschagrín F, Levesque RC, Brousseau R, Masson L, Harel J. Heterogeneity among virulence and antimicrobial resistance gene profiles of extraintestinal *Escherichia coli* isolates of animal and human origin. *Journal of Clinical Microbiology*. 2004;42(12):5444-5452.
38. Stine OC, Johnson JA, Keefer-Norris A, Perry KL, Tigno J, Qaiyumi S, Stine MS,

- Morris JG Jr. Widespread distribution of tetracycline resistance genes in a confined animal feeding facility. *Int. J. Antimicrob. Agents.* 2007;29:348–352.
39. Roberts MC. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility and distribution. *FEMS Microbiol. Rev.* 1996;19:1–24.
40. Roberts MC. Update on acquired tetracycline resistance genes. *FEMS Microbiol. Lett.* 2005;245:195–203.
41. Andrašević AT, et al. *Emerg. Infect. Dis.* 2002;8:14-18.
42. Adelowo OO, Fagade OE, Agero Y. Antibiotic resistance and resistance genes in *Escherichia coli* from poultry farms, southwest Nigeria. *The Journal of Infection in Developing Countries.* 2014;8(09):1103-1112.
43. Collignon P, Powers JH, Chiller TM, Aidara-Kane A, Aarestrup FM. World health organization ranking of antimicrobials according to their importance in human medicine: A critical step for developing risk management strategies for the use of antimicrobials in food production animals. *Clin Infect Dis.* 2009;49:132-141.

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