Prevalence, antibiotic sensitivity profile, and phylogenetic analysis of *Escherichia coli* isolated from raw dromedary camel milk in Matrouh Governorate, Egypt

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ABSTRACT

Objective: Most people in Matrouh Governorate consume camel milk as a treatment for many diseases in a raw state to obtain nutritive value. Raw dromedary camel milk can be contaminated by *Escherichia coli* through fecal matter at any point of milk handling; therefore, it may lose its value and safety specifications. This survey aimed to estimate the incidence of *E. coli* in fresh camel milk.

Materials and Methods: 100 fresh camel milk samples (50 from markets and 50 from farms) were randomly collected from different districts in Matrouh Governorate, Egypt, over 4 months for the detection of *E. coli* incidence through conventional bacterial isolation, molecular investigation, and gene sequencing.

Results: The prevalence rates of *E. coli* in the examined market and farm raw camel milk based on conventional methods were 24% and 8%, respectively, while those by molecular identification using *phoA* as an *E. coli* determinate gene were 4% and 6%, respectively. Moreover, *E. coli* *phoA* gene phylogenetic analysis revealed high sequence similarity to *E. coli* strain CP033158.1 in India and *E. coli* strain CP047594.1 in China. Antibiotic sensitivity of *E. coli* isolates showed high susceptibility to norfloxacin (10 µg) and cefoperazone (75 µg). On the other hand, high resistance was found in rifampicin (30 µg) and cefoxitin (30 µg).

Conclusion: The results indicate that market camel milk is more contaminated than the farms’ own. Additionally, antibiotic resistance is increasing due to antibiotic abuse.

Introduction

Raw dromedary camel milk is an essential factor in the nomadic diet for its medicinal properties in relation to many diseases and its richness in vitamins, minerals, antimicrobial factors, and antioxidants compared with other animal species [1].

Microbial contamination of raw camel milk may have multiple sources, such as the udder, utensils, droplets, cleaning water, dairymen, and dust. Furthermore, its nutritional value, which is good for microbial growth, depends on how long it has been stored [2]. *Escherichia coli* is a bacteria that is frequently found in human bowels and warm-blooded animals. Many *E. coli* strains are commensal. Nevertheless, certain strains, such as Shiga toxin-*E. coli* (STEC), may cause food poisoning, while others cause urinary and respiratory infections and other diseases [3]. It is passed to humans through contaminated food consumption, such as raw milk and raw food products [4].

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The occurrence rates of E. coli in raw dromedary camel milk in different countries were 8.1% of 24 samples in Harar and Dire Dawa, Eastern Ethiopia; 25.71% of 35 samples in Matrouh Governorate, Egypt; 7.44% of 215 samples from different regions of southern Iraq, but not detected in Giza, Egypt; and 8.5% of 104 samples from Garissa County, Kenya [6–10].

Food intoxication cannot occur in the absence of stx. E. coli needs the eaeA gene to attach, colonize, and then release the toxin, so eaeA is a significant virulence factor for E. coli pathogenesis and identification, which is responsible for the adherence of E. coli to the gut wall [11].

E. coli antimicrobial resistance has spread around the world, and its susceptibility forms show a lot of geographic variations and differences in people and the environment [12]. Many incidents of antibiotic resistance have been determined in microbes isolated from mastitic milk, such as E. coli, which is considered a warning to human and animal health nowadays [13].

The Center for Disease Control and Prevention [14] manages a nationwide network of sequences that are used to identify possible outbreaks. Sequencing provides investigators with data about the food poisoning bacteria. If the isolated bacteria from infected patients were genetically related, this would indicate that patients were infected by the same causative agent.

Sequencing is highly significant in the formation of the global database for foodborne pathogens. This is alarming because it is used to identify unknown genomes and sources of infection in multiyear and multistate outbreaks [15].

The current investigation is designed to assess the incidence of E. coli in raw dromedary camel milk gathered from different districts in Matrouh Governorate, Egypt, and screen for the possible presence of its determined gene, virulence genes (which are confirmed by gene sequencing and phylogenetic analysis), and antibiotic-resistant pattern.

Materials and Methods

Ethical approval

This study has prior approval from the animal care and use committee institution, Alexandria Uni. (ALEXU-IACUC) member of ICLAS. No. of agreement: AU 005 2019-07-15 MS (1) 02.

Sample collection

One hundred samples of raw camel milk were randomly gathered from Matrouh Governorate from various markets and farms (50 samples from each) in Siwa, Salloum, Almtani (Dardouma area), and Sidi Barani in the Matrouh desert regions in four consecutive months. Each sample (250 ml) was collected from markets as they were sold in their retail containers and from farms in sterile falcon tubes. The samples were transported to the laboratory of microbiology in a cool box at 4°C ± 1°C within 2–4 h. Each sample of milk was perfectly mixed before being subjected to bacteriological evaluation for E. coli.

Conventional identification of E. coli

Inoculation and incubation of the selective enrichment medium (lauryl sulfate broth) were carried out as described previously [16]. In brief, 1 ml of raw camel milk was added to each lauryl sulfate broth tube (each tube contains 6 ml lauryl sulfate broth and is supplied with overturned Durham tubes). Then, it was stored at 37°C ± 1°C/24 ± 2 h. The tubes were observed for opacity, cloudiness, and any visible gas; negative tubes were incubated for up to 48 ± 2 h.

Isolation of E. coli on eosin–methylene blue (EMB) agar [17]. A loopful of positive lauryl sulfate broth tube was streaked onto a pre-dried surface of EMB agar medium; then, the petri dishes were stored at 35°C ± 0.5°C/18–24 h. Ideal colonies of E. coli on EMB medium are flat colonies with a dark center, with or without metallic green shine.

Molecular identification using conventional polymerase chain reaction (PCR)

Molecular identification was conducted in the Lab. for Veterinary Quality Control on Poultry Production, Animal Health Research Institute (AHRI), Giza, Egypt. DNA extraction was performed using the QIAamp DNA Mini Kit (catalog no. 51304) in accordance with the pamphlets. The PCR procedures of each primer pair were conducted according to their parallel reference in Table 1. The products of PCR were subjected to gel electrophoresis [18] and then transferred into a UV cabinet. The gel was pictured using a gel recording system (Alpha Innotech), and the records were examined using software.

Sequencing

Sequencing was conducted at Elim Biopharmaceuticals, USA. An extracted conventional PCR product was sequenced in the forward and reverse directions on an Applied Biosystems 3130 Automated DNA Sequencer (ABI 3130 USA) using a ready reaction Big Dye Terminator V3.1 cycle sequencing kit (Perkin-Elmer/Applied Biosystems, Foster City, CA) (Cat. No. 4336817). BLAST® analysis [22] was used to create sequence characters for GenBank accessions. Sequence results were conducted corresponding to the guides. The results of nucleotide sequencing
were submitted to GenBank via Bankit (GenBank n.d.). The sequences were accepted and received accession numbers.

**Phylogenetic analysis**

Phylogenetic analysis was conducted using MEGA X [23] by comparing the resultant sequences with those available in GenBank. The phylogenetic tree was built according to UPGMA.

**Antibiotic susceptibility testing**

Antibiotic sensitivity testing of isolates was carried out by the disk diffusion technique [24]. The isolates were exposed to sensitivity tests against norfloxacin (10 µg), cefoperazone (75 µg), pefloxacin (5 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), tobramycin (10 µg), rifampicin (5 µg), streptomycin (10 µg), neomycin (30 µg), chloramphenicol (30 µg), ofloxacin (5 µg), levofloxacin (5 µg), piperacillin (100 µg), erythromycin (15 µg), and novobiocin (30 µg), which were used in the treatment of most mastitis cases in the city. The areas of complete inhibition were calculated and explained after incubation at 35°C ± 2°C for 24 h.

Multiple antibiotic resistance (MAR) was determined for all isolates through the formula $\text{MAR} = \frac{a}{b}$, where $a$ corresponds to the sum of antibiotics to which isolates were resistant and $b$ signifies the total number of antibiotics that were used for sensitivity [25].

**Results and Discussion**

*Escherichia coli* existence in raw dromedary camel milk is a threat to human health. The results recorded in Table 2 show that *E. coli* could be detected in 24% (12/50) and 8% (4/50) of the assessed market and farm raw camel milk samples, respectively, using conventional biochemical methods. Higher results (31.5%) were reported by [26].

The isolated *E. coli* was screened for *phoA* gene using molecular identification. As shown in Table 2 and Figure 1, the prevalence rates were 4% (2/50) in markets and 6% (3/50) in farm. All positive samples did not respond to the Egyptian standard [27], which stipulated that raw milk should be free from *E. coli*.

The market raw camel milk samples were contaminated more than the farms’ own, which indicates that the hazards occurred during filling and transportation through polluted containers and poor storage temperatures [28]. Ruminants are the main reservoirs of STEC. Milk is contaminated with it through mastitis, fecal matter, or contaminated milking utensils [29].

The subsistence of *E. coli* in raw camel milk is because of fecal contamination by either direct or indirect methods such as poor sanitation during handling, far markets, and lack of refrigerators, which lead to a high bacterial load in the market samples [30].

Raw camel consumption is usually followed by diarrheagenic *E. coli* outbreaks attributable to rough handling procedures. Additionally, a high incidence of pathogenic

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### Table 1. Sequences of primers used in conventional PCR.

| Objective bacteria | Objective gene segment | Oligonucleotide sequence (5’→3’) | Band (bp) | Reference |
|--------------------|------------------------|----------------------------------|-----------|-----------|
| *E. coli*           | *phoA*                 | 5’ CGATTCTGGAAATGGCAAAAG 3’      | 720       | [19]      |
|                     |                        | 3’ CGTGATCAGGGTGACTGAC 5’        |           |           |
|                     | *stx1*                 | 5’ ACACCTGGATGATCTCAGGG 3’       | 614       | [20]      |
|                     |                        | 3’ CTGAATCCTCCCTCATTG 5’         |           |           |
|                     | *stx2*                 | 5’ CCATGCAACGGACAGCATTG 3’       | 779       |           |
|                     |                        | 3’ CCTGCAACTGACGACTTTG 5’        |           |           |
|                     | *eaeA*                 | 5’ ATGCTTAGTGGCTGTTAGG 3’        |           |           |
|                     |                        | 3’ GCCTTCATCATTCGCCCTTC 5’       | 248       | [21]      |

### Table 2. Incidence of *E. coli* isolated from assessed raw dromedary camel milk samples.

| Source         | No. of examined samples | Conventional methods | Molecular identification |
|----------------|-------------------------|----------------------|-------------------------|
|                |                         | No.     | %   | No.     | %   |
| Market milk    | 50                      | 12       | 24  | 2        | 4   |
| Farm milk      | 50                      | 4        | 8   | 3        | 6   |
*E. coli* strains in fresh milk is detected in many countries all over the world [31].

**Figure 2** shows that the phylogenetic analysis of the forward *phoA* sequence of *E. coli*, which was isolated from raw dromedary camel milk (MT478119), showed high sequence similarity to *E. coli* strain CP033158.1 in India that were isolated from mastic milk and *E. coli* strain CP047594.1 in China that were isolated from deer feces by 59%. This similarity explains the various mechanisms of antibiotic resistance transmission and the different ways in which *E. coli* infects humans and animals.

**Figure 3** shows that *E. coli* isolates have the highest resistance to rifampycin (30 µg), cefoxitin (30 µg), streptomycin (10 µg), rifampicin (5 µg), erythromycin (15 µg), piperacillin (100 µg), and novobiocin (30 µg) and high susceptibility to norfloxacin (10 µg), cefoperazone (75 µg), tobramycin (10 µg), and ofloxacin (5 µg). Analysis of the antibiotic susceptibility of the isolated *E. coli* showed that all of them were multidrug-resistant as they showed resistance to more than three classes of antibiotics.

It was found that 100% of the *E. coli* isolates tested were resistant to cefixime, levofloxacin (87.1%), piperacillin (78%), and nitrofurantoin (58%) [32]. Only 15% of the *E. coli* isolates tested were resistant to nitrofurantoin. There are many different types of antibiotics that *E. coli* can be resistant to, but the most common one is β-lactamase production. This gives the bacteria broad-spectrum resistance to cephalosporin and co-resistance to other types like aminoglycosides and tetracyclines [33].

**Table 3** shows that the MAR ranged from 0.352 to 0.764 of the tested isolates (17 antibiotic agents). A calculated MAR > 0.2 indicated that the isolate came from a high-risk source of contamination and that there was abuse of antibiotics, while a calculated MAR < 0.2 indicated that this strain was identified from an area where antibiotics were used rarely or not used at all [25].

Multi-antimicrobial resistance in *E. coli* has become a perturbing topic that is threatening global public health. Improper choice of antibiotics, overuse, and consumption
without prescription are causes of high antibiotic resistance in human and veterinary medicine worldwide, which leads to a high morbidity and mortality rate due to the low accessibility of effective antibiotics [34–36].

Conclusion

Camel milk in Matrouh Governorate is consumed raw without processing, with a lack of refrigeration facilities in the desert during milking, handling, and transport until it reaches the consumers. To mitigate the risks posed by *E. coli* contamination of milk, good manufacturing practices must be followed. Additionally, an Egyptian standard must be established for raw camel milk.

List of abbreviations

AHRI, Animal Health Research Institute; bp, base pair; BLAST, Basic Local Alignment Search Tool; EMB, Eosin–methylene blue; *eaeA*, intimin gene; h, hour; MAR, multiple antibiotic resistance; µg, milligram; ml, milliliter; *phoA*, phosphokinase; *stx1*, Shiga toxin-1; *stx2*, Shiga toxin-2; STEC, Shiga toxin *E. coli*; UPGMA, Unweighted pair group method with arithmetic mean; USA, United States of America.

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Conflict of interest

The authors declare no conflict of interest.

**MAR index = a/b**, where *a* represents the sum of resisted antibiotics and *b* represents the total number of antibiotics used for sensitivity.

### Table 3. Antibiotic sensitivity report and MAR index of *E. coli* strains from examined samples.

| Organism/origin | Multidrug resistance MOs | Resistance pattern (α) | MAR index** |
|-----------------|--------------------------|------------------------|-------------|
| **E. coli**     |                          |                        |             |
| Market milk     | 1                        | PEF, CIP, F, RF, FOX, R, S, N, C, LEV, PRL, E, NV | 0.764       |
| Market milk     | 1                        | PEF, CIP, F, RF, FOX, R, S, N, C, PRL, E, NV | 0.647       |
| Farm milk       | 1                        | RF, FOX, R, S, N, PRL, E, NV | 0.471       |
| Farm milk       | 1                        | RF, FOX, R, S, PRL, E, NV | 0.412       |
| Farm milk       | 1                        | RF, FOX, R, PRL, E, NV | 0.352       |

PEF, pefloxacin; CIP, ciprofloxacin; F, nitrofurantoin; RF, rifamycin; FOX, cefoxitin; RD, rifampicin; S, streptomycin; N, neomycin; C, chloramphenicol; LEV, levofloxacin; PRL, piperacillin; E, erythromycin; NV, novobiocin; TOB, tobramycin.

### Authors’ contributions

AA and EK conceptualized the idea. EA performed the experiment and collected the data. AA and EK analyzed the data. AA, EA, EH, RS, and HK wrote and edited the manuscript. AA, EA, EH, RS, and EA made funds available.

### References

[1] Muthukumaran MS, Mudgil P, Baba WN, Ayoub M A, Maqsood S A. A comprehensive review on health benefits, nutritional composition, and processed products of camel milk. Food Rev Int 2022;1–37; https://doi.org/10.1080/87559129.2021.2088953
[2] Amrouche T, Mounier J, Pawtowski A, Thomas F, Picot A. Microbiota associated with dromedary camel milk from Algerian Sahara. Curr Microbiol 2020; 77(1):24–31; https://doi.org/10.1007/s00284-019-01788-4
[3] WHO. *E. coli*. 2018. Available via https://www.who.int/news-room/fact-sheets/detail/E-Coli
[4] Center for Disease Control and Prevention. *E. coli (Escherichia coli)*. 2020a. Available via https://www.cdc.gov/ecoli/
[5] Baschera M, Cernela N, Stevens MJ, Liljander A, Gorman VM, et al. Shiga toxin-producing *Escherichia coli* (STEC) isolated from fecal samples of African dromedary camels. One Health 2019; 7:100087; https://doi.org/10.1016/j.onehlt.2019.100087
[6] Adugnaa M, Seifub E, Kebededd A, Dolochitt R. Quality and safety of camel milk along the value chain in Eastern Ethiopia. Int J Food Stud 2013; 2:150–7; https://doi.org/10.7455/ijfs/2.2.2013.a2
[7] Ombarak RA, Elbagory AM, Governate M. Bacteriological quality and safety of raw camel milk. Kin Egypt. Egypt J Dairy Sci 2014; 42:95–103; https://doi.org/10.21608/JCVR.2015.34836
[8] Abbas BA, Khudad BY, Anad IT. Antibiotic resistance pattern of *Escherichia coli* isolated from camel milk and detection the presence of its gene. Sci Pract J Vet 2015; 2(42):220.
[9] Bassuony NI, Abdel-Salam AF, Abdel-Ghany ZM, El-Karamany AM, Atwa MA, Hassanein AM. Effect of camel milk on microbiological and chemical quality of soft cheese. J Food Dairy Sci 2014; 5(2):63–77.
[10] Noor M, Rotich V, Kiarie JW, Cheruiyot K, Kagira JM. Prevalence, risk factors associated with brucellosis and presence of pathogenic bacteria isolated from camel milk in Garissa County, Kenya. South Asian J Res Microbiol 2020;42–52; https://doi.org/10.9734/sajrm/2020/v6i430158
[11] Yang X, Sun H, Fan R, Fu S, Zhang J, Matussek A, et al. Genetic diversity of the intimin gene (eaeA) in non-O157 shiga toxin-producing

http://bdvets.org/javar/
Escherichia coli strains in China. Sci Rep 2020; 10(1):1–9; https://doi.org/10.1038/s41598-020-60225

[12] Rehman K, Niaz S, Tahir A, Akash M. Microorganisms and antibiotic production. In: Antibiotics and antimicrobial resistance genes in the environment, Elsevier, pp.1–6, 2020; https://doi.org/10.3389/fmicb.2018.02066

[13] Shah P, Shirivastava S, Gogpi P, Saxena S, Srivastava S, Singh RJ, et al. Wasp venom peptide (Polypia MP-1) shows antimicrobial activity against multi drug resistant bacteria isolated from mastitic cow milk. Int J Pept Res Therap 2022; 28(1):14; https://doi.org/10.1007/s10989-021-10355-0

[14] Center for Disease Control and Prevention. Outbreak of E. coli infections in 12 states. 2020b. Available via https://www.cdc.gov/ecoli/2020/o157h7-10-20b/index.html (Accessed 22 November 2020).

[15] Saeed E, Amer AA, Keshta HG, Khalifa E. Prevalence and antibiogram of Salmonella enterica isolated from raw dromedary camel milk in Matrouh Governorate, Egypt. Int J Vet Sci 2021; https://doi.org/10.47278/journal.jivs.2021.0108

[16] International Organization for Standardization. Microbiology of food and animal feeding stuffs—horizontal method for the detection and enumeration of presumptive Escherichia coli—most probable number technique 7251:2005.

[17] AOAC. Official methods of analysis. AOAC, Washington, DC, vol. 75, no. 2, pp. 257–60, 1992; https://doi.org/10.1093/jaoac/75.2.36A

[18] Sambrook J, Fritscgh EF, Mentiates. Molecular coloning. A laboratory manual. Cold Spring Harbor Laboratory press, New York, NY, 1989.

[19] Hu Q, Tu J, Han X, Zhu Y, Ding C, Yu S. Development of multiplex PCR assay for rapid detection of Riemerellaanatitpestifer, Escherichia coli, and Salmonella enterica simultaneously from ducks. J Microbiol Meth 2011; 86:64–9; https://doi.org/10.1016/j.jmim.2011.07.007

[20] Dipinetto L, Santaniello A, Fontanella M, Lagos K, Fioretti A, Menna LF. Presence of shiga toxin-producing Escherichia coli 0157:H7 in living layer hens. Lett Appl Microbiol 2006; 43:293–5; https://doi.org/10.1111/j.1472-765X.200601954.x

[21] Bisi-Johnson MA, Obi CL, Vasaikar SD, Baba KA, Hattori T. Molecular basis of virulence in clinical isolates of Escherichia coli and Salmonella species from a tertiary hospital in the Eastern Cape, South Africa. Gut Pathog 2011; 3:9; https://doi.org/10.1186/1757-4749-3-9

[22] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990; 215(3):403–10; https://doi.org/10.1016/S0022-2836(05)80360-2

[23] Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X. molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 2018; 35(6):1547–9; https://doi.org/10.1093/molbev/msy096

[24] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 28th edition, Cold Spring Harbor Laboratory Press, New York, NY, 2018.

[25] Krupmerman PH. Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. Appl Environ Microbiol 1983; 46(1):165–70; https://doi.org/10.1128/aem.46.1.165-170.1983

[26] Atera T, Legesse Y, Mummed B, Ugra B. Bacteriologial quality of raw camel milk along the market value chain in Fafen zone, Ethiopian Somali Regional State. BMC Res Notes 2016; 9(1):285; https://doi.org/10.1186/s13104-016-2088-1

[27] Egyptian Standard, ES. Milk and milk products part1: raw milk. Egyptian Organization for Standardization, 154-1/2005.

[28] Owusu-Kwarteng J, Akahanda F, Aygel D, Jepsersen L. Microbial safety of milk production and fermented dairy products in Africa. Microorganisms 2020; 8(5):752; https://doi.org/10.3390/microorganisms8050752

[29] Ayoub ES, Amer AA, Keshta HG, Sedeeek EK. Microbial assessment of raw dromedary camel milk in Matrouh Governorate, Egypt. Alexandria J Vet Sci 2020; 66(2); https://dx.doi.org/10.5455/ajves.94753

[30] Disassa N, Sibhat B, Mengistu S, Muktar Y, Belina D. Prevalence and antimicrobial susceptibility pattern of E. coli 0157: H7 isolated from traditionally marketed raw cow milk in and around Asosa Town, Western Ethiopia. Vet Med Int 2017; https://doi.org/10.1155/2017/758151

[31] Jones G, LeFevre S, Donguy MP, Nisavanah A, Terpant G, Fougere E, et al. Outbreak of Shiga-producing Escherichia coli (STEC) O26 paediatic haemolytic uraemic syndrome (HUS) cases associated with the consumption of soft raw cow’s milk cheeses, France, March to May 2019. Euro Surveillence 2019; 24(22); https://doi.org/10.2807/1560-7917.ES.2019.24.22.1900305

[32] Ejez H, Imran M, Zafar A, Javed H, Al Farraj DA, Younas S, et al. Phenotypic characterisation of carbapenem-producing Escherichia coli isolated from a tertiary care paediatric hospital. Int Medical J 2020; 27(2):1.

[33] Hammad AM, Eltahan A, Hassan HA, Hussien H, Shimamoto T. Loads of coliforms and fecal coliforms and characterization of the mototolerant Escherichia coli in fresh raw milk cheese. Foods 2022; 11:332; https://doi.org/10.3390/foods1103032

[34] Farhan ZA, AL-Iedani AA. Molecular detection of Shiga toxin (stx1 and stx2) and intimin (eaeA) genes in Escherichia Colisolated from fecal samples of cattle, sheep, and human in Basrah Governorate. Basrah J Vet Res 2019; 18(2):288–305.

[35] Irenge LM, Ambroise J, Bearzatto B, Durant JF, Chirimwami RB, Gala RL. Whole- genome sequences of multidrug-resistant Escherichia coli in South-Kivu Province, Democratic Republic of Congo: characterization of phylogenomic changes, virulence, and resistance genes. BMC Infect Dis 2019; 19(1):137; https://doi.org/10.1186/s12879-019-3763-3

[36] Yuan J, Wang X, Shi D, Ge Q, Song X, Hu W, et al. Extensive antimicrobial resistance and plasmid-carrying resistance genes in mcr-1-positive E. coli sampled in swine, in Guangxi, South China. BMC Vet Res 2021; 17(1):1–10; https://doi.org/10.1186/s12917-021-02750-4