agriculture

Communication

The Prevalence of *Escherichia coli* Derived from Bovine Clinical Mastitis and Distribution of Resistance to Antimicrobials in Part of Jiangsu Province, China

Tianle Xu [1,2,*], Wendi Cao [3], Yicai Huang [3], Jingwen Zhao [3], Xinyue Wu [3] and Zhangping Yang [1,3]

[1] Joint International Research Laboratory of Agriculture and Agri-Product Safety, Ministry of Education of China, Yangzhou University, Yangzhou 225009, China
[2] International Joint Research Laboratory in Universities of Jiangsu Province of China for Domestic Animal Germplasm Resources and Genetic Improvement, Yangzhou University, Yangzhou 225009, China
[3] College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, China
* Correspondence: tl-xu@yzu.edu.cn

Abstract: Bovine mastitis is often taken as one of the most common diseases in dairy farms, which its pathophysiology leads to a reduction of milk production and its quality. The penetration of pathogenic bacteria into the mammary gland, through either a contagious or environmental approach, has been determined the way of infection. The mastitis derived bacteria have become a challenge in practice, since the increasing exposure of antimicrobial. In order to identify characteristics of the epidemiological regulation and drug resistance of the pathogenic bacteria of bovine mastitis in northern Jiangsu, 156 clinical mastitis milk samples were collected from 3 large-scale farms for the epidemiological investigation and analysis of the drug resistance of *E. coli*. The bacteria were positively isolated in a total of 143 milk samples. The results showed that 78 strains of *E. coli* were detected, with a prevalence rate of 26.99%, followed by 67 strains of *K. pneumoniae*, with a prevalence of 23.19%, and 38 strains of *Staphylococcus*, with a prevalence of spp. 13.15%. In addition, 78 strains of *E. coli* isolated from bovine mastitis were tested for susceptibility to 8 kinds of antibiotics. It was shown that gentamicin and tetracycline were the most effective against *E. coli*, with the susceptibility rate of 83.3%, followed by streptomycin and ciprofloxacin, with 73.1% and 71.8% respectively. However, β-lactams including penicillin, cefothiophene, and amoxicillin showed serious resistance to *E. coli* isolates. There were 12 drug resistance genes detected by PCR, including β-lactam (blaTEM, blaCTX-M, and blaSHV), aminoglycoside (armA and armB), tetracycline (tetA, tetB, and tetC), and quinolone (qnrS, qepA, oqxA, and oqxB) related genes. Notably, all *E. coli* isolates carried blaTEM gene (100%). The detection rate of blaCTX-M was 53.8%, followed by the detection of blaSHV (20.5%), armA (9.0%), tetA (26.9%), tetB (2.6%), tetC (20.5%), qnrS (29.5%), oqxA (37.2%) and oqxB (1.3%). The present study provides crucial information on the distribution of bovine mastitis derived bacterial pathogens in Jiangsu province, as well as highlighting the antimicrobial resistance which might help to improve the efficiency of antibiotics treatment on bovine mastitis.

Keywords: *Escherichia coli*; drug resistance; bovine mastitis; β-lactams

Citation: Xu, T.; Cao, W.; Huang, Y.; Zhao, J.; Wu, X.; Yang, Z. The Prevalence of *Escherichia coli* Derived from Bovine Clinical Mastitis and Distribution of Resistance to Antimicrobials in Part of Jiangsu Province, China. *Agriculture* **2023**, *13*, 90. https://doi.org/10.3390/agriculture13010090

Academic Editor: Jiaqi Wang

Received: 19 December 2022
Revised: 25 December 2022
Accepted: 27 December 2022
Published: 29 December 2022

1. Introduction

Bovine mastitis is one of the diseases that cause great economic losses in the dairy industry [1,2]. It can lead to a decreased milk production, lowered milk quality, reproductive barriers, and a higher elimination rate [3]. The occurrence and development of mastitis over the past years appears to have changed, due to differences in the scales and management of the dairy farming industry, in China [4]. The pathogenic microorganisms that induce mastitis in dairy cows include bacteria, fungi, viruses, and mycoplasma, among which bacteria are the most important pathogens [4]. Mastitis derived pathogens can be divided into contagious pathogens and environmental pathogens [5]. The contagious pathogens

include coagulase-negative *Staphylococci*, *Streptococcus agalactiae*, and *Streptococcus dysgalactiae*, which can be further spread among dairy cows through milking equipment and other means [6]. The environmental pathogens include *Escherichia coli* (*E. coli*), *Streptococcus uberis*, *Klebsiella pneumoniae*, and coagulase-negative *Staphylococcus*, which mainly exist in habitats such as soil, feces, and beds, and are transmitted mainly through udder contact [7].

Studies have shown that *E. coli* infection is usually characterized with persistence and a higher possibility of causing recurrent infections [5]. Moreover, the cases of cow mastitis caused by *E. coli* have shown an increasing trend in recent years [8]. As *E. coli* is an opportunistic pathogen widely present in the environment, it can cause human and animal infections with zoonotic diseases [9]. Currently, antibiotics are usually used to treat *E. coli* infected mastitis with better efficiency in animal husbandry and veterinary clinics. However, studies have shown that *E. coli* capable of developing broad-spectrum resistance to commonly used antibiotics, along with rapid variation [10]. For a long time, under selective pressure, the antibiotic resistance of *E. coli* continues to rise, which brings great challenges to the prevention and treatment of clinical upper colibacillosis [11]. Therefore, it is of great significance for the prevention and control of colibacillosis and public health to carry out the detection of pathogenic bacteria of bovine mastitis and the systematic detection of antibiotic resistance of *E. coli* from mastitis, as well as the resistance genes carried [12].

Although studies have recently focused on tackling antimicrobial resistance by designing alternative treatment regimens to antimicrobial, the discussion of issues and the emergence of resistance are still encouraged [13]. Among the factors involved in antimicrobial resistance, the bacterial status, including susceptibility and resistance, tolerance, persistence, and biofilm formation, are the main drivers of resistance outcome [14,15]. *E. coli* has many accessory genomes of plasmids and chromosome gene loci. In Beijing, China, 87.1% of the 70 *E. coli* isolates obtained from bovine mastitis cases were resistant to streptomycin, kanamycin, and ampicillin [16]. With the phenotypes of antibiotic resistance, the *E. coli* isolates are known as efficient reservoirs for resistant genes and are capable of transfering the genes among pathogenic bacteria [17]. The common harbored resistance genes for *E. coli* in America, mentioned in previous reports, are blaCTX-M, bla-TEM, ampC, tetA, tetB, sulI, and sulII [18,19]. Moreover, 5.3% of the isolates from bovine mastitis cases in Ningxia, China, were found to be carrying rmtB, bla-TEM1, and bla-CTX-M-15 which are related to β-lactams resistance [20]. More recently, we have characterized the phenotypic resistance of *Klebsiella pneumonia* isolated from clinical bovine mastitis in some parts of Jiangsu province [21]. As a result, isolates were found resistant to β-lactams including penicillin and amoxicillin, with the rates of 85.3% and 67.6%, respectively [21]. However, the phenotypic resistance of *E. coli* in northern Jiangsu province is rarely investigated. The objective of the current study is to underscore the prevalence of *E. coli* derived from bovine clinical mastitis, as well as investigate the phenotypes of the multi-drug resistant *E. coli* isolates, in Jiangsu province, China.

2. Materials and Methods

2.1. Isolation and Identification

A total of 156 samples were collected from the milk of dairy cows with clinical mastitis during July to October, in 2021. All strains isolated from the selected farms were in three regions (Xinyi, Sihong, and Huai'an) of Jiangsu Province in China. The milk samples were obtained from cows with clinical mastitis, diagnosed by using visible symptoms and testing with the California Mastitis Test (CMT), as officially recommended [22,23]. A volume of 100 µL of collected milk samples was plated onto a cultivation with blood agar (made with 5% freshly prepared whole blood, collected from sheep, and aerobically incubated at 37 °C for 24 h). By checking the morphology of the colonies, a single colony with identical morphology from each milk sample was then re-cultured by streaking on MacConkey (MC) agar, for purification. The sub-cultured bacteria were picked and amplified in Luria-Bertani (LB) broth at 37 °C for 24 h. The suspected isolates were further

confirmed by PCR verification (Vazyme, Nanjing, China) with extracted genomic DNA from bacteria (Tiangen, Beijing, China), following 16S rDNA pyrosequencing (Tsingke., Ltd., Beijing, China), and sequences were proofread with SnapGene software (GSL Biotech, Chicago, IL, USA) [24]. Finally, the obtained sequences from the PCR products were analyzed using a BLAST sequence search, used for species identification, on the NCBI database. The confirmed isolates were stored in tubes containing 15% glycerol at −80 °C. The antimicrobial susceptibility of *E. coli* isolates was then determined using the Kirby–Bauer test and resistance gene detection.

2.2. Determination of Antimicrobial Susceptibility

The antimicrobial susceptibility was tested on a plate with Mueller–Hinton agar (MHA) by Kirby–Bauer (K-B) disk diffusion [25]. The 8 disks (Hangzhou Microbial Reagent Co., Hangzhou, China) used in the experiment were incubated with the following antibiotics: ciprofloxacin (CIP, 5 μg), streptomycin (STR, 10 μg), gentamycin (CN, 10 μg), tetracycline (TCY, 30 μg), cephalothin (KF, 30 μg), penicillin (PEN, 10 μg), amoxicillin (AML, 20 μg), and lincomycin (MY, 2 μg). The *E. coli* ATCC 25,922 strain was taken as the reference. The results of zone diameter for each disk were recorded as susceptible, intermedium, or resistant, on the basis of the interpretative standards of the Clinical and Laboratory Standards Institute [26]. When the recommended values for the antimicrobial disks were not available, following the manufacturer's instructions was advised.

2.3. Resistance Gene Detection

The detection of 12 resistance genes positively carried in *E. coli* was determined using PCR assays on 78 isolated *E. coli* isolates used in this study, including blaTEM, blaCTX-M, blaSHV, armA, armB, tetA, tetB, tetC, qnrS, qepA, oqxA, and oqxB [27,28]. Primers used in the current study has been listed in Table 1. The PCR was performed as follows: denaturing at 94 °C for 5 min; then 35 cycles, at 94 °C for 30 s, corresponding to extension and annealing, for 30 s and 72 °C for 1 min, along with an additional elongation at 72 °C for 10 min. The reference strains of ATCC 25,922 were taken for PCRs as well. PCR amplicons were then verified by running gels with 1% agar and performing DNA sequencing (Tsingke, Beijing, China). Visualization of bands on the gel images was used to identify the presence of each targeted gene.

Table 1. Primers designed for the PCR assay.

Genes	Primers (5′-3′)	Annealing (°C)	Products (bp)
blaTEM	F: TCGCCGCATACACTATTCTCAGAATGA R: ACGCTCACCGGCTCCAGATTTAT	50	445
blaCTX-M	F: ATGTGCAGACCAGTAAGTATGGC R: TGGGTAATAGTACCAGAACAG	57	593
blaSHV	F: ATGCGTTATATTCGCCTGTG R: TGCTTTGTTATTCGGGCCAA	57	747
armA	F: GGGTCTTACTATTCTGCCTAT R: ATTCCCTTCTCCTTTCCAG	50	503
armB	F: TTTCTGCGGGCGATGTAA R: AGTTCTGTTCCGATGGTCTTT	57	523
tetA	F: GCTACATCCTGCTTGCCTTC R: CATAGATCGCCGTGAAGAGG	60	210
tetB	F: TTGGTTAGGGGCAAGTTTTG R: GTAATGGGCCAATAACACCG	60	659
tetC	F: CTTGAGACCTTCAACCCAG R: ATGGTCGTCATCTACCTGCC	60	418
qnrS	F: ACATAAAGACTTAAGTGATC R: CAATTAGTCAGGATAAAC	52	619
qepA	F: CCAGCTCGGCAACTTGATAC R: ATGCTCGCCTTCCAGAAAA	60	570
oqxA	F: CTCGGCGCGATGATGCT R: CCACTCTTCACGGGAGACGA	57	392

Table 1. *Cont.*

Genes	Primers (5′-3′)	Annealing (°C)	Products (bp)
oqxB	F: TTCTCCCCCGGCGGGAAGTAC R: CTCGGCCATTTTGGCGCGTA	64	512
16SrDNA	TTCGGACCTCACGCTATCA GAAGGCACCAA TCCATCTC	62	824

3. Results

3.1. Isolation of Bacterial from Bovine Clinical Mastitis

Among the 156 milk samples, 12 species of bacteria were identified. However, 13 milk samples showed no bacterial detection, due to the non-visible growth of colonies on the cultured plates. The detectable rate of bacterial pathogens from clinical mastitis was 91.67% (Table 2). The infected quarters of bovine mammary glands are usually contaminated with various pathogens and the incidence is becoming more complicated to manage. As shown in Table 3, out of 143 samples that were culture-positive, the infected patterns from those samples showed 1 species of bacteria in 44 samples (30.77%), 2 species of bacteria in 52 samples (36.36%), and 3 species of bacteria in 47 samples (32.87%). Of the 289 strains of bacteria isolated, 8 species with 220 strains were closely related to mastitis, with a total detection rate of 76.12%. Among 289 isolated strains, *E. coli* was the most frequently isolated (78 strains, 26.99%), followed by 67 strains of *Klebsiella* spp. (23.19%), 38 strains of *Staphylococcus* spp. (13.15%), 8 strains of *Streptococcus agalactis* (2.77%), 4 strains of *Streptococcus uberis* (1.38%), 3 strains of *Streptococcus dysgalactiae* (1.04%), and 2 strains of *Pseudomonas aeruginosa* (0.69%) (Figure 1).

Table 2. Isolation of 156 clinical mastitis milk samples.

Items	Positive Isolates	Negative Isolates	Total Isolates
Samples (No.)	143	13	156
Ratio (%)	91.67	8.33	100

Table 3. Pathogen infection pattern of 143 positive isolation.

Items	Single Isolates	Double Isolates	Triple Isolates	Total Isolates
Samples (No.)	44	52	47	143
Ratio (%)	30.77	36.36	32.87	100

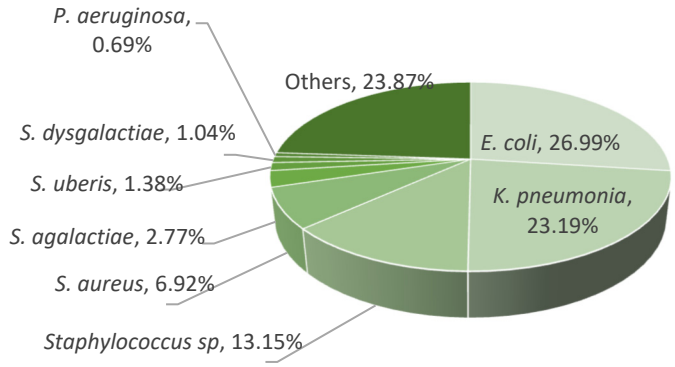

Figure 1. Analysis of 289 strains of pathogenic bacteria of bovine mastitis. All isolates were obtained and identified through bacterial isolation, purification, and 16S rDNA identification.

3.2. Phenotypic Resistance of E. coli Isolated from Bovine Clinical Mastitis

As shown in Figure 2, *E. coli* strains isolated from cow mastitis in northern Jiangsu have developed resistance to the eight antibiotics used, to varying degrees, with the resistance rate ranging from 11.6–66.7%. The resistance of *E. coli* isolates to lincomycin was the

most serious, with the resistance rate of 66.7%, followed by the resistance to β-lactams including penicillin, cefothiophene, and amoxicillin, with rates of 60.2%, 57.7% and 55.1%, respectively. In contrast, *E. coli* isolates were more sensitive to gentamicin, tetracycline, streptomycin, and ciprofloxacin than others tested, and the resistance rates were 16.7%, 11.6%, 26.9% and 26.9%, respectively.

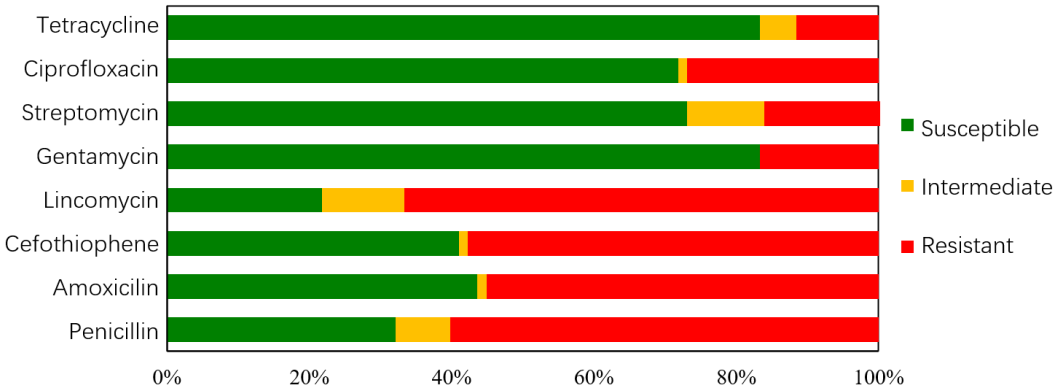

Figure 2. Phenotypic drug-resistance proportions of *E. coli*. All the 78 isolates of *E. coli* were analyzed with 8 antimicrobial agents. The bars in green, yellow, and red indicate the antimicrobial's susceptibility with susceptible, intermediate, and resistant, respectively.

E. coli isolates were found to be multi-drug resistant in farms of northern Jiangsu province. As shown in Figure 3, 22 strains were the most resistant to 5 antibiotics, accounting for 28.2%. Similarly, 16 strains resistant to an amount of 6 antibiotics, followed by 11 and 7 strains resistant to 1 and 2 antibiotics, respectively. However, only 2 strains appeared to be resistant to 4 or 7 antibiotics. Notably, major resistance to β-lactam drugs indicate that the isolated *E. coli* strains from bovine mastitis in northern Jiangsu are dominated by lactam drugs and are becoming relatively serious.

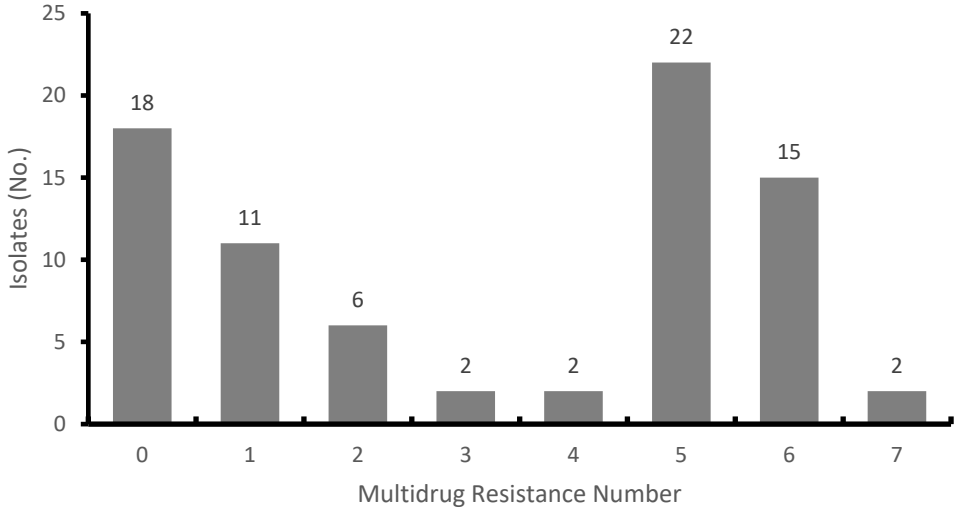

Figure 3. Multidrug Resistance of 78 *E. coli* strains to 8 Antibiotics. All 78 *E. coli* isolates were collected and analyzed for multi-drug resistance with tetracycline, ciprofloxacin, streptomycin, gentamycin, lincomycin, cefothiophene, amoxicilin, and penicillin.

3.3. Genotypic Analysis of Antimicrobial-Resistance in Isolated E. coli Strains

According to the resistance phenotype of isolated *E. coli* to various antibiotics in this study, and the clinical use of antibiotics in the farms, 12 drug resistance genes related to four types of antibiotics were detected. The results are shown in Figure 4. For β-Lactams: 78 strains of *E. coli* have the detected blaTEM gene (100%). Moreover, nearly half of the

E. coli carried the blaCTX-M gene, and had a detection rate of 53.8%. A total of 16 strains of *E. coli* had the detectable blaSHV gene, accounting for 20.5% of the total. For aminoglycoside: only 7 strains had the detectable armA gene: however, no armB gene was detected. For tetracyclines: The detection rates of tetA and tetC genes were 26.9% and 20.5% of the total isolates, respectively, and only two strains were detected with tetB gene. For quinolone: The detection rates of oqxA and qnrS genes were 37.2% and 29.5%, respectively. Only one *E. coli* strain was detected with the oqxB gene, but no strain carried the qepA gene. The detection rate of each drug resistance gene was not significantly different from the corresponding antibiotic resistance rate.

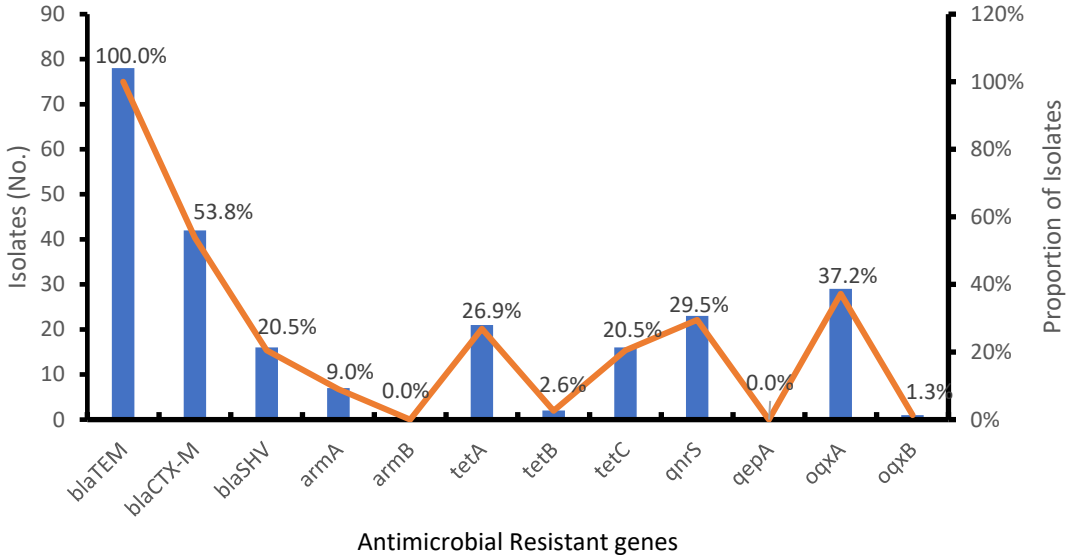

Figure 4. Detection of drug-resistant genes in *E. coli*. PCR was performed to identify the carriage of β-lactam (blaTEM, blaCTX-M, and blaSHV), aminoglycoside (armA and armB), tetracycline (tetA, tetB, and tetC), and quinolone (qnrS, qepA, oqxA, and oqxB) related genes in 78 *E. coli* isolates.

4. Discussion

In this study, 156 clinical mastitis milk samples collected from three dairy farms in Xinyi, Suqian, and Xuyi in northern Jiangsu were isolated and identified. The results showed that the pathogenic bacteria were mainly dominated by *E. coli*, *Klebsiella* spp., *Staphylococcus* spp., *Staphylococcus aureus*, *Streptococcus agalactis*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, and *Pseudomonas aeruginosa*, and the detection rate of gram-negative bacteria was high. However, it has been reported in an earlier publication that the isolated bacteria from farms in Ningxia, China displayed different distributions of pathogens as compared to the current study [29]. The reason for the difference may be attributed to the sampling time and region, since the patterns of the infection for bovine mastitis are region specific. The sampling time in this study was from July to October, which was hot and humid. Cows are prone to heat stress, which will cause the decline of cow resistance, accelerate the growth and reproduction of bacteria, and impact mammary tissue. Therefore, this period is associated with a high incidence of clinical mastitis in cows. During the sampling and investigation, it was also found that although these farms are large-scale between each other, the environmental sanitation, milking methods, and other feeding management aspects of these farms in northern Jiangsu still need to be improved. This is indicative that external factors such as farm management have an important impact on the induction of cow mastitis and its infection types. In addition, of 143 milk samples shown to be bacteria positive, 99 had mixed infection, accounting for 69.23% of the total samples. The variety of bacterial pathogens isolated further indicates that the environmental sanitation and feeding management of these dairy farms need to be improved. Attention should be paid to the timely cleaning of urine and feces during the

rainy season, and the regular disinfection of the environment, to prohibit the growth of environmentally derived pathogens such as *E. coli*.

E. coli is one of the main pathogens that cause bovine mastitis. However, due to the increased incidence of antibiotic resistant bacterial infections, the effect of antibiotic treatment is becoming limited. By comparing and analyzing the drug susceptibility of 78 *E. coli* strains from bovine mastitis, we found that at present, coliform bacteria from bovine mastitis in northern Jiangsu Province is highly resistant to β- Lactam antibiotics. However, the susceptibility of the coliforms to tetracycline and gentamicin are still maintained. By referring to relevant data, we found that *E. coli* from bovine mastitis in some regions of China, maintained high resistance to β-Lactam antibiotics, which is consistent to the current research. As reported in a previous study, the main pathogenic bacteria isolated from three farms in Jiangsu Province was tested for drug susceptibility, where almost all isolates of *E. coli* were shown to be resistant to penicillin, ampicillin, and lincomycin, but sensitive to gentamycin, ciprofloxacin, and streptomycin [30]. It has been reported that *E. coli* from dairy cows in Henan Province showed the highest drug resistance rate of β-lactam antibiotics, followed by the resistance of ampicillin sodium, cefotaxime, and ceftiofur, which exceeded 60%. In contrast, a more comprehensive study revealed that 75 (22.9%) out of 328 *E. coli* isolates were confirmed as quinolone-resistant, in a nationwide sampling investigation [8]. However, *E. coli* from bovine mastitis in Tianjin and Ningxia areas has low sensitivity to streptomycin, which is inconsistent with the current study [31]. During the sampling, we noticed that lincomycin, penicillin, and cephalosporin are routine drugs used in farms, which also explains why *E. coli* from bovine mastitis in northern Jiangsu province is highly resistant to these antibiotics. In addition, 26.9% of the 78 strains of *E. coli* isolated in the present study were resistant to quinolone (ciprofloxacin), which was much higher than the data collected from developed countries. The prevalence of quinolone resistant *E. coli* in bovine mastitis cases reported by Finland, Canada, and the United States were 0.7% and 0.0%, respectively [32,33]. It is speculated that the widespread addition of quinolones in the animal feeding diet is related to the high drug resistance rate of quinolones in China [34].

The resistance genes can be horizontally transferred by inserting sequence, plasmids, transposons, integrons, bacteriophages, and other movable genetic elements [35]. Extended-Spectrum β-Lactamases (ESBLs) producing enzymes are important factors for the resistance of *E. coli* to cephalosporins, of which blaCTX-M has the highest prevalent gene in ESBL pathogens. It has been evidenced that more than half of the 70 *E. coli* strains were found to be blaCTX-M positive in four regions of China's farms, which was consistent with the results of this study [10]. The efflux pump is determined as the main drug resistance mechanism of tetracyclines [36]. The predominant genes tetA and tetB are in charge of the resistance of *E. coli* to tetracyclines, in which the current study detected tetA (26.9%) and tetB (2.6%) in tetracycline-resistant strains, which further proved the conclusion. Quinolones are effective antibacterial agents for the treatment of various infections caused by *E. coli*. Unfortunately, due to the widespread use of quinolone drugs, the number of quinolone resistant *E. coli*, derived from animals produced for food in China has increased [36–38]. Multiple mutations that cause amino acid changes in the targeted enzyme related to the quinolone resistance determining region (QRDR) is the most common mechanism of high-level resistance of clinical strains to quinolones [38]. In addition, the common resistance of β-lactam and quinolone drugs is a common phenomenon, due to the presence of β-lactamases and the substitution mutations in QRDR, regardless of whether the plasmid mediated quinolone resistance (PMQR) gene is present [39]. In this study, 21 ciprofloxacin resistant strains were all resistant to β-lactam, including penicillin, amoxicillin, and cefothiophene, which further confirmed this theory.

Collectively, the dominant pathogens causing bovine mastitis in 3 dairy farms in northern Jiangsu are *E. coli* and *K. pneumoniae*, which provides an experimental basis for the prevention and treatment of bovine mastitis and clinical medication. Among them, the resistance of isolated *E. coli* to β-lactams was the most serious in the selected farms.

The detection rate of resistance genes blaTEM (100%) and blaCTX-M (53.8%) was high, which was consistent with the β-lactam drug resistance phenotype. Our study highlights the distribution and characteristics of pathogenic *E. coli* derived from bovine mastitis, and provides reference of antimicrobial selection for the treatment.

Author Contributions: Z.Y. and T.X. performed study concept and design; W.C., Y.H. and T.X. performed experiment and writing, review of the paper; T.X., J.Z. and X.W. provided acquisition and interpretation of data; Z.Y. and W.C. provided material. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the National Natural Science Foundation of China (32102731). the Open Project Program of Joint International Research Laboratory of Agriculture and Agri-Product Safety, the Ministry of Education of China, Yangzhou University (JILAR-KF202204). The Open Project Program of International Joint Research Laboratory in Universities of Jiangsu Province Of China for Domestic Animal Germplasm Resources and Genetic Improvement: (IJRLD-KF202214).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: We appreciate the support of the milk sample provisions from the dairy farms located in the 3 correlated farms.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bar, D.; Tauer, L.W.; Bennett, G.; González, R.; Hertl, J.A.; Schukken, Y.H.; Schulte, H.F.; Welcome, F.L.; Gröhn, Y.T. The cost of generic clinical mastitis in dairy cows as estimated by using dynamic programming. *J. Dairy Sci.* **2008**, *91*, 2205–2214. [CrossRef] [PubMed]
2. Hertl, J.A.; Groehn, Y.T.; Leach, J.; Bar, D.; Bennett, G.J.; Gonzalez, R.N.; Rauch, B.J.; Welcome, F.L.; Tauer, L.W.; Schukken, Y.H. Effects of clinical mastitis caused by gram-positive and gram-negative bacteria and other organisms on the probability of conception in New York State Holstein dairy cows. *J. Dairy Sci.* **2010**, *93*, 1551–1560. [CrossRef] [PubMed]
3. Gao, J.; Barkema, H.W.; Zhang, L.; Liu, G.; Deng, Z.; Cai, L.; Shan, R.; Zhang, S.; Zou, J.; Kastelic, J.P. Incidence of clinical mastitis and distribution of pathogens on large Chinese dairy farms. *J. Dairy Sci.* **2017**, *100*, 4797–4806. [CrossRef] [PubMed]
4. He, W.; Ma, S.; Lei, L.; He, J.; Li, X.; Tao, J.; Wang, X.; Song, S.; Wang, Y.; Wu, C.; et al. Prevalence, etiology, and economic impact of clinical mastitis on large dairy farms in China—ScienceDirect. *Vet. Microbiol.* **2020**, *242*, 108570. [CrossRef]
5. Zhao, X.; Lacasse, P. Mammary tissue damage during mastitis: Causes and controls. *J. Dairy Sci.* **2005**, *88*, 2.
6. Esener, N.; Green, M.J.; Emes, R.D.; Jowett, B.; Davies, P.L.; Bradley, A.J.; Dottorini, T. Discrimination of contagious and environmental strains of Streptococcus uberis in dairy herds by means of mass spectrometry and machine-learning. *Sci. Rep.* **2018**, *8*, 17517. [CrossRef]
7. White, D.G.; Zhao, S.; Simjee, S.; Wagner, D.D.; McDermott, P.F. Antimicrobial resistance of foodborne pathogens. *Microbes Infect.* **2002**, *4*, 405–412. [CrossRef]
8. Yang, F.; Zhang, S.; Shang, X.; Wang, L.; Li, H.; Wang, X. Characteristics of quinolone-resistant *Escherichia coli* isolated from bovine mastitis in China. *J. Dairy Sci.* **2018**, *101*, 6244–6252. [CrossRef]
9. Blanco, M.; Blanco, J.E.; Mora, A.; Dahbi, G.; Bernárdez, M. Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from cattle in Spain and identification of a new intimin variant gene (eae-xi). *J. Clin. Microbiol.* **2004**, *42*, 645. [CrossRef]
10. Yu, Z.N.; Wang, J.; Ho, H.; Wang, Y.T.; Huang, S.N.; Han, R.W. Prevalence and antimicrobial-resistance phenotypes and genotypes of *Escherichia coli* isolated from raw milk samples from mastitis cases in four regions of China. *J. Glob. Antimicrob. Resist.* **2020**, *22*, 94–101. [CrossRef]
11. Bradley, A.J.; Green, M.J. Adaptation of *Escherichia coli* to the Bovine Mammary Gland. *J. Clin. Microbiol.* **2001**, *39*, 1845–1849. [CrossRef] [PubMed]
12. Li, J.; Xie, S.; Ahmed, S.; Wang, F.; Gu, Y.; Zhang, C.; Chai, X.; Wu, Y.; Cai, J.; Cheng, G. Antimicrobial Activity and Resistance: Influencing Factors. *Front. Pharmacol.* **2017**, *8*, 364. [CrossRef] [PubMed]
13. Xu, T.; Zhu, H.; Liu, R.; Wu, X.; Chang, G.; Yang, Y.; Yang, Z. The protective role of caffeic acid on bovine mammary epithelial cells and the inhibition of growth and biofilm formation of Gram-negative bacteria isolated from clinical mastitis milk. *Front. Immunol.* **2022**, *13*, 1005430. [CrossRef] [PubMed]

14. Tyerman, J.G.; Ponciano, J.M.; Joyce, P.; Forney, L.J.; Harmon, L.J. The evolution of antibiotic susceptibility and resistance during the formation of Escherichia colibiofilms in the absence of antibiotics. *BMC Evol. Biol.* **2013**, *13*, 22. [CrossRef] [PubMed]
15. Gędas, A.; Olszewska, M.A. Chapter 1—Biofilm formation and resistance. In *Recent Trends in Biofilm Science and Technology*; Simoes, M., Borges, A., Chaves Simoes, L., Eds.; Academic Press: Cambridge, MA, USA, 2020; pp. 1–21.
16. Liu, Y.; Liu, G.; Liu, W.; Liu, Y.; Ali, T.; Chen, W.; Yin, J.; Han, B. Phylogenetic group, virulence factors and antimicrobial resistance of *Escherichia coli* associated with bovine mastitis. *Res. Microbiol.* **2014**, *165*, 273–277. [CrossRef]
17. Hayer, S.S.; Lim, S.; Hong, S.L.; Elnekave, E.; Alvarez, J. Genetic determinants of extended spectrum cephalosporin and fluoroquinolone resistance in *Escherichia coli* isolated from diseased pigs in the U.S.A. *mSphere* **2020**, *5*, 920–990. [CrossRef]
18. Srinivasan, V.; Gillespie, B.E.; Lewis, M.J.; Nguyen, L.T.; Headrick, S.I.; Schukken, Y.H.; Oliver, S.P. Phenotypic and genotypic antimicrobial resistance patterns of *Escherichia coli* isolated from dairy cows with mastitis. *Vet. Microbiol.* **2007**, *124*, 319–328. [CrossRef]
19. López Meza, J.E.; Higuera Ramos, J.E.; Ochoa Zarzosa, A.; Chassin Noria, O.; Valdez Alarcón, J.J.; Bravo Patiño, A.; Baizabal Aguirre, V.M. Molecular characterization of *Staphylococcus* spp. isolates associated with bovine mastitis in Tarímbaro, Michoacán, Mexico. *Técnica Pecu. México* **2006**, *44*, 91–106.
20. Yu, T.; He, T.; Yao, H.; Zhang, J.B.; Wang, G.Q. Prevalence of 16S rRNA Methylase Gene rmtB Among *Escherichia coli* Isolated from Bovine Mastitis in Ningxia, China. *Foodborne Pathog. Dis.* **2015**, *12*, 770–777. [CrossRef]
21. Xu, T.; Wu, X.; Cao, H.; Pei, T.; Zhou, Y.; Yang, Y.; Yang, Z. The Characteristics of Multilocus Sequence Typing, Virulence Genes and Drug Resistance of Klebsiella pneumoniae Isolated from Cattle in Northern Jiangsu, China. *Animals* **2022**, *12*, 2627. [CrossRef]
22. Hogan, J.S.; Gonzalez, R.N.; Harmon, R.J.; Nickerson, S.C.; Smith, K.L. *Laboratory Handbook on Bovine Mastitis*; National Mastitis Council: Madison, WI, USA, 1999.
23. Blum, S.; Heller, E.D.; Krifucks, O.; Sela, S.; Hammer-Muntz, O.; Leitner, G. Identification of a bovine mastitis *Escherichia coli* subset. *Vet. Microbiol.* **2008**, *132*, 135–148. [CrossRef]
24. Frank, J.A.; Reich, C.I.; Sharma, S.; Weisbaum, J.S.; Wilson, B.A.; Olsen, G.J. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl. Environ. Microbiol.* **2008**, *74*, 2461–2470. [CrossRef]
25. Ceriotti, F.; Zakowski, J.; Sine, H.; Altaie, S.; Horowitz, G.; Pesce, A.J.; Boyd, J.; Horn, P.; Gard, U.; Horowitz, G. Clinical and Laboratory Standards Institute (CLSI). 2012. Available online: https://www.scienceopen.com/document?vid=1d021ccc-8583-4e6b-a47c-8735734df154 (accessed on 1 December 2022).
26. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing, M100*, 31st ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2021.
27. Turton, J.F.; Perry, C.; Elgohari, S.; Hampton, C.V. PCR characterization and typing of Klebsiella pneumoniae using capsular type-specific, variable number tandem repeat and virulence gene targets. *J. Med. Microbiol.* **2010**, *59*, 541. [CrossRef] [PubMed]
28. Yu, W.L.; Ko, W.C.; Cheng, K.C.; Lee, C.C.; Lai, C.C.; Chuang, Y.C. Comparison of prevalence of virulence factors for Klebsiella pneumoniae liver abscesses between isolates with capsular K1/K2 and non-K1/K2 serotypes. *Diagn. Microbiol. Infect. Dis.* **2008**, *62*, 1–6. [CrossRef]
29. Dong, W.; Fawan, L.; Xuqing, W.; Jianbo, W.; Jianming, P.; Linjun, D.; Junhong, L.; Dengqiang, H. Isolation and identification of mastitis pathogens in dairy cows from some areas of Ningxia. *Prograss Vet. Med. Chin.* **2012**, *33*, 4. [CrossRef]
30. Dan, W.; Feng, Y.; Xinpu, L.; Jinyin, L.; Longhai, L.; Zhe, Z.; Yaru, Z.; Hongsheng, L. Isolation, identification and drug resistance detection of the pathogenic bacteria causing dairy cow mastitis in Suzhou and capsular polysaccharide typing test of the pathogens. *Chin. J. Prev. Vet. Med.* **2018**, *40*, 7. [CrossRef]
31. Qian, S.; Juyun, D.; Yina, L.; Jinlian, S.; Zongshuai, L.; Xingxu, Z.; Yong, Z. Isolation, Identification and Drug Resistance Analysis of Main Pathogens ofDairy Cow Clinical Mastitis in Wuzhong Area of Ningxia. *Prog. Veterianry Med. Chin.* **2022**, *43*, 6.
32. Saini, V.; Mcclure, J.T.; Léger, D.; Keefe, G.P.; Scholl, D.T.; Morck, D.W.; Barkema, H.W. Antimicrobial resistance profiles of common mastitis pathogens on Canadian dairy farms. *J. Dairy Sci.* **2012**, *95*, 4319–4332. [CrossRef]
33. Metzger, S.A.; Hogan, J.S. Short communication: Antimicrobial susceptibility and frequency of resistance genes in *Escherichia coli* isolated from bovine mastitis. *J. Dairy Sci.* **2013**, *96*, 3044–3049. [CrossRef] [PubMed]
34. Xiao, Y.H.; Giske, C.G.; Wei, Z.Q.; Shen, P.; Heddini, A.; Li, L.J. Epidemiology and characteristics of antimicrobial resistance in China. *Drug Resist. Updates* **2011**, *14*, 236–250. [CrossRef]
35. Zhang, S.; Abbas, M.; Rehman, M.U.; Wang, M.; Jia, R.; Chen, S.; Liu, M.; Zhu, D.; Zhao, X.; Gao, Q.; et al. Updates on the global dissemination of colistin-resistant Escherichia coli: An emerging threat to public health. *Sci. Total Environ.* **2021**, *799*, 149280. [CrossRef] [PubMed]
36. Neela, F.A.; Nonaka, L.; Rahman, M.H.; Suzuki, S. Transfer of the chromosomally encoded tetracycline resistance gene tet(M) from marine bacteria to *Escherichia coli* and Enterococcus faecalis. *World J. Microbiol. Biotechnol.* **2009**, *25*, 1095–1101. [CrossRef]
37. Wang, G.Q.; Wu, C.M.; Du, X.D.; Shen, Z.Q.; Song, L.H.; Chen, X.; Shen, J.Z. Characterization of integrons-mediated antimicrobial resistance among *Escherichia coli* strains isolated from bovine mastitis. *Vet. Microbiol.* **2008**, *127*, 73–78. [CrossRef] [PubMed]

38. Zhao, H.X.; Zhao, J.L.; Shen, J.Z.; Fan, H.L.; Guan, H.; An, X.P.; Li, P.F. Prevalence and molecular characterization of fluoroquinolone resistance in *Escherichia coli* isolates from dairy cattle with endometritis in China. *Microb. Drug Resist.* **2014**, *20*, 162–169. [CrossRef] [PubMed]
39. Bajaj, P.; Kanaujia, P.K.; Singh, N.S.; Sharma, S.; Kumar, S.; Virdi, J.S. Quinolone co-resistance in ESBL- or AmpC-producing *Escherichia coli* from an Indian urban aquatic environment and their public health implications. *Environ. Sci. Pollut. Res. Int.* **2016**, *23*, 1954–1959. [CrossRef]