Outbreak of Colibacillosis in Intensive Chicken Farms: Pathogenicity and Molecular Characterization

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Abstract

Avian pathogenic E. coli (APEC) are generally considered to be the reservoir for human extraintestinal pathogenic E. coli (ExPEC): they share similar genetic characteristics and pathogenicity with no or minimal host specificity. In this study, we successfully isolated and identified an E. coli strain as the culprit responsible for serious colibacillosis outbreaks in intensive chicken farms in China in 2016. We investigated its phylogenetic classification (A, B1, B2, C, D, E, and F) by PCR analysis; its virulence and host range using challenge experiments with different animals; and its virulence factors, drug resistance genes, sequence type (ST), and related biological information through high-throughput sequencing. This isolate was found to belong to ST95, group B2, and serotype O18. The E. coli strain shows strong virulence in chickens with a minimum lethal dose (MLD) of 3 × 103 CFU/chicken and a strong virulence in mice with an MLD of 3 × 102 CFU/mouse and in rabbits with an MLD of 3 × 103 CFU/rabbit. Whole-genome sequencing showed that it consists of six types of prevalent resistance genes, 33 antibiotic efflux genes, and nine recognized virulent factors. The detailed data are available from GenBank (SRR13005645) for further study.

Outbreak of Colibacillosis in Intensive Chicken Farms: Pathogenicity and Molecular Characterization

Running Title: Characterization of a highly pathogenic avian

E. coli

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Abstract

Avian pathogenic *E. coli* (APEC) are generally considered to be the reservoir for human extraintestinal pathogenic *E. coli*(ExPEC): they share similar genetic characteristics and pathogenicity with no or minimal host specificity. In this study, we successfully isolated and identified an *E. coli* strain as the culprit responsible for serious colibacillosis outbreaks in intensive chicken farms in China in 2016. We investigated its phylogenetic classification (A, B1, B2, C, D, E, and F) by PCR analysis; its virulence and host range using challenge experiments with different animals; and its virulence factors, drug resistance genes, sequence type (ST), and related biological information through high-throughput sequencing. This isolate was found to belong to ST95, group B2, and serotype O18. The*E. coli* strain shows strong virulence in chickens with a minimum lethal dose (MLD) of 3×10^3 CFU/chicken and a strong virulence in mice with an MLD of 3×10^2 CFU/mouse and in rabbits with an MLD of 3×10^3 CFU/rabbit. Whole-genome sequencing showed that it consists of six types of prevalent resistance genes, 33 antibiotic efflux genes, and nine recognized virulent factors. The detailed data are available from GenBank (SRR13005645) for further study.

KEY WORDS

Avian *E. coli* , China, mice, pathogenicity, rabbit

INTRODUCTION

Avian pathogenic *E. coli* (APEC), a subclass of extraintestinal pathogenic *E. coli* (ExPEC), causes acute avian colibacillosis with respiratory and systemic infections in poultry; therefore, it is a disease of great economic significance to poultry producers worldwide [Manges, et al., 2019]. Because APECs are opportunistic pathogens, outbreaks of avian colibacillosis are frequently associated with infections by*Mycoplasmagallisepticum* or respiratory viruses, such as Newcastle virus or infectious bronchitis virus [Van, et al., 2020].

Epidemiological studies have isolated some APEC strains from poultry and poultry meat that are genetically similar to those responsible for human infections [Zhuge, et al., 2019; Borges, et al., 2019; Zhang, et al., 2019; Cui H, et al., 2020]. Such organisms could be transmitted to humans through the consumption of contaminated meat and by direct human–animal contact [Borges, et al., 2019]. APECs are generally suspected to be sources of bacteria capable of causing human ExPEC infections. Hence, we should pay more attention to the characterization of APEC isolates from avian colibacillosis outbreaks and determine their virulence in experimental models of chickens and mammals.

THE STUDY

In China, large number of young chickens died in succession in intensive chicken farms in 2016, and the morbidity and mortality were 40% and 25%, respectively. The resident veterinarian first tested whether

the deaths were caused by viral infectious diseases; however, the tests for avian influenza virus, Newcastle disease virus, and infectious bronchitis virus were negative. On anatomical observation of the dead chickens, the veterinarian found swollen livers and spleens as well as broken follicles. After eliminating the possibility of viral infectious disease involvement, *E. coli* strains were isolated from the livers of the diseased chickens. Following the combined use of two to three antibiotics to treat the affected chickens, the epidemic was controlled, further verifying that bacteria were behind these outbreaks.

We identified the serotype and phylogenetically classified the bacterial strain via morphological and biochemical tests and PCR analysis. The isolate, named Z0105, was obtained from the liver of a dying layer. After analysis, the isolate was identified as *E. coli* serotype O18. Triplex PCR targeting *chu* A, *yja* A, and TspE4.C2a was used to further classify the *E. coli* O18 to the B2 group. The definitions of the different of *E. coli* phylogroups (A, B1, B2, C, D, E, and F) are based on triple PCR, which was developed by Clermont and colleagues in 2000 [Clermont, et al., 2013]. Most ExPEC isolates belong to phylogroup B2 and some to a lesser group, D. The strains assigned into B2 or D are generally recognized as having a stronger virulence in animals and humans.

Whole-genome sequencing was performed on the *E. coli* O18 using the PacBio sequel II platform (Shanghai OE Biotech Co., Ltd.), and the genome was in the order of 5,642,598 bp in size with a G +C content of 50.49%. Genomic sequences were compared using Diamond [Akhter, et al., 2012] software to obtain genes with the annotation e<1e-5. A total of eight common databases were used, including NR annotation, COG/KOG function annotation, GO classification, Swissprot, eggNOG, KEGG, and Pfam (Figure 1). To search for antimicrobial resistance genes and virulent factors, protein-coding genes were aligned against the Comprehensive Antibiotic Resistance Database (CARD; https://card. mcmaster.ca/) and the Virulence Factors of Pathogenic Bacteria (VFPB; http: //www. mgc. ac.cn/VFs/main.htm) using recommended similarity thresholds.

Using VFPB, the *E. coli* isolate Z0105 was analyzed for the presence of known APEC virulence factors. The virulence-associated genes (and their encoded proteins) harbored by Z0105 included *iut* A (*Aerobactin siderophore* receptor),*foc* G (F1C fimbriae), *pap* C (P-fimbriae), and*fyu* A (yersiniabactin receptor), *Kps* M (K1 capsule),*Vat*(vacuolating autotransporter toxin), *fim* H (type I fimbrial adhesion), *hly* (hemolysins), and *usp* (uropathogenic specific protein). CARD analysis showed the presence of$bla_{\text{CTX-M-65}}$, $bla_{\text{OXA-1}}$, and$bla_{\text{CMY-47}}$ (beta-lactamase),*Fos* A3(fosfomycin), *Tet* G (tetracycline), *aad* A3 (aminoglycoside nucleotidyltransferase), *Sul* 2 and *Sul* 1 (sulfonamide resistant dihydropteroate synthase), and *Qnr* S2 (quinolone resistance protein). Additionally, there were 33 predicted antibiotic efflux genes in *E. coli* O18.

For multilocus sequence typing (MLST), seven house-keeping genes, namely*adk* , *fum* C, *gyr* B, *icd* , *mdh* ,*pur* A, and *rec* A, were sorted from the draft genome sequence and submitted to the MLST database (*http://pubmlst.org/)* to compare their allele profiles and define the bacterial sequence type (ST). MLST is currently the most powerful typing system for the discrimination of bacterial population genetics. Furthermore, we PCR-amplified the seven house-keeping genes and sequenced the products to compare with the seven genes from the whole-genome sequencing. On the basis of the gene allele number, the *E. coli* isolate from this outbreak belongs to ST95, which is a predominant ST type in ExPEC strains.

The Z0105 isolate belongs to ST95, group B2, and serotype O18 and shows strong virulence in chickens, mice, and rabbit. Seventy-day-old chickens (obtained from Jiangsu Lihua Animal Husbandry Co. Ltd., Changzhou, China) were challenged intramuscularly with the Z0105 isolate, while 6-week-old female BALB/c mice (obtained from the Comparative Medicine Center of Yangzhou University, Yangzhou, China) were challenged intraperitoneally. Two-month-old New Zealand rabbit (obtained from Jiangsu Jinling Rabbit Farm, Nanjing, China) were challenged intravenously. The experimental and control groups each contained 10 chickens, 10 mice, and 5 rabbits. All animals were injected with a serially diluted bacterial culture of 3×10^6 CFU to 3×10^2 CFU in 0.2 mL PBS, and *E. coli* MG1655 was used as the negative control strain. The animals were observed daily over 14 days and the mortality rate was recorded.

All animals in the negative control groups were alive and healthy. The chickens in the experimental groups had

a 100% mortality rate when the challenge dose was 3×10^4 CFU or above. The chickens in the experimental groups showed 40% mortality when challenged with 3×10^3 CFU in this study. The mice in the experimental groups showed a 100% mortality rate when the challenge dose was 3×10^4 CFU or above and 60% and 10% mortality when challenged with 3×10^3 CFU and 3×10^2 CFU, respectively. The rabbits in the experimental groups showed 100% mortality when the challenge dose was 3×10^5 CFU or above; while they showed 60% and 20% mortality when challenged with 3×10^4 CFU and 3×10^3 CFU, respectively (Figure 2). In previous reports, APEC of group B2 had high strong virulence to young chicks within a couple of weeks and the lethal dose was generally equal to or greater than 10^7 CFU [Gao, et al., 2018]. For mice, UPEC of group B2 also showed a certain degree of virulence, but the lethal dose was generally 10^6~10^8 CFU [Stromberg, et al., 2019; Gao, et al., 2017]. The Z0105 isolate belongs to ST95, group B2, and serotype O18 and shows stronger virulence than reported before. The dead animals were examined by necropsy, and the Z0105 were successfully isolated and identified as *E. coli*O18. APEC O18 was the cause of the serious colibacillosis in this outbreak and is a considerable risk to the poultry industry and a potential threat to human health and food safety.

CONCLUSION

With the implementation of a ban on antibiotic overuse in industrial livestock by the government, poultry colibacillosis will pose an increasingly serious threat to the poultry industry. The culprit in this outbreak was *E. coli* O18, belonging to ST95 and group B2, which are predominant pathogenic bacteria in APEC. The isolate Z0105 presented high lethality not only to chickens but also to mice and rabbits, and the potent virulence threatens the poultry industry. The current cases of serious antibiotic resistance and the emergence of highly virulent pathogens will undoubtedly pose increasingly greater threats to human health. There should be careful monitoring of avian colibacillosis epidemiology and the evolution of the causative strains.

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DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

ETHICAL STATEMENT

The study protocols for sample collection were approved by the Science and Technology Agency of Jiangsu Province. Approval was also granted by the Jiangsu Academy of Agricultural Sciences Experimental Animal Ethics Committee (approval ID NKYVET 2016-2017).

DATA AVAILABILITY STATEMENT

None declared.

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