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Article

Characteristics of *Escherichia albertii* Strains Isolated from Industrial Quails of the Russia

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Abstract: Background: *Escherichia albertii* is a lactose-negative escherichia that causes gastritis and enteritis in humans. Analysis of possible sources of infection points out that poultry may be a significant reservoir for this pathogen. The question of whether *E. albertii* can cause infections in poultry is still unanswered. Our article describes the isolation of *E. albertii*, for the first time in Russia, from the intestines of birds on a quail farm and characterization of obtained cultures. Methods: We isolated different bacteria from pathological poultry material using bacteriological methods and ruled out them as probable causes for enteritis. Biochemical identification of *E. albertii* and antibiotic sensitivity were performed using a Vitek-2 Compact instrument. Bacterial identification was done using the MALDI-TOF Biotyper instrument (Bruker, Germany). *E. albertii*-specific genes, virulence factor genes and microcin genes were determined real-time PCR. Results: Concluded that *E. albertii* isolated from sites of intestinal inflammation are a potential cause of enteritis and high poultry mortality – up to 15% of total livestock for 10- to 20-days quails. One of the *E. albertii* culture differed from the main group of *Escherichia* by its biochemical properties, and subsequent PCR analysis showed a lack of the intimin gene (*eae*). However, we have to perform model experiments, which we will conduct later, to prove that the bird is not just a reservoir for the pathogen but is also sick itself. Conclusions: We describing the first occasion of infection caused by *E. albertii* in industrial quails. During the study it was found that according to the molecular-genetic and phenotypic properties of isolated strains in quails there were at least 2 clonal groups of *E. albertii* differing in antibiotic resistance, biochemical indices and presence of the *eae* (intimin) gene.

Keywords: *Escherichia albertii*; *eae* genes; bird infections; APEC; antibiotic resistance

1. Introduction

Escherichia albertii is a Gram-negative, immobile, nonspore-forming, facultatively anaerobic, lactose-negative bacillus belonging to the family *Enterobacteriaceae* [1]. *E. albertii* was first isolated in diarrhea cases in children from Bangladesh, tentatively misidentified as *Hafnia alvei*, and later classified as a new *Escherichia* species in 2003. [2].

Often *E. albertii* is misidentified as enteropathogenic *E. coli* (EPEC) or enterohemorrhagic *E. coli* (EHEC) because of its genetic and phenotypic similarities to these pathogens. For example, *E. albertii* usually carries the *eae* gene encoding intimin, an important virulence factor also shared by pathogenic subgroups of *E. coli* [3]. This result probably led to an erroneous underestimation of the number of infections caused by *E. albertii*. For example, in several outbreaks of gastroenteritis, the causative agent was misdiagnosed as EPEC instead of *eae*-positive *E. albertii* [4].

The lack of a clear differential diagnosis remains a major problem, as there is no simple and accurate diagnostic protocol, especially for PCR typing, such as multilocus sequence typing (MLST) and O-genotyping. [5].

To date, three biogroup of *E. albertii* have been distinguished according to their phenotypic features: biogroup 1 – indole-negative, lysine-positive; biogroup 2 – indole-positive, lysine-negative; biogroup 3 – indole-positive, lysine-positive. This method may be useful for the identification of *E. albertii* in diagnostic laboratories or in the context of phylogeny [6].

Strains of *E. albertii* have been isolated from various animal sources, such as poultry, pigs, cats, dogs, bats, and raccoons, and raw meat of animal origin [7], but its natural reservoirs and routes of transmission to humans remain uncertain.

Nevertheless, an analysis of possible sources indicates that birds are the most significant reservoir for this pathogen [1]. Thus, the isolation of *E. albertii* cultures from migratory birds of the Asian-Australasian flyway (EAAF), which affects the territory of Russia, has been reported [8]. The genetic diversity of *E. albertii* isolates from migratory birds was demonstrated, and some isolates can potentially cause disease in humans. A large-scale study conducted on 9 farms in Mississippi and Alabama showed that of 270 cloacal swabs (30 per farm) tested by PCR diagnostics, 43 were positive for the presence of *E. albertii*, and 12 strains of *E. albertii* were isolated with varying degrees of isolation on individual farms from 0% to 23.3% [9]. Phylogenetically, isolates from different farms were distant but clonally similar from the same farm, including those with antibiotic and plasmid resistance. Importantly, 9 out of 12 *E. albertii* strains exhibited multiple drug resistance; one strain was even resistant to imipenem, a clinically important carbapenem antibiotic. In addition, comparative genomics analysis showed that two clusters of *E. albertii* strains isolated from chickens exhibited very close evolutionary relationships and similar virulence gene profiles to *E. albertii* strains isolated from humans.

A study in Switzerland on the detection of *E. albertii* in wild birds showed that out of 280 fecal samples (collected from 26 bird species), *E. albertii* isolates were obtained from 11 bird species belonging to eight families. Four of the eleven species were waterfowl. The other 7 species, including raptors and raven, often inhabit agricultural lands [10]. In the same study, pooled fecal samples from 150 broiler flocks (more than one million birds) did not reveal the presence of *E. albertii* in any of the samples.

It should be noted that the review studies cited were aimed at detecting *E. albertii* carriage, but not at detecting infection caused by this bacterium. Whether *E. albertii* can cause infection in poultry remains an open question.

Our paper describes the isolation of *E. albertii* isolates, for the first time in the Russian Federation, from the intestines of birds of a quail farm and the phenotypic and molecular genetic characterization of the obtained cultures. In addition, we show that *E. albertii* is the cause of lethal infection in birds. The listed provisions were the objective of our work.

2. Materials and Methods

2.1. Object of the Study

Live quails came from a quail farm in the Moscow region with 25 animals. Anamnesis indicated a significant loss of livestock (up to 15% of the total livestock) at the age of 10 to 20 days of life with signs of gastrointestinal tract damage. Keeping birds on the farm is cage-based. Crosses of three lines – Japanese, Pharaoh, and Manchurian gold. Quails were killed under chloroform anesthesia, after which they were isolated using a nutrient media.

2.2. Nutrient Media and Seeding

Using dactyloscopic smears (see below), quail parenchymatous organs were seeded on nutrient agar #1 (Nutrient Media, Obolensk), Endo agar (Nutrient Media, Obolensk), BHI agar (brain-heart infusion, HiMedia M-211, India) and addition of 10 µg/ml NADH (A1393.0001, AppliChem, Spain) 5% (w/v), fresh yeast extract, 1% (wt/volume) glucose (A0883.0500, AppliChem, Spain) and 0.001% (wt/volume) L cysteine (LLC NPP "PanEco"). To obtain parenchymatous smears, parenchymatous organs were briefly cauterized with ethanol, the organ was excised, and the cut surface was pressed against agar several times. To obtain scrapings from the suborbital sinuses, the upper part of the beak was excised with sterile scissors and scraped with a 2-µl loop. The loop content was seeded on Petri dishes containing dense agar. The trachea and oviducts were scraped with loops and the loop contents were seeded onto agar dishes. The intestinal contents were sown with a 10 µl loop on the agar surface and further spread on the surface of the medium with new loops using the Drigalsky method to isolate colonies. *Clostridium perfringens* agar (HiMedia, India) supplemented with nutrients was used to isolate clostridia. The seeded Petri dishes were placed in an anaerostat (Schuett-biotec GmbH D-37079, Göttingen, Germany) and cultured under anaerobic conditions using anaerobic bags cultured at 42 °C for 24 h.

Cups of crops were also duplicated. Part of the dishes was placed in an anaerostat under microaerophilic conditions (with a candle) at 37 °C. The other part was cultured under anaerobic conditions, using anaerobic bags, at 42 °C for 24 h.

Culture typing. The grown colonies were evaluated culturally and morphologically and then analyzed by mass spectrometry.

2.3. Mass Spectrometric Analysis

Sample preparation was performed as previously described [11]. Protein extraction was performed according to the protocol proposed by Bruker (Daltonics GmbH, Germany). The results were summarized using the FlexAnalysis software (Bruker Daltonics GmbH, Bremen, Germany).

2.4. PCR Analysis of *Escherichia coli*

DNA was isolated from bacterial culture grown on nutrient agar dishes (HiMedia, Mumbai, India) using simple cell lysis. The PCR-mix for 20 µL reactions contained 0.4 mM dNTP (Thermo Fisher Scientific, USA), 2.5 µL DreemTaq Green Buffer 10x (Thermo Fisher Scientific, USA), 10 mM of each primer, 0.1 units/µL DreemTaq DNA polymerase (Thermo Fisher Scientific, USA). The amplification products were analyzed using the ChemiDoc XRS+ system (Bio-Rad, USA).

E. albertii strains were identified by searching for *E. albertii*-specific genes such as *mdh*, *lysP*, and *clpX* (Table 1). We amplified the malate dehydrogenase gene (*mdh*), lysine-specific transporter gene (*lysP*) and heat shock protein gene (*clpX*) using multiplex PCR (Table 1) [12].

In addition, we used real-time PCR differentiation of *E. coli* pathogroups (EPEC, ETEC, EIEC, EHEC, EAgrEC) with the AmpliSens® Escherichiose-FL kit (AmpliSens, Moscow, Russia) for isolated *E. albertii* and *E. coli* cultures.

Identification of the intimin gene – *eae* (common between *E. albertii* and EPEC) in *E. albertii* cultures was performed according to the European Union reference laboratory methodology for *E. coli* "Identification and characterization of verocytotoxin-producing *Escherichia coli* (VTEC) by real-time PCR amplification of major virulence genes and genes associated with serogroup mainly associated with severe human infections" [13].

2.5. CDT Typing

Cytotoxic cell distension toxin (CDT) and cell cycle inhibitory factor (*cif*), types of cyclomodulins of pathogenic *Escherichia coli* that are not associated with any specific phylogroup, were investigated using specific primers (Table 1) [14].

Virulence factor genes of APEC (avian pathogenic *Escherichia coli*) were investigated according to the protocols proposed in the articles (Table 1) [15,16].

Microcin genes were determined according to the conditions proposed in [17].

2.6. Serotyping of *Escherichia coli*

The serotyping of *Escherichia coli* was performed according to the standard protocol recommended by the manufacturer (JSC "Biomed" named after. I.I. Mechnikov, Russian Federation).

2.7. Biochemical Identification and Susceptibility to Antimicrobials

Biochemical identification of *E. albertii* and antibiotic sensitivity were performed using GN cards on a Vitek-2 Compact instrument.

In addition, antibiotic sensitivity was studied by the disk-diffusion method using NICF disks (St. Petersburg), with the results recorded automatically on a Scan 500 device (Interscience).

Table 1. List of primers used in the study.

The <i>mdh</i> , <i>lysP</i> and <i>clpX</i> genes are specific to <i>E. albertii</i>					
Gene	Primer	5'-3'	Annealing, °C	Product size (bp)	Reference
<i>Mdh</i> malatedehydrogenase	mdh-F	CTGGAAGGCGCAGATGTGGTACTGATT	55	115	[Ошибка! Источник ссылок и не найден.]
	mdh-R	CTTGCTGAACCAGATTCTTCACAATACCG			
	lysP-F	GGGCGCTGCTTTCATATATTCTT			
<i>Lys</i> Lysine-specific transporter	lysP-R	TCCAGATCCAACCGGGAGTATCAGGA	55	252	[Ошибка! Источник ссылок и не найден.]
	clpX-F	TGGCGTCGAGTTGGGCA			
<i>Clp</i> Heat shock protein	clpX-R	TCCTGCTGCGGATGTTTACG	55	384	[Ошибка! Источник ссылок и не найден.]
Pre-denaturation 95°C – 5 min; 25 cycles: denaturation 95°C – 1 min, annealing 55°C – 1 min, elongation 72°C – 1 min-. Final elongation 72°C – 3 min.					
<i>E. coli</i> APEC virulence genes					
<i>cva</i> colicin V plasmid	cvaF	CACACACAAACGGGAGCTGTT	63	672	[Ошибка! Источник ссылок и не найден.]
	cvaR	CTTCCGCAGCATAGTTCCAT			
<i>omp</i> episomal outer membrane protease	ompF	TCATCCCGGAAGCCTCCCTCACTACTAT	63	496	[Ошибка! Источник ссылок и не найден.]
	ompR	TAGCGTTTGCTGCACTGGCTTCTGATAC			

	ironF	AAGTCAAAGCAGGGGTTGCCCCG			ник ссылк и не найде н.] [Ошиб ка! Источ
<i>iroN</i> salmochelinsiderophore receptor	ironR	GATCGCCGACATTAAGACGCAG	63	667	ник ссылк и не найде н.] [Ошиб ка! Источ
	fimF	GGATAAGCCGTGGCCGGTGG			ник ссылк и не найде н.] [Ошиб ка! Источ
<i>fim</i> fimbriae	fimR	CTGCGGTTGTGCCGAGAGG	63	331	ник ссылк и не найде н.] [Ошиб ка! Источ
	iutF	GGCTGGACATCATGGGAAGTGG			ник ссылк и не найде н.] [Ошиб ка! Источ
<i>iut</i> Aerobactinsiderophore receptor	iutR	CGTCGGGAACGGGTAGAATCG	63	302	ник ссылк и не найде н.] [Ошиб ка! Источ
	issF	CAGCAACCCGAACCACTTGATG			ник ссылк и не найде н.] [Ошиб ка! Источ
<i>iss</i> Episomal gene for increased survival in serum	issR	AGCATTGCCAGAGCGGCAGAA	63	323	ник ссылк и не найде н.] [Ошиб ка! Источ
	hlyF	GGCCACAGTCGTTTAGGGTGCTTACC			ник ссылк и не найде н.] [Ошиб ка! Источ
<i>hly</i> hemolysin	hlyR	GGCGGTTTAGGCATTCCGATACTCAG	63	450	ник ссылк и не найде н.] [Ошиб ка! Источ
	eaeF	CATTGATCAGGATTTTCTGGTGATA			ник ссылк и не найде н.] [Ошиб ка! Источ
<i>eae</i> intimin	eaeR	CTCATGCGGAAATAGCCGTTA	63	102	ник ссылк и не найде н.]
Pre-denaturation 95°C – 5 min; 35 cycles: denaturation 95°C – 30 sec, annealing 63°C – 45 sec, elongation 72°C – 1 min 45 sec. Final elongation 72°C – 5 min.					
<i>E. coli</i> microcin genes					
Microcin B17	mcc B17-F	TCACGCCAGTCTCCATTAGGTGTTGGCATT	60	135	[Ошиб ка! Источ
	mcc B17-R	TTCCGCCGCTGCCACCGTTTCCACCACTA C			

Microcin C7	mcc C7-F	CGTTCAACTGTTGCAATGCT	60	134	ник ссыл и не найд н.]
	mcc C7-R	AGTTGAGGGGCGTGAATTG			
Microcin E492	mcc E492-F	GTCTCTCCTGCACCAAAAGC	60	291	
	mcc E492-R	TTTTCAGTCATGGCGTTCTG			
Microcin H47	mcc H47-F	CACTTTCATCCCTTCGGATTG	60	227	
	mcc H47-R	AGCTGAAGTCGCTGGCGCACCTCC			
Microcin J25	mcc J25-F	TCAGCCATAGAAAGATATAGGTGTACCAA T	60	175	
	mcc J25-R	TGATTAAGCATTTTTCATTTTAATAAAGTGT GGTAAATGATATATGAGAGAAATAACGTT			
Microcin L	mcc L-F	A	60	233	
	mcc L-R	TTTCGCTGAGTTGGAATTCCTGCTGCATC			
Microcin V	mcc V-F	CACACACAAAACGGGAGCTGTT	60	680	
	mcc V-R	TTTCGCTGAGTTGGAATTCCTGCTGCATC			
Microcin M	micM-4-F	CGTTTATTAGCCCGGGATT	60	166	
	micM-4-R	GCAGACGAAGAGGCACTTG			
Pre-denaturation 95°C – 5 min; 35 cycles: denaturation 95°C – 30 sec, annealing 60°C – 30 sec, elongation 72°C – 30 sec. Final elongation 72°C – 5 min.					
Primers encoding different types of <i>cdt</i> genes and <i>cif</i> gene in <i>E. coli</i>					
<i>cdtB-II, cdtB-III, cdtB-V</i>	CDT-s1	GAAAGTAAATGGAATATAAATGTCCG	60	467	[Ошиб ка! Источ ник ссыл и не найд н.]
	CDT-as1	AAATCACCAAGAATCATCCAGTTA			
<i>cdtB-II*</i>	CDT-IIas	TTTGTGTTGCCGCCGCTGGTGAAA	62	556	
<i>cdtB-III, cdtB-V*</i>	CDT-IIIas	TTTGTGTCGGTGCAGCAGGGAAAA	62	555	
<i>cdtB-I, cdtB-IV</i>	CDT-s2	GAAAATAAATGGAACACACATGTCCG	56	467	
	CDT-as2	AAATCTCCTGCAATCATCCAGTTA			
<i>cdtB-I</i>	CDT-Is	CAATAGTCGCCCACAGGA	56	411	
	CDT-Ias	ATAATCAAGAACACCACCAC			
<i>cdtB-IV</i>	CDT-IVs	CCTGATGGTTCAGGAGGCTGGTTC	56	350	
	CDT-IVas	TTGCTCCAGAATCTATACCT			
<i>cdtC-V</i>	P105	GTCAACGAACATTAGATTAT	49	748	
	c2767r	ATGGTCATGCTTTGTTATAT			
<i>cif</i>	cif-int-s	AACAGATGGCAACAGACTGG	55	383	
	cif-int-as	AGTCAATGCTTTATGCGTCAT			
Pre-denaturation 95°C – 5 min; 35 cycles: denaturation 95°C – 30 sec, annealing – 30 sec, elongation 72°C – 30 sec. Final elongation 72°C – 5 min.					

* Reverse primers, used with the CDT-s1 primer.

3. Results

3.1. Characteristics of *E. albertii* and Other Microorganisms

The study aim was to determine the causes of increased mortality of quails 10-20 days old with signs of gastrointestinal disorders and diarrhea. The mortality rate, depending on the workshop, ranged from 8% to 20%. Antibiotic therapy with a complex preparation including enrofloxacin and colistin before laboratory testing showed no obvious improvement in quail health.

According to the results of the study, most quails carried *Salmonella enterica* variant bredeney, which is the causative agent of avian salmonellosis. *Salmonella* cultures were not isolated from the gastrointestinal tract. There was respiratory salmonellosis, which was localized mainly in the trachea and suborbital sinuses. The identity of *Salmonella* was confirmed using PCR and agglutinating serum. Cultures of *Streptococcus pluranimalium* causing avian streptococcosis, *Bordetella hinzii* and *Gallibacterium anatis*, the causative agents of pasteurell-like infection in birds, were isolated from the same localization.

At the same time, viral infections such as avian influenza, infectious bronchitis, and Newcastle virus were excluded by PCR analysis. In isolated cultures of *Clostridium perfringens*, PCR analysis did not detect the NetB toxin, which has a leading role in the development of necrotizing enteritis, and the cultures were classified as toxotype A according to the table proposed by Van Immersee [16].

E. coli cultures isolated from the intestine were not assigned to any of the *Escherichia* pathogroups (EPEC, ETEC, EIEC, EHEC, EAgEC) by real-time PCR-RT, and only 4 isolated cultures had genes that could be assigned to the APEC pathogroup. 75% of cultures of this pathogroup were isolated from the tracheas.

The intestine was found to have the following lesions: greenish feces, injected intestinal and mesenteric vessels, wall thickening, necrotic, hemorrhagic and ulcerated areas (Figure 1). Cultures were isolated from poultry with these features in one of the workshops and classified them as *E. albertii* during the study. The results of the bacteriological examination are summarized in Table 2.



Figure 1. Pathological anatomical image of the dissection of the affected intestine with the site from which cultures of *E. albertii* were isolated.

Table 2. Summary of the results of the bacteriological isolation of pathogenic microorganisms from poultry.

No. of sample, workshop, age		Parenchymatous organs (liver, lungs, heart, spleen)	The contents of the sinuses, trachea	Intestines
1 line, 21d	1	<i>Staphylococcus piscifermentans</i>	<i>Salmonella bredeney</i> , <i>Streptococcus pluranimalium</i> , <i>Bordetella hinzii</i>	<i>Clostridium perfringens</i>
	2	<i>Staphylococcus piscifermentans</i>	<i>Salmonella bredeney</i> , <i>Bordetella hinzii</i>	<i>Clostridium perfringens</i>
	3	<i>Staphylococcus piscifermentans</i>	<i>Salmonella bredeney</i>	<i>Clostridium perfringens</i>
	4	<i>Staphylococcus piscifermentans</i>	<i>Salmonella bredeney</i> , <i>Streptococcus pluranimalium</i> , <i>Bordetella hinzii</i>	
	5		<i>Salmonella bredeney</i> , <i>Bordetella hinzii</i>	
2 line, 21d	1	<i>Staphylococcus piscifermentans</i>	<i>Salmonella bredeney</i> , <i>Streptococcus pluranimalium</i>	
	2	<i>Staphylococcus piscifermentans</i>	<i>Gallibacterium anatis</i> , <i>Staphylococcus piscifermentans</i>	<i>Clostridium perfringens</i>
	3	<i>Staphylococcus piscifermentans</i>	<i>Streptococcus pluranimalium</i>	

3 line, 18d	4	<i>Staphylococcus piscifermentans</i>	<i>Gallibacterium anatis</i> , <i>Staphylococcus piscifermentans</i>	<i>Clostridium perfringens</i>
	5	<i>Staphylococcus piscifermentans</i>	<i>Gallibacterium anatis</i> , <i>Staphylococcus piscifermentans</i>	
	1	<i>Staphylococcus piscifermentans</i>	<i>Gallibacterium anatis</i> , <i>Streptococcus pluranimalium</i> , <i>Bordetella hinzii</i>	
	2		<i>Salmonella bredeney</i> , <i>Streptococcus pluranimalium</i> , <i>Bordetella hinzii</i>	
	3	<i>Staphylococcus piscifermentans</i>	<i>Salmonella bredeney</i> , <i>Streptococcus pluranimalium</i> , <i>Bordetella hinzii</i> , <i>Gallibacterium anatis</i>	
4 line, 18 d	4	<i>Staphylococcus piscifermentans</i>	<i>Streptococcus pluranimalium</i> , <i>Bordetella hinzii</i>	<i>Escherichia albertii</i>
	5		<i>Gallibacterium anatis</i> , <i>Salmonella bredeney</i>	
	1		<i>Gallibacterium anatis</i> , <i>Streptococcus pluranimalium</i> , <i>E.coli</i>	
	2	<i>Staphylococcus piscifermentans</i>	<i>Gallibacterium anatis</i> , <i>Streptococcus pluranimalium</i> , <i>Staphylococcus piscifermentans</i>	
	3		<i>Gallibacterium anatis</i>	
m/f, 18 d	4	<i>Staphylococcus piscifermentans</i>	<i>Gallibacterium anatis</i> , <i>Streptococcus pluranimalium</i> , <i>Staphylococcus piscifermentans</i>	<i>Escherichia albertii</i> , <i>Clostridium perfringens</i>
	5	<i>Staphylococcus piscifermentans</i>	<i>Staphylococcus piscifermentans</i>	
	1		<i>Salmonella bredeney</i>	
	2	<i>Staphylococcus piscifermentans</i>	<i>E.coli</i>	
	3		<i>E.coli</i>	
	4		<i>Salmonella bredeney</i>	<i>Escherichia albertii</i>
	5		<i>Salmonella bredeney</i>	<i>Escherichia albertii</i>

3.2. Identification of *E. albertii*

The cultures isolated on Endo agar were lactose-negative and were initially considered to be *E. coli*/*Salmonella* spp. because no significant cultural and morphological differences from colonies of *E. coli* strains were detected and the bird was a carrier of *Salmonella* spp. The mass spectral analysis did not provide an unambiguous interpretation, with close ID values for both *E. albertii* and *E. coli*.

Vitek2 analysis using GN maps showed that all cultures were *E. coli* with a probability of more than 90%. At the same time, it was determined that, conditionally, they are divided into two groups according to the atypical features of *E. coli*. Uncharacteristic for *E. coli* features of the first group: AGAL (Alpha-galactosidase), BGAL (Beta-galactosidase), BGUR (Beta-glucuronidase) – most cultures, for the second group – AGAL, BGAL, BGUR, PHOS (Phosphatase). Further characterization of antibiotic resistance using Vitek2 device and disk diffusion method showed that they differed in antibiotic sensitivity as well (Table 3, Figure 2). Thus, culture-genetic differences were observed in strains of poultry from the same housing.

Table 3. Summary of the results of the bacteriological isolation of pathogenic microorganisms from poultry.

Antibiotics by Vitek2	<i>E. albertii</i> group 1 with the <i>eae</i> gene (in parentheses MPC mg/l)	<i>E. albertii</i> group 2 without <i>eae</i> gene
BLRS	-	-
Ampicillin	S (8)	R (16)
Amoxicillin/clavulonic acid	S (8)	R (16)
Cefotaxime	S (≤ 0,25)	S (≤ 0,25)
Ceftazidime	S (≤ 0,12)	S (≤ 0,12)
Cefipime	S (≤ 0,12)	S (≤ 0,12)
Eatapenem	S (≤ 0,12)	S (≤ 0,12)
Meropenem	S (≤ 0,25)	S (≤ 0,25)
Amikacin	S (≤ 2)	S (≤ 2)
Gentamicin	S (≤ 1)	S (≤ 1)
Netilmicin	ND (≤ 1)	ND (≤ 1)
Ciprofloxacin	R (1)	R (1)
Tigecycline	S (≤ 0,5)	S (≤ 0,5)
Fosfomycin	≤ 16	≤ 16
Nitrofurantoin	S (≤ 16)	S (≤ 16)
Trimethoprim/sulfamethoxazole	S (≤ 20)	S (≤ 20)
Antibiotics using the disk-diffusion method		
Amoxicillin/clavulonic acid, 30	S	R
Ampicillin, 25	S	R
Ceftazidime, 10	S	S
Gentamicin, 10	R	R
Colistin, 10.	R	R
Cefepime, 30.	R	R
Chloramphenicol, 30	S	S
Imipenem, 10	S	S
Trimethoprim/sulfamethoxazole	S	S
Amikacin, 30	R	R
Fosfomycin, 50	R	R
Norfloxacin,	R	R
Tigecycline	R	R

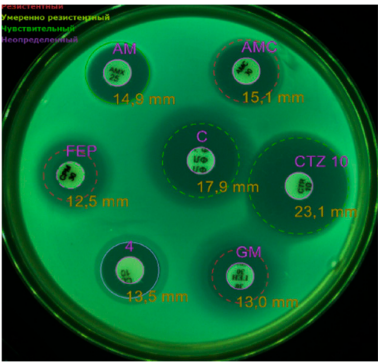


Figure 2. Antibiotic resistance of *E. albertii* cultures using the disk diffusion method (automatic detection using Scan 500 Interscience).

It should be noted the discrepancies in the readings of the two methods for cefepime and tigecycline, for the other antibiotics the readings were the same. The difference between the two clonal groups *E. albertii* was in their relationship to the antibiotics ampicillin and amoxicillin/clavulonic acid – in the first group the cultures were sensitive to them, whereas in the second group they were resistant. Otherwise, the antibiotic resistance profile coincided, and cultures

were resistant to fluoroquinolones (ciprofloxacin, norfloxacin), polymyxins (colistin), aminoglycosides (amikacin, gentamicin, tigecycline), and fosfomycin. Given the widespread use of fluoroquinolones and colistin in poultry farming (including on the livestock of this farm), the resistance of cultures to these groups is natural.

3.3. Results of PCR Analysis

The AmpliSens® Escherichiosis-FL PCR-RT kit showed that all isolated cultures of *E. albertii* belonged to the EPEC (enteropathogenic *Escherichiae*) pathogroup and contained the *eae* gene (encoding intimin), except for one culture, which we designated as group 2, because it also differed from the others in biochemical parameters and antibiotic resistance.

According to the multiplex PCR data for the presence of *E. albertii*-specific genes *mdh*, *lysP* and *clpX*, all tested cultures had all three genes and thus it was confirmed that they belonged to the species *E. albertii*.

O-serotyping of cultures. All tested *E. albertii* cultures reacted simultaneously with sera for O18 and O101.

CDT- and *cif*- typing. According to the results, both strains contained *cdtB*-II, *cdtB*-III, *cdtB*-V, *cdtB*-I, *cdtB*-IV genes. The presence of *cdtB*-II, *cdtB*-III, *cdtB*-V genes was confirmed by positive results for all three primer pairs – CDT-s1+CDT-as1, CDT-s1+CDT-IIas, CDT-s1+CDT-IIIas (Table 1). The presence of the *cdtB*-I, *cdtB*-IV genes was confirmed by positive results only using the primer pair CDT-s2+CDT-as2 multiplex (*cdtB*-I, *cdtB*-IV). Specific primers for these types separately did not provide results. The presence of *cdtB*-I, *cdtB*-IV genes homologous to *E. coli* are doubtful and should be verified by full-genome sequencing. The *cdtC*-V and *cif* genes were not detected.

In addition, the obtained *E. albertii* cultures were tested for the presence of genes specific to pathogenic APEC and microcin genes (Table 1).

According to the results of the study, genes of the APEC pathogenicity group, such as the fimbriae gene (*fim*) and episomal serum survival enhancement gene (*iss*) were detected in *E. albertii* strains of both groups. The microcin M4 and microcin B17 genes were also identified, which apparently increased the competitive advantage of *E. albertii* among closely related species.

4. Discussion

We examined quails from an industrial farm for bacterial infections to determine the causes of mass mortality based on signs of gastrointestinal tract damage. A significant proportion of quails (48% of the total number of quails examined) were carriers of salmonellosis caused by *Salmonella enterica* serovar bredeney (serovar O:4 H:Lv 1,7). However, the localization of all isolated *Salmonella* cultures in the upper respiratory tract indicates that they are not the cause of gastrointestinal tract lesions in poultry. For industrial poultry, the respiratory tract is often the "entry gate" for *Salmonella*. Moreover, it is possible that the respiratory route of *Salmonella* infection in birds is preferable over the fecal-oral route [19,20].

Although in the above studies, in experimental infections with *Salmonella* by intratracheal or aerosolized methods, during the course of time, *Salmonella* colonization also occurred in the cecum, but not in organs (except lungs). In our case, we observed the only respiratory carriage of *Salmonellae*. *Salmonella* bredeney serovar itself is not described as a causative agent of salmonellosis infection in birds, whereas consumption of poultry meat contaminated with this serovar can cause gastroenteritis outbreaks in humans [21]. Thus, we excluded *Salmonella* as a possible cause of poultry mortality in this case.

Other isolated bacteria of importance in the poultry industry include *Gallibacterium anatis*. This bacterium is widely spread in poultry at industrial poultry farms in the Russian Federation [22,23]. However, the cultures *G. anatis* of isolated by us, although they have the RTX-toxin gene (*GtxA*) – the key virulence factor, are not hemolytic (do not belong to the haemolytica biovar), and it is known that hemolytic variants of *G. anatis* are capable of causing enteritis [24]. Therefore, *G. anatis* was not an etiologic agent of GI lesions in our case.

Another possible cause of enteritis is *E. coli*. However, this study determined that the isolated *E. coli* strains did not belong to any of the pathogroups leading to GI lesions. Avirulent *E. coli* strains and APEC pathogens were detected. It is known that APEC are extraintestinal *E. coli* with a leading intratracheal route of infection [25]. This was confirmed in our studies, as three of 4 cultures of the APEC pathogroup were isolated from the trachea (Table 2).

Thus, we hypothesized that *E. albertii* is a possible pathogen responsible for GI lesions in quail. Many cases of human gastroenteritis caused by *E. albertii* have been described, but information on avian diseases caused by this pathogen is scarce. For example, the authors [26], describe the death of wild birds of the Vesjurk family (*Carduelis flammea*) caused by *E. albertii*. According to their data, the cause of death was more than 8000 birds (out of those identified) in Alaska.

Previously, in the late 1990s, *E. albertii* was determined to be the cause of wild bird mortality in Scotland, but the authors incorrectly identified it as *E. coli* O86:K61[27].

We herein describe the first case of infection caused by *E. albertii* in industrial quail and industrial poultry in general. In the process of typing *E. albertii*, the biochemical properties of the cultures were determined. In general, the results showed that the differences in the biochemical properties of *E. coli*/*E. albertii* were characteristic of previously described *E. albertii* cultures [26]. At the same time, one isolated *E. albertii* culture exhibited a slight difference compared with the other cultures. Further analysis of antibiotic resistance and diagnosis using molecular genetic methods confirmed the heterogeneity of this culture with the others. Thus, it can be stated that there were two clonal groups of *E. albertii* in birds – containing and not containing the intimin *eae* gene. CDT-toxin genes were present in both groups, but no shigatoxin genes were detected. Apparently, the strain not carrying the intimin gene (and in the absence of shigatoxin genes) is avirulent in humans. For the remaining *E. albertii* strains, the virulence potential will be studied later after full-genome sequencing data is performed. In addition, it is necessary to obtain in vivo data on laboratory animals for a definitive conviction that they are virulent to birds because the mechanisms of pathogenesis in humans and birds may be different [27]. This work will be continued in the future.

It is interesting to note the presence of microcin M4 and B17 genes in the isolated cultures. M4 microcin of class IIb (siderophore peptide) [28] has antimicrobial activity against *Escherichia coli* and *Salmonellae* which may explain the fact that *S. enterica* is not found in the intestine. B17 is a class I microcin, a post-translationally modified peptide that inhibits bacterial DNA gyrase [29] has an even broader spectrum of antimicrobial activity affecting most *Enterobacteriaceae*. It is possible that the presence of these microcins may increase the distribution of *E. albertii* in the intestine ultimately increasing the risk of infection caused by it.

5. Conclusions

Significant mortality of poultry on quail farms with signs of GI lesions is caused, in our opinion, by *E. albertii*. During the study it was found that according to the molecular-genetic and phenotypic properties of isolated strains in quails there were at least 2 clonal groups of *E. albertii* differing in antibiotic resistance, biochemical indices and presence of the *eae* (intimin) gene. CDT-toxin genes were present in all strains and shigatoxin genes were absent. The described infection is the first evidence of *E. albertii* as a cause of death in industrial poultry.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, MGT and NNK; methodology, MGT and NNK; software, MGT and AAA; validation, MGT, NNK and AAA; formal analysis, MGT and YPS; investigation, MGT, AAA and OIT; resources, MGT and OEK; data curation, MGT, AAA, OIT and NNK; writing—original draft preparation, MGT and AAA; writing—review and editing, MGT, NNK, YPS and OEK; visualization, MGT and AAA; supervision, MGT; project administration, MGT and NNK; funding acquisition, MGT and NNK All authors have read and agreed to the published version of the manuscript.

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