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Study of the Prevalence of *E. Coli* in Poultry and Selection of Specific Bacteriophages

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ABSTRACT

Avian colibacillosis is one of the leading causes of mortality and morbidity associated with economic losses in the industry throughout the world. The problem is enhanced by the growing resistance of pathogenic pathogens towards antibiotics. This situation has strongly stimulated a renewal of scientists' interest in bacteriophages (phages), viruses of bacteria. They are abundant in nature, and accompany bacteria in each environment they colonize, including human microbiota. The aim of our work was to study the prevalence of pathogenic E-coli in poultry, isolate, characterize, and evaluate the potential use of isolated bacteriophages to control E. coli infections in poultry. 35 pathogenic E. coli strains were isolated from 90 sample of poultry excreta. The most common phylogenetic groups were A1 (25%), A2 (15%), B1 (35%), and B2 (25%). E. coli was 100% resistant to five antibiotics (Bacitracin, Clindamycin, Carbapenem, Cephalexin, Clarithromycin). E. coli was least resistant to oxacillin (40%), followed by tetracycline (48.6%). The prevalence of multidrug resistance was 91.4%. Such high levels of resistance in E. coli isolated from poultry excreta could pose a serious threat to humans. Five novel phages against pathogenic E. coli were isolated from sewage water and characterized in vitro. The electron microscopic analysis showed that two phages belonged to the Myoviridae family and three bacteriophages belonged to the *Siphoviridae* family, in the order Caudovirales.

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Introduction

Escherichia coli is a Gram-negative bacillus, a normal inhabitant of the digestive tract of birds, which is widely disseminated with feces. Most strains are nonpathogenic, however, certain pathogenic serotypes (avian pathogenic Escherichia coli-APEC) may induce disease, leading to mortality and condemnations. This opportunistic pathogen can act as both a primary and secondary pathogen. E. coli-associated infections are widely distributed among poultry of all ages and categories. Some strains, such as, enterohemorrhagic E. coli (EHEC), and its subgroup of Shiga toxin (Stx)-producing E. coli (STEC), are food-borne pathogens responsible for serious human diseases worldwide [1]. In the past decades, the emergence of multidrug antibiotic-resistant bacteria (MDR) has been reported as a result of too common and frequent use of antibiotics in human and veterinary medicine as well as in industry and agriculture. Moreover, MDR can be transmitted from food-producing animals to humans via direct contact between animals and humans, or through the food chain and the environment [2,3]. Poultry production is one of the worldwide sectors which utilizes many antibiotics.

Reducing antibiotic use is one of the biggest challenges to the poultry industry globally. Due to the increasing risk of antibiotic-resistant bacteria, the European Union in 2006 has imposed a ban on the use of antibiotics as growth promoters in food-producing animals, but they are still used in other parts of the world. Following the ban, many countries reported a negative impact on animals' well-being, the re-emergence of old infectious diseases in poultry, and an increase in the usage of antibiotics in poultry for therapeutic purposes. Nowadays, foodborne bacterial pathogens have been considered as the leading bacterial causes of human diseases. In the era of the increasing emergence of multidrug-resistant bacteria and a lack of new effective antibiotics, it is natural that much scientific effort has been put into developing and implementing new technologies to combat bacteria. In this context, bacteriophages (phages) have been proposed as an alternative strategy to antibiotics for poultry, and thus for food safety and public health [4]. Phages have many advantages over antibiotics. Phages are natural bacterial killers and are considered the most abundant microbial entities; about 1031 phage virions are present on Earth [5]. In addition, they are particular to bacterial targets without adverse impacts on normal microbiota as antibiotics do [6,7]. The effectiveness and safety of phage therapy in comparison to antibiotics is partially due to the specificity of bacteriophages for particular bacteria, manifested as the ability to infect only one species, serotype or strain. This mechanism of action does not cause destruction of the commensal intestinal flora. Self-replication of bacteriophages takes place during treatment, which eliminates the need to apply them repeatedly. Another advantage of phages is that they cannot bind to and replicate in eukaryotic cells, which causes a decrease in their titre, correlated with a marked reduction in the number of pathogenic bacteria inducing a given infection in the organism. An equally important advantage is that

phages are not toxic, because most of them are composed mainly of proteins and nucleic acids [8]. Many previous studies on the application of bacteriophages in veterinary medicine reported the successful use of bacteriophages in treating farm animal pathogens such as *E. coli, Salmonella*, Campylobacter [9-12]. Therefore, isolation and characterization of new phages is potentially useful for phage therapy applications in both human and veterinary pathogens.

Phage therapies are an effective tool in eliminating bacterial infections in various species of animals. Bacteriophages have also proven successful in treating diseases in poultry. One of the objectives of phage therapy in animals is to assess the suitability of bacterial viruses for control of pathogens having an important influence on animal productivity and health. Bacteriophages used in treatment have been effective in preventing infections and in treatment of colibacteriosis in poultry [8].

Materials and Methods

Sample Collection

Samples were collected from four farms located in different geographical regions of Georgia. From the birds housed in a confined environment, a fresh excreta sample without any contamination of other scavenging materials was collected randomly in a sterile plastic container. Samples of excreta were collected from birds aged 8 weeks to 16 weeks. These excreta samples were transferred with proper labels to the laboratory within 24 h. for identification of bacterial isolates and antibiotic sensitivity patterns. Total 90 samples were taken during the 6 months, 40 – farms of south part of Georgia and 50 – farms of west part of Georgia.

Identification of Bacterial Isolates

The transferred excreta samples were thoroughly homogenised and one gram of excreta was transferred through swab sticks into sterile conical flasks. There were several types of agar developed for the purpose of this study, namely: XLD, MacConkey, Chromagar E. coli, Chromagar STEC, Brain-Hearth Agar and BHI broth. To ensure an aseptic sterilised environment, all procedures were carried out in a laminar flow cabinet. the stirred faecal matter from the sterile conical flask was transferred to the BHI broth for enrichment and incubated at 37C for 24 h. After incubation of 24 h. samples were inoculated to different selective media in order to identify E. coli colonies and incubated at 37C for another 24 h. The selected colony growth was processed using gram stains and examined under the microscope. As the morphologies of gram-negative bacteria were not specific for E. coli alone, further confirmation was obtained by a Microgen Biochemical Identification Kits to perform standard biochemical testes to identify the bacterial sub species.

Antibiotic Sensitivity Pattern

The culture plates that demonstrated colonisation were inoculated, and the following 13 antibiotic discs were added with the specific concentrations: bacitracin, clindamycin, tobramycin, sisomicin, tetracycline, kanamycin, ertapenem, ampicillin, fosfomycin, azithromycin, cephalexin, clarithromycin, oxacilin. After 24 h of the disc diffusion process, the Minimum Inhibitory Concentrations (MICs) for break points were identified. These MIC break points were determined based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) [13].

Pulsed-Field Gel Electrophoresis

PFGE of all isolates was performed using the protocol provided by Bio-Rad (Pulsed Field Electrophoresis Systems Instruction Manual and Applications Guide, BIORAD). Briefly, isolates were grown on brain heart infusion agar plates at 37°C for 24 h. Bacterial cultures were embedded in 1% agarose plugs (Agarose LF, VWR), lysed, washed, and digested separately with the restriction enzyme XbaI (Neb, BioLabs), for at least 12 h at 50°C, respectively. XbaI-digested Salmonella enterica serotype Braenderup DNA was used as a reference size standard.

Bacteriophage Isolation

Bacteriophages were isolated from the sewage water. 90 ml of the sewage water was enriched with 10 ml concentrated broth, and 1 m of respective 18-hour E. coli culture. Then the whole mixture was placed into the incubator for 24 hours, at 37°C. After about 18-24 hours the incubated material was filtered via Millipore filters and the phage present assessed. To isolate pure phage stocks, phage plaques were purified by picking up a single phage plaque with sterile micropipette tips and repeating the process at least five times. To achieve higher phage stocks, the isolated phages were enriched and propagated as follows: in TSB, an indicator host (100 mL, 107 CFU/mL) was infected separately with each phage and incubated at 37°. Next, the lysates were centrifuged at 6000 g for 15 min at 4°C to eliminate any leftover bacterial cells and debris. The phage-containing supernatant was then centrifuged at 15,000 g for 45 min. Finally, the phage pellets were resuspended in SM buffer (100 mM $MgSO_4/7H_2O$; 10 mM NaCl; 50 mM Tris-HCl; pH 7.5) and filtered using 0.22 m syringe filters. A double-layer agar method was used to determine the phage titers and confirm the presence of lytic phages in the filtrate.

Bacteriophage Host Ange

The isolated phages were investigated for host range specificity and lysis efficiency against all isolated strains of *E. coli* using a spot assay. The host bacteria grown overnight and then 100 µl of cell suspension was added to 5 ml of soft LB agar (0.6% agar), which was pre-heated to 42°C in a water bath. The mixture was gently vortexed, poured over LB agar plates (1.5% agar), and allowed to solidify at room temperature during 30 min to produce bacterial lawns. Then, 10 µl of phage stock dilutions (10-fold serial dilutions in SM buffer) was spotted onto the upper agar layer, and the plates were dried at room temperature for 30 min. The plates were incubated overnight at 37°C, and looked for single plaques or bacterial growth inhibition zones after 24 hours. A double-layer agar method was used to determine the phage titers and confirm the presence of lytic phages in the filtrate.

PFGE of Isolated Phages

We used pulsed field gel electrophoresis to estimate the phage genome mass. We added 0.05 ml of phage suspension to 0.45 ml of 50°C temperature 1% melted agarose gel (Agarose LF, VWR) and applied in special forms. After gel solidification, we incubated in lysis buffer (0.5M EDTApH8.0; 10mM Tris-HCl pH8.0; 1% SDS; 0.2mg/ml proteinase K) at 55°C for 18 hours. After incubation, we washed the phage-containing gel with TE buffer.

Electrophoresis was performed in a 1% LF agarose gel with the appropriate electrophoresis buffer (5x Tris-Borate-EDTA) using the Gene Navigator TM System (Amersham). A molecular mass marker (Puls Marker TM 50-1000kb, Sigma) was used for electrophoresis. The electrophoresis pulse parameters corresponded to the following conditions: 10-50 seconds, 200 volts (6V/sm) 20 hours, 140C. We stained the gel in ethidium bromide solution (0.5 mg/ml) for 20 minutes. For visualization of DNA fragments, we used UV-transilluminator (LKB 2011 MACROVUE Transilluminator) and photographed (Kodak Gel Logic 112) [14].

Transmission Electron Microscopy

We used electron microscopy to study phage virion morphology. For this purpose, pure phage lines with a titer of 10^9 PFU/ml was prepared. All samples were stained with 1% uranyl acetate and viewed by electron microscopy in a JEOL JEM 1400 TEM at 80kV. We received electronic photographs in various magnifications. According to the morphology of phages, classification and assignment to the appropriate family according to the classification of the International Committee on Taxonomy of Viruses was performed.

Results

Bacterial Isolates

A total of 90 poultry excreta samples were tested across four commercial poultry farms to identify bacterial isolates. Of the 90 samples, *E. coli* was isolated in 35 (38.9%). 20 *E. coli* isolates were characterized further. The majority of the isolates belonged to the phylogenetic group B1 (35%), followed by groups B2 (25%), and A (25%) and A2 (15%) (Figure 1, 2). Study of biochemical properties showed that all isolated strains were characterized with lactose fermentation ability. There was one isolate identified as STEC/Shiga toxin-producing *E. coli*.

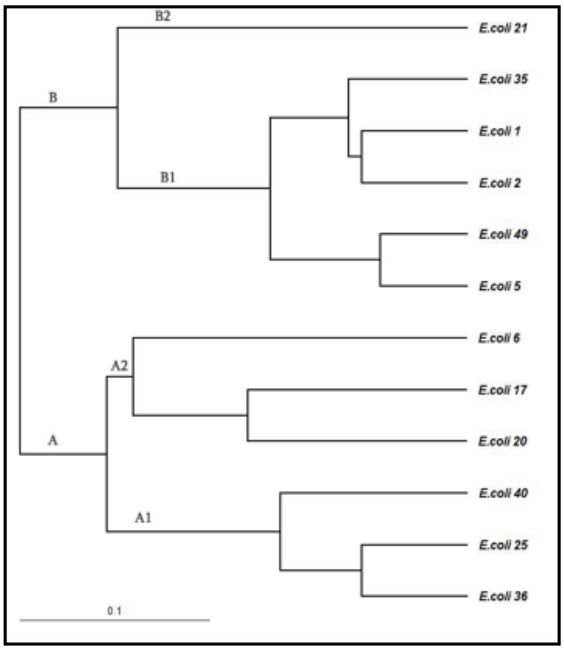

Figure 1: PFGE-based Phylogenetic Dendrogram of *E.coli* Strains

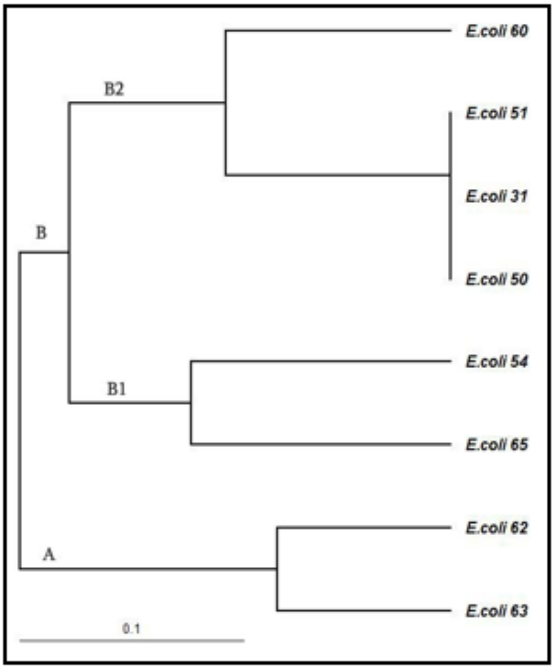

Figure 2: PFGE-based Phylogenetic Dendrogram of *E.coli* Strains

Antimicrobial Susceptibility Testing

All identified *E. coli* samples were tested against 13 antibiotics. All isolates had resistance to at least one of the 13 tested antibiotics. Isolated *E. coli* strains were 100% resistant to five antibiotics (Bacitracin, Clindamycin, Carbapenem, Cephalexin, Clarithromycin). *E. coli* strains were least resistant to oxacillin (40%), followed by tetracycline (48.6%) (Table 1). Overall, 91.4% (32/35) of the isolates showed resistance to at least eight antimicrobials.

Table 1: Antibiotic Susceptibility of Isolated *E. coli* Strains

Name of Antibiotic	No. of Isolates Tested for Sensitivity	Antibiogram					
		Sensitive		Intermediate		Resistant	
		n	%	n	%	n	%
Bacitracin	35	0	(0)	0	(0)	35	(100)
Clindamycin	35	0	(0)	0	(0)	35	(100)
Tobramycin	35	1	(2.8)	13	(37.1)	21	(60)
Sisomicin	35	1	(2.8)	11	(31.4)	23	(65.7)
Tetracycline	35	9	(25.7)	8	(22.8)	18	(51.4)
Kanamycin	35	1	(2.8)	12	(34.3)	22	(62.8)
Ertapenem	35	0	(0)	0	(0)	35	(100)
Ampicillin	35	0	(0)	2	(5.7)	33	(94.3)
Fosfomycin	35	0	(0)	1	(2.8)	34	(97.1)
Azithromycin	35	0	(0)	3	(8.5)	32	(91.4)
Cephalexin	35	0	(0)	0	(0)	35	(100)
Clarithromycin	35	0	(0)	0	(0)	35	(100)
Oxacilin	35	9	(25.7)	12	(34.3)	14	(40)

Isolation and Characterization of Bacteriophages

Nine novel bacteriophages against nine *E. coli* bacterial hosts were isolated from sewage water samples. The phages produced round clear plaques with their respective host isolates after overnight incubation at 37˚C which confirmed them as being lytic. Highly resistant strains from all farms were selected as host strains. Clear plaques appeared after 18 h incubation at 37°C. Five effective phages identified as Φ6, Φ31, Φ40, Φ54 and Φ70 were selected for further characterization.

Host Range Determination

The phage host range, as exhibited by lytic activity against 35 isolates varied from five (14.2%) to 23 (65.7%). Phage Φ40 had the broadest host range, inhibiting 23 (65.7%) isolates followed by Φ54 at 21 (60%) isolates, then Φ70 at 17 (48.5%) isolates, Φ6 at 15 (42.8%) isolates; while Φ31 had the narrowest host range of 5 (14.2%) isolate. Out of the 34 isolates sensitive to the phages, 32 were multidrug resistant. The phage sensitivity pattern of the selected phages on the *E. coli* isolates is presented in Table 2.

Table 2: Spectrum of Activity of Isolated Bacteriophages

Strains	Bacteriophages				
	Φ 6	Φ 31	Φ 40	Φ 54	Φ 70
E. coli 1	n/l	n/l	n/l	scl	n/l
E. coli 2	cl	n/l	scl	scl	n/l
E. coli 5	n/l	n/l	n/l	scl	n/l
E. coli 6	cl	n/l	scl	scl	n/l
E. coli 17	n/l	n/l	n/l	n/l	n/l
E. coli 20	n/l	n/l	cl	n/l	n/l
E. coli 21	scl	scl	n/l	n/l	n/l
E. coli 25	scl	n/l	n/l	n/l	n/l
E. coli 31	n/l	cl	n/l	scl	n/l
E. coli 35	cl	scl	scl	scl	n/l
E. coli 36	n/l	n/l	scl	scl	n/l
E. coli 37	scl	n/l	scl	scl	n/l
E. coli 38	scl	n/l	scl	scl	scl
E. coli 39	n/l	n/l	n/l	n/l	scl
E. coli 40	scl	n/l	cl	n/l	n/l

E. coli 46	n/l	n/l	scl	scl	scl
E. coli 47	n/l	n/l	scl	scl	scl
E. coli 48	n/l	n/l	n/l	n/l	scl
E. coli 49	scl	scl	scl	scl	scl
E. coli 50	n/l	n/l	cl	n/l	n/l
E. coli 51	n/l	n/l	cl	n/l	n/l
E. coli 53	n/l	n/l	n/l	scl	scl

Characterization of Bacteriophages Genome

Through pulsed-field gel electrophoresis (PFGE), bacteriophages Φ31, Φ40, and Φ54 had a double-stranded DNA genome of around 40-45 kbp, which is equivalent to the values suggested by the International Committee on Taxonomy of Viruses (ICTV) for bacteriophages in the *Siphoviridae* family. Bacteriophages Φ6 and Φ70 had a double-stranded DNA genome of around 145 and 190 kbp, respectively, which is equivalent to the values bacteriophages in the *Myoviridae* family. The phylogenetic trees showed that the investigated five phages were genetically different from one another (Figure 3).

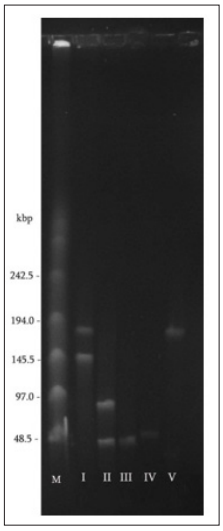

Figure 3: PFGE Profiles of Selected Bacteriophages

Bacteriophage Morphology

The electron micrograph revealed that the three phages Φ31, Φ40, and Φ54 had typical morphology of *Siphoviridae* family with an icosahedral head with diameter 50 nm and long thin tail with length 116-140 nm. Phages Φ6 and Φ70 had a head diameter with approximately 50-60 nm and the tail length of approximately 75-85 nm (Figure 4-8), which is typical for *Myoviridae* family, in the order *Caudovirales*.

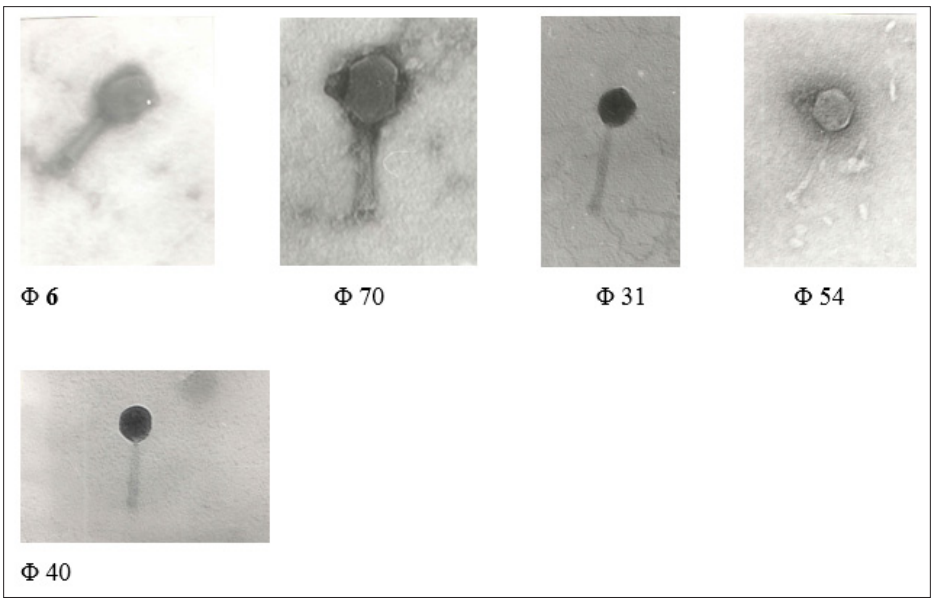

Figure 4-8: Electron Micrography of Bacteriophages

Discussion

In this study, we investigated the prevalence and antimicrobial resistance of *E. coli* strains isolated from poultry farms in Georgia. Our findings show different resistance patterns to several antibiotics that have been commonly used in human medicine and veterinary practice. Susceptibility to antibiotics such as ampicillin, chloramphenicol and tetracycline are widely reported across several studies. Antibiotics such as streptomycin and erythromycin are classified as critically important antibiotics for human use, and these antibiotics are under the high prioritisation category of P3 based on their potential to transfer antibiotic resistance from *E. coli* isolates of animals to humans [15]. Occurrence of resistance is likely to be as a result of irrational drug use, especially among the poultry farmers and use of antibiotic supplemented feeds.

The prevalence of tetracycline resistance in the present study was 48.6%. This is contrary to other studies from Sierra Leone, which reported resistance of 100% but other studies reported similar results as our study, which varied from 40–89% [16-18]. Out of the 35 isolates, none belonged to the serogroups O1, O2 and O78 which were reported to be the most common found in poultry. This means that the above serogroups are not common among APEC infecting chicken in Georgia. Ewers et. al also demonstrated that colibacillosis can be associated with serogroups other than O1, O2 and O78 [19]. Antimicrobial resistance (AMR), currently the second leading cause of deaths worldwide, killing 700.000 people a year, is expected to reach a mortality rate of 10 million deaths by the year 2050 and might even exceed cancer [20]. This necessitates conglomerate efforts toward the development of alternative therapies to antibiotics within a narrow time frame. Bacteriophage is the most promising alternative to antibiotics for treating infections. Bacteriophages are currently addressed at all stages of the poultry production "from farm to fork", however, their implementation into live birds and food products still provokes discussions especially in the context of the current legal framework, limitations, as well as public health and safety.

The effectiveness and safety of phage therapy in comparison to antibiotics is partially due to the specificity of bacteriophages for particular bacteria, manifested as the ability to infect only one species, serotype or strain. This mechanism of action does not cause destruction of the commensal intestinal flora [8]. Phage therapy has also proven to be an effective therapeutic tool in fighting pathogenic strains of Escherichia coli, particularly in preventing the development of colibacillosis, which initially develops in the respiratory tract and air sacs and then takes the form of sepsis, causing considerable mortality in poultry. Phage suspensions applied directly to the air sac in 3-day-old birds in a range of titres from 10^6 to 10^3 PFU to treat *E. coli* infections substantially reduced mortality rates to 5% and 25%, respectively. Similar results were obtained after inoculation of a bacteriophage suspension in the drinking water of birds at 1 week of age (103 or 10^4 PFU of bacteriophages per/mL) followed by air sac challenge with 10^3 CFU of E. coli phages. Mortality was decreased to 25% and 5%, respectively. No mortality was observed in chickens treated with 10^8 PFU of an E. coli bacteriophage mixture [21]. Authors also demonstrated that the use of bacteriophages at titres of 10^4–10^2 PFU in the form of an aerosol in chicks with symptoms of colibacillosis significantly reduced the mortality of the chicks and prevented infections in other birds. Aerosol administration of bacteriophage SPR02 at a titre of 10^8 PFU/mL combined with challenge with 10^4 CFU/mL of *E. coli* completely protected the birds against infection [8]. The results obtained from out experiments showed that, no single phage was able to lyse all the studied APEC strains. The maximum number that could be lysed was 23 out of 35 (65.7%). This is due to specificity of bacteriophages towards their hosts. This is in agreement with other studies that demonstrated that phages usually have a limited host range [22,23]. The three phages, Φ40, Φ54 and Φ70, which had a high lytic activity against 17 to 23 *E. coli* isolates, are better candidates for formulation of cocktails for therapeutic intervention compared to the others. Lysis of the 32 resistant *E. coli* isolates by the phages demonstrates the potential of bacteriophages in controlling infections caused by multidrug resistant bacteria.

Conclusion

The increasingly observed acquisition of antibiotic resistance by bacteria necessitates new strategies for combating drug-resistant bacteria. The results of research on bacteriophages, indicating that they can be an alternative means of eliminating pathogens posing a threat to humans and animals, justify its continuation, particularly in view of increasing drug-resistance in bacteria and restrictions on the use of antibiotics. The development of adequate phage preparations may in the future prove to be one of the most effective methods for fighting bacteria that are pathogenic for humans and animals, and will also make it possible to obtain products that are safe and free of antibiotics.

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References

1. Nolan LK, Barnes HJ, Vaillancourt JP, Abdul Aziz T, Logue CM at al. (2013) Colibacillosis in Diseases of Poultry. https://onlinelibrary.wiley.com/doi/abs/10.1002/9781119421481.ch18.
2. Kirbis A, Krizmana M (2015) Spread of antibiotic resistant bacteria from food of animal origin to humans and vice versa. Procedia Food Sci 5: 148-151.
3. Lin DM, Koskella B, Lin HC (2017) Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. World J Gastrointest Pharmacol Ther 8: 162-173.
4. Żbikowska K, Michalczuk M, Dolka B (2020) The Use of Bacteriophages in the Poultry Industry. Animals 10: 872
5. Batinovic S, Wassef F, Knowler SA, Rice DTF, Stanton CR, et al. (2019) Bacteriophages in natural and artificial environments. Pathogens 8: 100.
6. Abdelsattar AS, Abdelrahman F, Dawoud A, Connerton IF, El-Shibiny A (2019) Encapsulation of E. coli phage ZCEC5 in chitosan–alginate beads as a delivery system in phage therapy. AMB Express 9: 87.
7. Mu A, Mc Donald D, Jarmusch AK, Martino C, Brennan C, et al. (2021) Assessment of the microbiome during bacteriophage therapy in combination with systemic antibiotics to treat a case of staphylococcal device infection. Microbiome 9: 92.
8. Andrzej Wernicki, Anna Nowaczek, Renata Urban-Chmiel (2017) Bacteriophage therapy to combat bacterial infections in poultry. Andrzej Virology Journal 14: 179.
9. Huff W, Huff G, Rath N, Balog J, Donoghue A (2002) Prevention of Escherichia coli infection in broiler chickens with a bacteriophage aerosol spray. Poult Sci 81: 1486-1491.
10. Higgins JP, Higgins SE, Guenther KL, Huff W, Donoghue AM, et al. (2005) Use of a specific bacteriophage treatment to reduce Salmonella in poultry products. Poult Sci 84: 1141-1145.
11. Atterbury RJ, Van Berge, Ortiz MAP, F Lovell MA, Harris JA et al. (2007) Bacteriophage Therapy to Reduce Salmonella

Colonization of Broiler Chickens. Appl Environ Microbiol 73: 4543-4549.

12. Loc Carrillo C, Atterbury RJ, El Shibiny A, Connerton PL, Dillon E, et al. (2005) Bacteriophage Therapy to Reduce Campylobacter jejuni Colonization of Broiler Chickens. Appl Environ Microbiol 71: 6554-6563.
13. European Committee on Anti Microbial Susceptibility Testing. Clinical Break Points and Dosing of Antibiotics. Available online: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Guidance_documents/To_clinical_colleagues_on_recent_changes_in_clinical_microbiology_susceptibility_reports_9_July2021.pdf.
14. Lingohr E, Shelley Frost, Roger P Johnson (2009) Determination of Bacteriophage Genome Size by Pulsed-Field Gel Electrophoresis. Methods Mol Biol 502: 19-25.
15. WHO (2019) Advisory Group on Integrated Surveillance of Antimicrobial Resistance. https://www.who.int/publications/i/item/9789241515528.
16. Alie HD, Mansaray Dennis PY, Yankson Raymonda AB, Johnson Francis L Moses, Joseph Sam Kanu, et al. (2022) Bacterial Isolates and Antibiotic Resistance of Escherichia coli Isolated from Fresh Poultry Excreta Used for Vegetable Farming in Freetown, Sierra Leone. Int J Environ Res Public Health 19: 5405.
17. Abdu HU (2021) Isolation and Characterization of Multidrug-resistant Escherichia coli from Poultry Litter samples from Selected Farms in Kano metropolis Nigeria. Niger J Microbiol 35: 5623-5629.
18. Eyasu A, Moges F, Alemu A (2017) Bacterial isolates from poultry litters and their antimicrobial susceptibility patterns in Gondar, Northwest Ethiopia. Int J Microbiol Res Rev 6: 197-204.
19. Ewers C, Jan ßen T, Kießling S, Philipp HC, Wieler LH (2004) Molecular epidemiology of avian pathogenic Escherichia coli (APEC) isolated from colisepticemia in poultry. Vet Microbiol 104: 91-101.
20. Scarafile G (2016) Antibiotic resistance: Current issues and future strategies. Rev Health Care 7: 3.
21. Huff WE, Huff GR, Rath NC, Balog JM, Xie H, at el. (2002) Prevention of Escherichia coli respiratory infection in broiler chickens with bacteriophage. Poult Sci 81: 437-41.
22. Carey Smith GV, Billington C, Cornelius AJ, Hudson JA, Heinemann JA (2006) Isolation and characterization of bacteriophages infecting Salmonella spp. FEMS Microbiol Lett 258: 182-186.
23. Lu Z, Breidt F, Fleming HP, Altermann E, Klaenhammer TR (2003) Isolation and characterization of a Lactobacillus plantarum bacteriophage, ΦJL-1, from a cucumber fermentation. Int J Food Microbiol 84: 225-235.