

The reason for undertaking the research presented below is the widespread occurrence of infections in humans and animals by pathogenic *E. coli* strains showing resistance to antibiotics and chemotherapeutics. The global problem of drug resistance among bacterial strains in humans and animals is the most important challenge for the control of bacterial infections in the 21st century. Bacterial resistance to antibiotics appeared as the result of the widespread use of growth promoters as feed additives and uncontrolled distribution for treatment of bacterial infections in humans and animals. Increasing antibiotic resistance among bacteria significantly limits effective treatment and control of infections. Difficulties in effective control of infections also result from that fact that nearly 65% of bacterial infections in humans involve a biofilm formed by *E. coli* bacteria, which has been detected as the main cause of many intestinal infections in humans. The failure of chemotherapeutics to treat bacterial infections in humans and animals has led to the search for alternative methods, among which phage therapies play an important role. In many cases they are the basis for new targeted treatments applied in humans or experimental treatments in animals, or they are a component of preparations used to eliminate pathogens from the environment or from food.

The aim of the research will be to isolate and characterize bacteriophages exhibiting bactericidal activity against pathogenic strains of *E. coli* isolated from poultry farming environments and from cases of diarrhoea in humans.

The planned research will include identification of *E. coli* strains isolated from humans with symptoms of diarrhoea and from poultry using standard microbiological techniques, i.e. cultures on basic media (BHI, LB) and selective differential media (McConkey, TBX, Karmali); determination of phenotypic traits; determination of the antibiotic resistance of the strains; and genotypic analysis of drug resistance based on the presence of selected resistance genes. The strains will also be assigned to phylogenetic groups based on detection of genes such as *chuA* and *yjaA* and fragment *TspE4.C2* and by testing adhesion capacity and biofilm forming capacity using classical methods and molecular PCR with particular emphasis on Whole Genome Comparative Analysis (WGS). Next, the bacteria will be differentiated on the basis of genetic profiles to confirm the toxicity of isolates based on the presence of specific virulence genes.

For all pathogenic strains of *E. coli* bacteriophages will be isolated from cattle faeces and their bactericidal properties for the strains will be assessed by a standard plaque test. Morphological analysis of the phages will be performed using a transmission electron microscope, and the type and size of the phages will be determined based on photographic documentation. Genetic similarity between phages will be assessed by complex analysis of genetic material using the molecular techniques of PCR, PFGE and MALDI-TOF mass spectrometry. The ability of bacteriophages to inhibit biofilm formation will be analysed, and the ability to eradicate a mature biofilm formed by pathogenic *E. coli* strains will be determined.

The proposed project includes innovative research involving the isolation and characterization of pathogenic strains of *E. coli* in terms of their drug resistance and virulence, as well as evaluation of the antibacterial activity of bacteriophages specific for the analysed pathogenic *E. coli* strains obtained from humans and poultry. The results will contribute to assessment and comprehensive characterization of phages and will be presented at international conferences and published in JCR-indexed journals with a high IF. In the case of 'new' phages, their genetic profile will be submitted to GenoMed databases. Another unquestionable effect will be the development of a methodology for evaluating the kinetics of the activity of bacteriophages in inhibiting the formation of a biofilm by pathogenic strains of *E. coli* and eradicating such biofilms, which will make it possible to submit a patent application to the Polish Patent Office.

Due to the lack of a species barrier, it will be possible to use the isolated bacteriophages as components of formulations for eliminating these pathogens in humans and animals, especially as many studies have confirmed that a mixture of bacteriophages in the form of a cocktail is much more effective at eliminating infections by destroying the bacterial biofilm. The simultaneous use of phage and antibiotic therapy has also proven to be much more effective at reducing the number of *E. coli* cells in a biofilm than an antibiotic alone. An additional measurable effect will be a database created in the form of our own collection of both pathogenic *E. coli* strains isolated from humans and cattle and the corresponding bacteriophages, which can be used by epidemiologists in alternative therapies in both human and veterinary medicine and also as components of disinfectants.