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Authors	Bolocan, Andrei Sorin;Callanan, Julie;Forde, Amanda;Ross, R. Paul;Hill, Colin
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University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

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Phage therapy targeting *Escherichia coli* – a story with no end?

Andrei Sorin Bolocan, Julie Callanan, Amanda Forde, Paul Ross, Colin Hill

APC Microbiome Institute, University College Cork, Cork, Ireland

Corresponding author

Colin Hill

E: c.hill@ucc.ie

T: +353-21-490-3000

F: +353 21 490 3000

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ABSTRACT

Bacteriophages (phages) or bacterial viruses have long been proposed as an alternative therapy against antibiotic resistant bacteria such as *E. coli*. Even though poorly documented in the scientific literature, a long clinical history of phage therapy in countries such as Russia and Georgia suggests potential value in the use of phages as antibacterial agents. *E. coli* is responsible for a wide range of diseases, intestinal (diarrhoea) and extra-intestinal (UTI, septicaemia, pneumoniae, meningitis), making it an ideal target for phage therapy. This

review discusses the latest research focusing on the potential of phage therapy to tackle *E. coli* related illnesses. No intact phages are approved in EU or USA for human therapeutic use, but many successful *in vitro* and *in vivo* studies have been reported. However, additional research focused on *in vivo* multi-species models and human trials are required if phage therapy targeting *E. coli* pathotypes can be a story with happy end.

INTRODUCTION

Bacteriophage (phage) therapy describes the therapeutic use of bacterial viruses to treat bacterial infections. The story of phage therapy first began over a hundred years ago when bacteriologists such as Hankin, Gamaleya, Twort and d'Herelle (Keen 2015) each made separate observations of anti-bacterial activities now believed to associated with viruses. A century later, the literature is still populated with articles detailing the promise of phage therapy either as an alternative to antibiotic therapy or for use in patients for whom drug treatments have already failed. A long history of apparently successful bacteriophage therapy for humans has been reported in Eastern Europe (ELIAVA Institute in Tbilisi, Georgia; Russia), but the studies are often insufficiently documented, preventing their rigorous evaluation (Sulakvelidze, Alavidze and Morris 2001). If we are to generate success stories in phage therapy, we must first understand the potential benefits and challenges associated with the field. For this review, we focus on phage therapy directed towards one of the one of the most common bacteria known to medicine, *Escherichia coli.*

E. coli is a Gram-negative bacterium of many diverse types, the majority of which are part of the normal flora of the intestine and are believed to be relatively harmless. However, some strains have evolved mechanisms of pathogenicity, meaning they can cause disease in humans and animals. Such diseases can be intestinal (diarrhoea) or extra-intestinal, (urinary tract infection, septicaemia, pneumonia and meningitis) (Cabal *et al.* 2016; Millar *et al.* 2016). Based on pathogenicity profiles (virulence factors, clinical disease and phylogenetic

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profile), *E. coli* causing intestinal disease have been divided into six pathotypes: Enteropathogenic *E. coli* (EPEC), Enterohaemorrhagic *E. coli* (EHEC), Enteroinvasive *E. coli* (EIEC, including *Shigella* sp), Enteroaggregative *E. coli* (EAEC), Enterotoxigenic *E. coli* (ETEC) and Diffusely Adherent *E. coli* (DAEC). Two further pathotypes have also recently emerged, the Adherent Invasive *E. coli* (AIEC), often associated with inflammatory bowel disease (IBD) disease, and the Shiga toxin (Stx)-producing Enteroaggregative *E. coli* (STEAEC) (Mora *et al.* 2011; Agus *et al.* 2014; Conte *et al.* 2014). Extra-intestinal *E. coli* are separated into groups determined by disease association, including uropathogenic *E. coli* (UPEC), neonatal meningitis-associated *E. coli* (NMEC), and sepsis-causing *E. coli* (SEPEC) (Clements *et al.* 2012).

Current antibiotic treatments used to treat drug-resistant or/and adherent-invasive *E. coli* can result in severe alterations to an individual's microbiota as well as continuous relapses of disease (Langdon, Crook and Dantas 2016). As an alternative to antibiotics, phages have regained increasing attention. Therapeutic approaches typically use individual phages or cocktail of phages to specifically infect and kill target bacteria. Because of their limited species-specific host range, bacteriophages have the potential to impact pathogenic bacteria without causing collateral damage to commensal microbiota (Brüssow 2005; Tomat *et al.* 2013; Niu *et al.* 2014; Khan Mirzaei and Nilsson 2015). While the potential of phage therapy cannot be denied, the emergence of phage-resistant bacteria cannot be overlooked (Sulakvelidze, Alavidze and Morris 2001). An approach using a "phage cocktail" of multiple phage types with different host range specificities can help to alleviate potential problems with phage resistant 'mutants' (Dalmasso *et al.* 2015; Chadha, Katare and Chhibber 2016; Melo *et al.* 2016). Different mathematical models provide information on the maximum therapeutic effect of a phage population and quantitatively examine mutation rates of phage resistant bacteria (Kysela and Turner 2007; Beke, Stano and Klucar 2016). But phages can

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also mutate, such that they then successfully eliminate the bacterium that acquired resistance in the first place, conferring a potential advantage to phage therapy over antibiotic treatment (Dufour *et al.* 2015).

E. coli phages (coliphages) are commonly isolated from sewage, hospital waste water, polluted rivers and faecal samples of humans or animals (Song *et al.* 2007; Jamalludeen *et al.* 2009; Walker, Clokie and Kropinski 2009; Dalmasso *et al.* 2016; Snyder, Perry and Yousef 2016). Reports on phage therapy targeting various pathogenic *E. coli* have been described in the literature (Dufour *et al.* 2015, 2016; Sarker *et al.* 2015) and this review presents some of the recent findings of trials specifically targeting ExPEC, intestinal AIEC and EHEC.

BACTERIOPHAGE TARGETING EXTRA-INTESTINAL PATHOGENIC *ESCHERICHIA COLI* (EXPEC)

Extra-intestinal pathogenic *E. coli* (ExPEC) causes a diverse range of clinical diseases (bacteraemia, meningitis, urinary tract infections), and global morbidity and mortality rates due to ExPEC infections are on the rise.

Extra-intestinal uropathogenic *E. coli* (UPEC) is the leading cause of urinary tract infections (UTIs), and is responsible for approximately 75% of cases of over 150 million clinical cases reported annually (Kakkanat *et al.* 2015). The highest prevalence is in females, with 40-50% developing a UTI in their lifetime, compared to only 5% of males. UTIs are usually managed with antibiotic therapy, but over the years, antibiotic-resistant strains UPEC have emerged. The formation of biofilms further complicates treatment options (Chibeu *et al.* 2012; Soto 2014), encouraging more research into phage therapy against UPEC's.

Perepanova *et al.* (1994) described the efficiency of both local and oral administration of a multi-species bacteriophage cocktail to treat patients with acute and chronic urogenital inflammation, but the study is poorly documented for use as a model for human phage

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therapy. Chibeu *et al.* (2012) recently assessed the ability of three phages to eradicate UPEC biofilms and in 2016, Sybesma *et al.* described the lytic activity of commercial bacteriophage cocktails on *E. coli* and *Klebsiella pneumoniae* strains isolated from UTI patients. They also demonstrated the potential of 'bacteriophage adaptation' experiments to increase the lytic activity of bacteriophage cocktails. Also in 2016, Galtier *et al.* isolated three virulent phages from wastewater that target an antibiotic resistant strain of UPEC. Phage efficacy was characterised both *in vitro* and *in vivo*, and it was found that a single dose of the three-phage cocktail was able to dramatically reduce gut carriage of UPEC. These studies are among many that hold promise for the potential of bacteriophage therapy for the treatment of *E. coli* acquired UTI's.

The UPEC pathotype *E. coli* ST131-025b:H4 has been identified as a pandemic strain with potential worldwide spread and serious implications as a nosocomial infection, which has been recorded in cystitis as well in life-threating meningitis. Part of the B2 phylogenetic group, *E. coli* ST131-025b:H4 has a large number of virulence factors which are also linked to high levels of resistance to β-lactams and fluoroquinolones. The plasmid-mediated colitis resistant gene (*mcr-1*) has also been recently associated with ST131(Hasman *et al.* 2015). Pouillot *et al.* (2012) first reported phage therapy against a ciprofloxacin resistant clone of ST131-025b:H4 (designated *E. coli* S242) isolated from a neonate with fatal meningitis. A phage ($EC200^{PP}$) was isolated from sewage water from France, and which belongs to the *Podoviridae* family of phages, was able to treat sepsis and meningitis infections induced by drug-resistant *E. coli*. *In vivo* activity of phage $EC200^{PP}$ was not hampered by phage-resistant mutants of *E. coli* S242. Pharmacokinetic studies of phage $EC200^{PP}$ also showed it to be stable in urine.

Dufour and his colleagues (2016) provided evidence of the efficiency of bacteriophage LM33-P1, that exclusively infects O25b *E. coli* strains, which are highly resistant to

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betalactams and fluoroquinolones. Bacteriophage LM33-P1 is very efficient *in vitro* (short eclipse and latent periods of 7 and 9 minutes respectively, burst size 320 pfu, fast adsorption) and also *in vivo*, based on different animal models (pneumonia, septicaemia and UTI). Dufour *et al.* (2016) also showed that the mutually exclusive and specific interaction of phage LM33-P1 with O25b strains was lipopolysaccharide (LPS)-dependent.

ExPECs have been associated with ventilator-associated pneumonia (VAP), a common life-threatening hospital-acquired infection (Messika *et al.* 2012). The most predominant phylogenetic group is B2, with strains possessing a large number of virulence factor genes such as *sfa* and *iroN* that are essential for adhesion and iron uptake (Messika *et al.* 2012; Dufour *et al.* 2015). The use of bacteriophage to treat VAP offers an interesting alternative to conventional antibiotic therapies (Lynch, Clark and Zhanel 2013). In 2015, Doufour's team published the first study on the efficient treatment of *E. coli*-induced pneumonia with two bacteriophages (536_P1 and 536_P7) isolated from sewage. They showed that a combination of antibiotic treatment and phage therapy resulted in a 100% survival rate in VAP infected mice. The majority of mice were not rescued by treatment with phage 536_P7 alone, but adaptation of this phage toward the VAP-causing *E. coli* strain resulted in a variant which significantly improved treatment efficacy *in vivo*. The study of Cao *et al.* (2015) supported the efficacy of bacteriophage treatment of pneumonia caused by multi-drug resistant *K. pneumoniae*. The lytic phage, KLPN1, was shown to infect and lyse capsular type K2 strains, and genome sequence analysis revealed the phage to encode proteins (e.g. lysins, holins) that have potential applications in phage-associated therapies.

BACTERIOPHAGE TARGETING ADHERENT INVASIVE *E. COLI* (AIEC)

Bacteriophage therapy targeting adherent invasive *E. coli* (AIEC), long suggested as an etiological agent of Crohn's disease (CD), is gaining much academic and commercial interest

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as a therapeutic approach for the treatment of inflammatory bowel disease (IBD). While the role of AIEC in IBD is still subject to controversy, it does seem to have a higher prevalence in patients with IBD, as determined from faecal and biopsy samples (Glasser *et al.* 2001; Conte *et al.* 2014; da Silva Santos *et al.* 2015; O'Brien *et al.* 2016). It adheres to the ileal epithelium, invades the lamina propria and then proliferates within macrophages. While AIEC is a candidate target for antibiotic treatment of patients with Crohn's Disease, the downside is that antibiotics may also target the beneficial microflora of the gut. While examples of phage therapy targeting IBD are sparse, it is interesting that bacteriophages, especially those from *Caudovirales* family, have been identified in a higher abundance in gut and ileal biopsies of CD patients (Jin, Zhang and Sun 2014), perhaps providing a potential basis for improvements in IBD diagnoses. Danglas and Debarbieux (2014) described eight different phages isolated from sewage which infect AIEC strain LF82, which is the reference strain for IBD studies. In a murine model with a derivative of LF82 engineered to have resistance to streptomycin and kanamycin (LF82KS), the levels of LF82(KS) recovered from stool were significantly lower when a cocktail of the eight phages was introduced. In 2014, Tsui, Jacobs and Braun.set out to prove the specificity of five AIEC phages, with the finding that each of the phages effectively targeted both non-adherent and adherent *E. coli*. When a combination of the phages was tested, it did not prove to be any more effective than the most potent of the phages alone, and the researchers are currently aiming to find AIEC mutants that have acquired resistance to the phages.

Whilst preliminary data are promising for the place of phage therapy in IBD, one of the major outstanding issues is to specifically identify the correct target. In addition to AIEC, over-represented bacteria in IBD have included *Mycobacterium avium* ssp. *paratuberculosis, Clostridium difficile*, *Campylobacter concisus*, *Salmonella* spp. and *Enterococcus* spp. Furthermore, the development of appropriate pre-clinical models for phage therapy studies

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relating to IBD are challenging because in many cases there is a difficulty in achieving efficient colonisation of the model with the test bacterium (Macfarlane, Steed and Macfarlane 2009; Jin, Zhang and Sun 2014; Naser *et al.* 2014; Dulai *et al.* 2015; Zhang 2015). Multi-species mouse models are therefore recommended for advancing phage therapy utilisation in IBD (Sartor and Mazmanian 2012; Eun *et al.* 2014).

BACTERIOPHAGES TARGETING THE ENTEROHEMORRHAGIC PATHOTYPE OF *E. COLI* (EHEC)

Intestinal pathogenic *E. coli* are generally divided into those that cause diarrhoea by expressing heat-labile or heat-stable toxin (enterotoxigenic *E. coli* (ETEC)) or Shiga toxin (Shiga toxin-producing *E. coli* (STEAEC), including enterohemorrhagic *E. coli* (EHEC). The most common EHEC, *E. coli* O157:H7, is usually community-acquired via contaminated food or water end is well known as a causative agent of diarrhoea and urinary problems (haemolytic uremic syndrome) in humans. It is evident that phage treatment of O157:H7 is not without its challenges, with only a modest 10-fold reduction in O157:H7 reported, even when phages were applied at a high multiplicity of infection (Sheng *et al.* 2006). In a separate trial, Sarker and team (2015) found that the oral application of phages to children hospitalised with acute diarrhoea failed to improve the diarrhoeal outcome, possibly due to insufficient ability of the phages to target the wide range of *E. coli* genetically variance. In addition, it was not clear whether *E. coli* was in fact responsible for the diarrhoea, as stool samples were largely dominated by faecal streptococci. Reduced effectiveness of phage titres following passage through gastric acid was identified as another possible reason for the trial failure. Furthermore, possible differences between physiological state of *E. coli* in faeces and in the intestine, and a low faecal titre of the pathogen, may not have allowed the replication of *E. coli* phages. These results confirm that far more knowledge is needed on *in vivo* phage-

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bacterium interactions if we are to design effective phage therapy trials. On a positive note, the coliphages administered during the trial were found to safely transit the gut, helping to demonstrate the safety aspects of phage therapy.

CONCLUSIONS

Future studies focusing on phage therapy should answer the following questions: Is phage therapy efficient *in vivo* against a pathogen as complex as *E. coli*, with its high versatility and ability to change rapidly in time and space? Or should the concept of phage therapy against *E. coli* be consigned to history? Despite some of the challenges presented, the data is still compelling for future applications of *E. coli* phage therapy, based on successful *in vitro* and pre-clinical *in vivo* demonstrations. However, we need robust data from more human clinical trials to fully assist development of phage therapy against the various *E. coli* pathotypes described.

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