Using Antibodies, Probiotics, Phages To Pressure Pathogens

Antibiotic resistance helps to drive efforts to evaluate these biologically based items as alternatives for combating infectious agents

Harald Brüssow

Although antibiotics remain the principal therapeutic approach to treating bacterial infections, because of increasing antibiotic resistance among pathogens, some governmental agencies such as the Canadian Institutes of Health Research are urging more efforts to seek alternatives to conventional antibiotics. Some researchers are studying novel antimicrobial compounds from plant or other sources, while others are exploring whether synthetic chemicals will work against new target proteins from pathogenic bacteria.

In particular, researchers in the food industry are intensively investigating alternative approaches to preventing or treating infectious diseases. One focus in the food industry is to develop new means for reducing the burden of pathogens that may contaminate products. For instance, although phages might not eliminate pathogens, they can help to change the equilibrium in a particular environment, putting pathogens at a disadvantage.

Another strategy is to introduce commensal bacteria to replace other species, thereby gaining more sustained control over a particular pathogen. Providing an individual with even temporary relief from an infectious agent might allow the immune system to control and then eliminate the pathogen. In addition, researchers are studying whether there are ways to control common hospital infections, such as those caused by *Staphylococcus aureus*, which often carries high levels of antibiotic resistance.

Summary

- Researchers in health care and the food industry are intensively investigating alternative approaches to preventing or treating infectious diseases, particularly those agents that cause diarrhea.
- Cow-produced antibodies proved effective in treating children with diarrhea caused by rotaviral but not bacterial infections.
- The use of probiotics as a bacterial replacement therapy appears to be moving toward clinical use but may entail deploying probiotics that target only specific infectious agents.
- Phages appear to have great potential as biological antimicrobials—in part because, unlike antibiotics, phages are species specific and thus do not damage commensal bacteria.

Roots of the Quest for Alternative Means for Combating Pathogens

The idea for biologically based alternatives to what became conventional antibiotics goes back to the late 19th century. Paul Ehrlich championed this approach, despite his role as a pioneer of chemotherapeutic agents. He is perhaps best known for developing arsenic compounds against syphilis. Its medical success was so impressive that he spoke of a "zauberkugel," or magic bullet, which then was no exaggeration considering the historical impact of syphilis. In addition, Ehrlich was a founder of immunology—demonstrating, for example, that passive antibodies are transferred from mother to infant by breast feeding. He also prepared animal antisera against bacterial toxins for use in treating toxin-mediated infectious diseases.

Ehrlich shared the Nobel Prize in 1908

Harald Brüssow is a Senior Research Scientist at the Nestlé Research Centre Nutrition and Health Department/Food and Health Microbiology, Lausanne, Switzerland.

FIGURE 1

Diarrheal diseases are the second major cause of morbidity and mortality in children from developing countries. This picture from the world's largest diarrhea research clinic in Dhaka/Bangladesh vividly illustrates the extent of the problem. (Photo: Dr. Sarker/ICDDR,B.)

with Elie Metchnikoff, who discovered phagocytosis. Late in his life, Metchnikoff turned his attentions to lactic acid bacteria as probiotics. He reasoned that probiotic bacteria, carrying out useful fermentations instead of putrefaction in the gut, could have beneficial effects, including increasing human longevity.

Another early champion of biologically based approaches to combating infectious diseases was Félix d'Herelle, who codiscovered bacteriophages. Unlike Metchnikoff and Ehrlich, d'Herelle was both a scientist and an entrepreneur. In 1919, he began using bacteriophage to treat patients with dysentery. Since that time, phages have had a colorful medical history. However, despite enjoying some popularity during the 1930s, they did not attract much interest from the pharmaceutical industry, possibly because this sector developed more from chemistry than biology.

Potential Advantages in Using Alternatives to Conventional Antibiotics

Antibiotics are chemicals that target specific biochemical reactions or structural features in bacteria that differ from those found in human or other host species. Penicillin, for example, interferes with bacterial cell wall synthesis, a structure and process that are absent in humans. Even so, using penicillin to treat infections has drawbacks because the drug also affects nontarget commensal bacteria. Moreover, penicillin causes allergic reactions among some individuals.

Further, bacterial pathogens readily develop resistance to penicillin through at least one of four independent mechanisms—namely, import block, export facilitation, antibiotic modification, and enzyme changes. Each of these mechanisms arises through relatively simple changes, involving genes that predate the medical use of antibiotics. Resistance development is further facilitated because some of those genes may be carried on mobile elements such as plasmids and transposons that can shuttle the resistance genes among bacterial species. Another important problem is that antibiotic-resistant pathogens typically maintain their virulence.

Faced with such challenges, it makes sense to evaluate biologically based alternatives to antibiotics, including antibodies, probiotics, and phages. Each of these classes of antimicrobial materials has been actively antimicrobial over millions of years, presumably gaining ground against pathogens during this coevolutionary period.

Take probiotics, which can be defined as health-promoting bacteria. These commonly commensal bacteria typically colonize the same anatomic sites where facultative pathogens also gain a foothold. The outcome of whether a pathogen or probiotic species prevails when competing for such niches typically will depend in large part on the expression of complex gene sets rather than any single advantageous gene. Ecological success therefore is the cumulative effect of many genes working in combination.

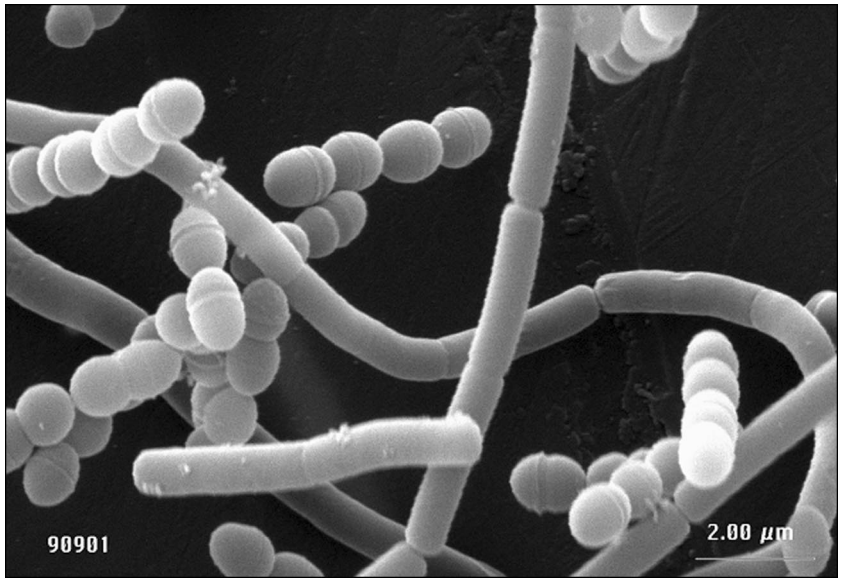
Because commensal bacteria compete along several lines with a pathogen, this set of imprecise interactions makes it difficult for microbiologists to precisely define probiotic activity. Nonetheless, using probiotics can put pathogens under pressure from multiple sides, making it difficult for them to evade this competition through simple mutations that affect only one or

a few biochemical reactions. Even if the pathogen can adapt to such competitive pressures, it might be pushed along a different trajectory, becoming less virulent.

Components of the immune system provide another means for withstanding pathogens. For example, neutralizing antibodies typically will bind to specific surface structures or secreted proteins from pathogens, subjecting them to immune selection pressures.

Yet another means for dealing with pathogens depends on bacterial phages that can be selected to recognize particular bacterial virulence factors such as capsular polysaccharides as receptors. In therapy trials in a veterinary setting, phage were used to treat calves with severe *Escherichia coli* diarrhea. This phage recognized the K1 capsular antigen as cellular receptor. Very quickly during the course of that trial, phage-resistant clones appeared in bacterial samples from the treated animals. The *E. coli* pathogen acquired resistance simply through loss of the K1 capsule antigen. However, because this antigen serves as a virulence factor, the phage-resistant clones proved less virulent for the calves.

FIGURE 2



The concept of probiotic bacteria was historically developed from lactic acid bacteria used in milk fermentation. The picture shows *Lactobacillus bulgaricus* (long rods) and *Streptococcus thermophilus* (chains of cocci) used in the production of yogurt. In dairy products large amounts of live bacteria are thus naturally ingested by humans and have been discussed in relation with human health. (Photo: M. Rouvet, NRC.)

Cow-Produced Rotavirus Antibodies Move into Clinical Testing

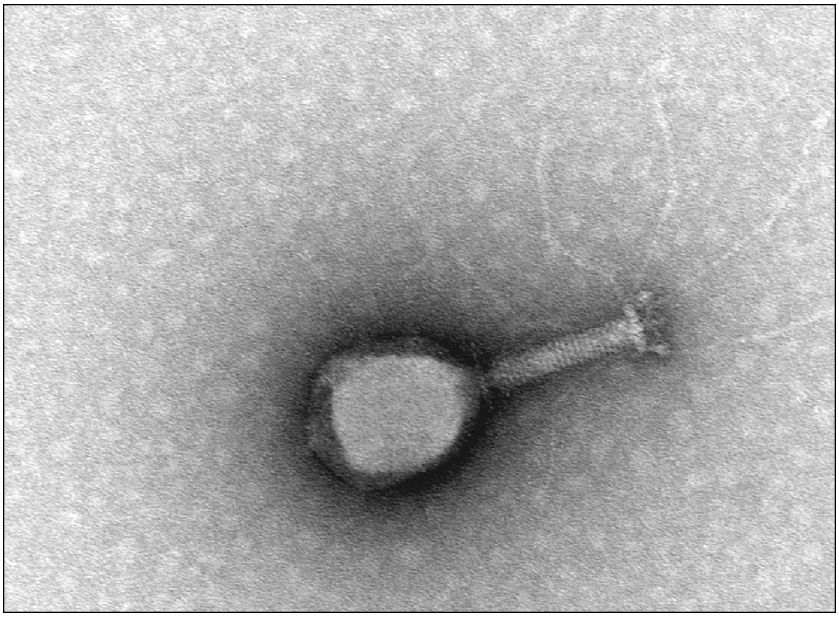
Before biologically based antimicrobials can be considered commercially viable treatments for infectious diseases, they must be evaluated in clinical trials. The most extensively studied examples of biological approaches are those for treating infectious agents that cause diarrhea, which is the second most frequent cause of childhood mortality in developing countries. Although many different kinds of microbes can cause diarrhea, *E. coli* and rotavirus infections are the leading causes of diarrhea among children. *E. coli* is also the prime cause of travelers' diarrhea among adults.

Rotaviruses are also a major cause of diarrhea in newborn calves, which carry no maternal antibodies in their blood and depend on receiving them in the early milk, or colostrum, from mother cows. Because cows were subject to rotavirus infections as calves, the mature animals are immunologically primed and thus are a promising choice as a source for antibodies that could be used for treating diarrhea-causing infections not only in calves but also in humans. This approach to producing alternative treatments is also noteworthy because it is based on common dairy-farming and milk-processing practices that are widely available, even among countries within the developing world. Because milk is a conventional food, its use as a dietary supplement, albeit containing additional and specific antibodies, raises no major safety concerns.

From a production standpoint, this approach looks practical. For example, when vaccinated, cows produce substantial amounts of neutralizing rotavirus antibodies in colostrum—specifically, 10 kg of polyclonal IgG antibody from 1 ton of milk. Because these IgG antibodies provide passive immunity through transfer to calves, they prove remarkably stable during gut passage, even in children.

How well do cow colostrum-borne antibodies perform when tested in clinical trials? Milk-produced immunoglobulin preparations were

FIGURE 3



Portrait of an obligate bacterial killer: this T4-like bacteriophage of *Escherichia coli* was isolated from the stool of a pediatric diarrhea patient in Dhaka. Note the six tail fibers in their search position for a target cell. The fibers are fixed to the baseplate, the "landing module" of this virus. (Photo: M.-L. Dillmann and S. Chibani-Chennoufi, NRC.)

well tolerated among individuals who received milk containing increasing anti-rotavirus titers. Patients with rotavirus diarrhea who received milk from unvaccinated cows did not improve. However, when children with rotavirus diarrhea were treated with a high-titered milk antibody preparation produced in cows vaccinated with rotavirus, their rotavirus infections were at least partly attenuated.

The high-titer preparation was tested subsequently in a double-blinded placebo-controlled clinical trial, following World Health Organization (WHO) criteria, such as quantitative stool output. The trial focused on children who were hospitalized because of severe rotavirus-caused diarrhea, and it was conducted at the The International Centre for Diarrhoeal Disease Research in Dhaka, Bangladesh, a world-class facility that treats more than 100,000 such patients each year.

From the first day that the young patients began receiving milk containing the rotavirus antibodies, they experienced a significant decrease in stool output, whereas those patients in the control group experienced no such change. Rotavirus clearance from the stool and the recovery of the patients also were significantly accelerated among antibody-treated children.

However, in a similar effort involving major O serotypes of diarrhea-causing *E. coli*, such treatments were not effective, at least among children. In this case, milk cows were vaccinated with killed whole bacterial cells supplemented with toxin antigens, leading the animals to produce high antibody titers in their milk. When milk containing matched antibodies was administered to volunteers who were infected with a single diarrhea-causing *E. coli* serotype, their diarrhea subsided. However, these same antibody-containing milk preparations were not effective when tested in children with *E. coli*-induced diarrhea, including those cases in which the milk antibody specifically matched the *E. coli* serotype.

Probiotic Bacteria

"Probiotics appear to be a useful adjunct to rehydration therapy in treating acute, infectious diarrhea in adults and children," according to a meta-analysis that was published by the Cochrane Library several years ago (http://www.cochrane.org/reviews/en/ab003048.html). Nonetheless, "more research is needed to inform on the use of particular probiotic regimens in specific patient groups."

For example, based on several European clinical trials, bouts of diarrhea were reduced among patients who were infected with rotavirus when they consumed *Lactobacillus rhamnosus* strain GG. However, similar clinical trials in South America failed to show a beneficial effect. Similar observations were made in prevention studies.

A different picture emerged when 230 children from Bangladesh, hospitalized with acute diarrhea, were treated orally with *L. paracasei* strain ST11. This strain had no significant effect on patients who were infected with rotavirus. However, it had a significant impact reducing stool frequency and output among patients with "non-rotavirus" diarrhea from the first day of treatment. These patients also recovered more quickly than did those who received no probi-

otic. The clinical data suggest distinct probiotic actions for these two *Lactobacillus* strains.

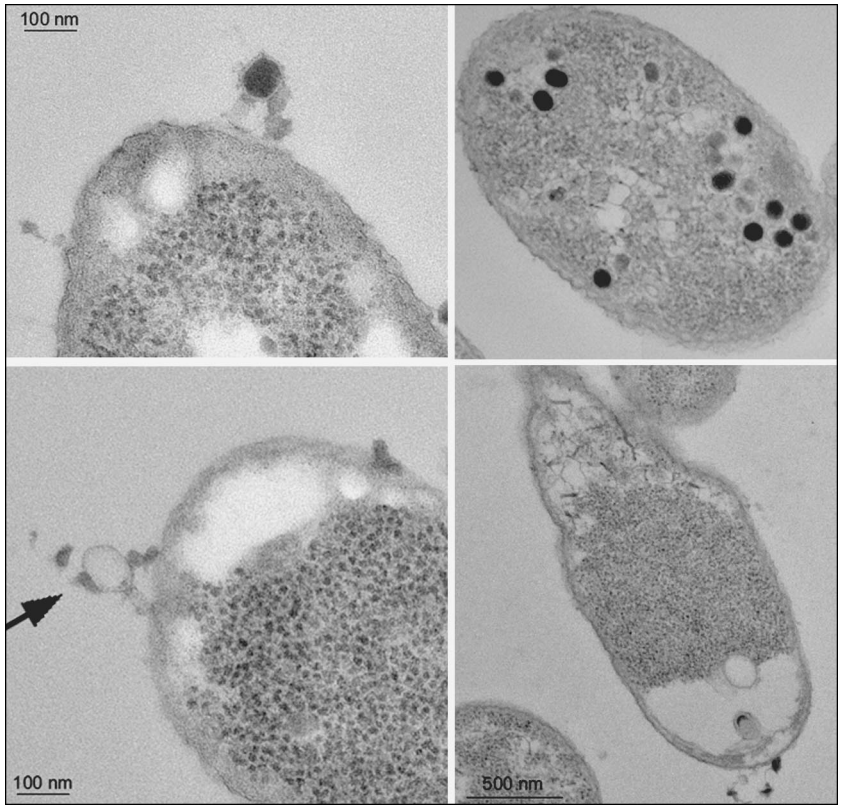
Probiotics also look promising when used against infections in the oropharynx and the nasopharynx. For example, researchers report an inverse relationship between colonization with commensal α-streptococci and the presence of group A streptococci in the throat. In a Swedish trial, patients with tonsillitis who received both antibiotics and a spray application of commensal streptococci showed reduced recurrence of infection compared to patients who received only antibiotics.

In other studies, researchers found an inverse relationship in the nasopharynx between commensal α-streptococci and *Haemophilus influenzae*, whose presence increases the risk of acute otitis media. In clinical trials, when otitis media patients were treated with antibiotics plus commensal α-streptococci application, the recurrence rate of their infections was reduced significantly compared to patients who received only antibiotics. However, α-streptococci had no effect for those patients who were not treated with antibiotics, indicating the commensal alone could not chase the pathogen from its niche.

Bacterial replacement therapy appears to be moving beyond its interesting theoretical basis and toward use that is backed by evidence from clinical trials. However, despite claims of multiple beneficial effects from single probiotic strains, recent clinical trials suggest that specific probiotics will be useful against only specific infections.

Despite that restriction, however, the potential of probiotics is substantial. For instance, lactobacilli can protect the natural microbiota of the vagina against *Candida* and other urogenital infectious agents, with several clinical trials providing evidence of their potential. In practical terms, the dairy industry has substantial experience producing lactic acid bacteria on a massive scale in fermented milk products such as yogurt, meaning it will be easy to meet future demands for this important constitutent of human commensal flora.

FIGURE 4



T4 phage infection of E. coli. Left: T4 phage with full (top) and empty (bottom) head adsorbed to an *E. coli* cell. Right: Intracellular production of progeny T4 phage within an infected cell (top) and the burst of an infected cell (bottom). T4 phages are found in environmental water, stool, and sewage samples; humans are thus naturally exposed to viable T4 phage. (Photos: M.-L. Dillmann and M. Weiss, NRC.)

Despite Skepticism, Potential for Phage Therapy Appears Substantial

Phages appear to have great potential as biological antimicrobials. Most bacterial pathogens have phages. Furthermore, phages are easily isolated from the environment, can be produced cheaply, and have a clear-cut mode of action, namely lysis of the target cell following their own intracellular replication. Moreover, the biotechnologically produced phage protein lysin by itself can kill target cells from the outside.

Unlike antibiotics, phages are very species specific, avoiding collateral damage to non-target bacteria. In the absence of target bacterial species, phages are quickly eliminated from the body. However, when the target species is present above a threshold titer, phage is a self-amplifying antimicrobial, providing a pharma-

cological property that is not found in ordinary drugs.

In parts of Eastern Europe, phages are being used as a standard therapy for treating diarrheal diseases and wound infections. For instance, in Russia, standardized phage preparations are sold over the counter and have been used widely—and safely—for more than 20 years.

In a large clinical trial that was conducted in 1963 in the Republic of Georgia when it was still part of the Soviet Union, orally administered phage significantly reduced the incidence of dysentery and diarrhea. However, neither the details of those clinical trials nor the composition of the phage cocktails was ever documented. Absent more concrete evidence, there is widespread skepticism as to whether phage is as effective as claimed.

Meanwhile, the evidence for phage therapy being effective seems on firmer ground in veterinary settings, and traces to seminal studies from the 1980s of *E. coli* diarrhea in calves by the late Williams Smith, who was from the Houghton Poultry Research Station in England, and his collaborators. At the time, their efforts failed to arouse much interest from industry.

More recently, several biotechnology companies began studying the use of phage as a means for preventing foodborne infections by several bacterial pathogens, including *E. coli* O157:H7, *Campylobacter*, *Salmonella*, and *Listeria*. For example, officials of the Food and Drug Administration in 2006 approved a *Listeria* phage cocktail to use with meat products (*Microbe*, October 2006, p. 455). That approval boosted food industry interest in phages. This industry already had a good knowledge base on phages since they are the major cause of food fermentation failures. Several food companies therefore conduct phage research programs.

However, what is the potential of phages in clinical medicine generally and, specifically, for treating *E. coli* diarrhea in children? T4-like phages from stool specimens of patients with diarrhea show broad in vitro lytic activity against diarrhea-associated *E. coli* O serotypes. Sequencing analysis indicates these T4-like phage carry no genes that make them virulent in humans.

Thus T4 is the prototype "lytic" phage that only kills its target bacterium, digesting the bacterial genome to obtain nucleotide precursors for phage DNA synthesis without exchanging genes with its bacterial host. This behavior is important since many lambdoid phages carry bacterial virulence genes. Moreover, temperate phages should be avoided for phage therapy purposes because, during its lysogenic phase, the phage integrates its genome into the host bacterial chromosome. There is good evidence that sequential prophage acquisitions shaped human pathogens, such as *Streptococcus pyogenes*, enabling them to acquire phage-encoded virulence genes.

When T4 phage were fed either to mice or to human volunteers, the phage survived gastrointestinal passage without causing observable adverse health effects. In both cases, T4 phage did not decrease fecal *E. coli* counts, meaning its passage through the gut does not lead to collateral damage of the commensal microbiota. Controlled phage treatment trials are pending.

SUGGESTED READING

Allen, S. J., B. Okoko, E. Martinez, et al. 2005. Probiotics for treating infectious diarrhea. The Cochrane Library. Wiley and Sons, Chichester, p. 1–65.

Brüssow, H., C. Canchaya, and W.-D. Hardt. 2004. Phages and the evolution of bacterial pathogens: from genomic rearrangement to lysogenic conversion. Microbiol. Mol. Biol. Rev. **68**:560–602.

Brüssow, H. 2005. Phage therapy: the *Escherichia coli* experience. Microbiology **151**:2133–2140.

Brüssow, H. 2007. The quest for food: a natural history of eating. Springer Scientific Publisher, New York.

Kutter, E., and A. Sulakvelidze. 2004. Bacteriophages: biology and applications. Taylor and Francis, CRC Press.

Sarker, S. A, T. H. Casswall, D. Mahalanabis, et al. 1998. Successful treatment of rotavirus diarrhea in children with immunoglobulin from immunized bovine colostrum. Pediatr. Infect. Dis. J. **17**:1149–1154.

Sarker, S. A., S. Sultana, G. J. Fuchs, et al. 2005. *Lactobacillus paracasei* strain ST11 has no effect on rotavirus but ameliorates the outcome of nonrotavirus diarrhea in children from Bangladesh. Pediatrics **116:**e221–228.

Smith, H. W., M. B. Huggins, and K. M. Shaw. 1987. The control of experimental *Escherichia coli* diarrhoea in calves by means of bacteriophages. J. Gen. Microbiol. **133**:1111–1126.

Tacket, C. O., G. Losonsky, H. Link, et al. 1988. Protection by milk immunoglobulin concentrate against oral challenge with enterotoxigenic *Escherichia coli*. N. Engl. J. Med. **318**:1240–1243.

Wilson, M. 2005. Microbial inhabitants of humans: their ecology and role in health and disease. Cambridge University Press.