

Another group of phages that are unacceptable for phage therapy is a large group of temperate phages – transposable phages. One of the possible scenarios of phage evolution during phage therapy, which can affect human health, may be associated with the activity of transposable phages. The first phage transposon active on *P. aeruginosa* – phage D3112 – was described long ago [2], but the study of phages of this species still reveals unexpected effects, including an amazing ability to integrate into its own genome [3]. Another feature of this phage is the peculiarity of its expression during interspecies transfer. When transferring the D3112 genome in the hybrid plasmid RP4 :: D3112 to *E. coli* (strain B), the *E. coli* exconjugants which received RP4::D3112 retained viability only at 42°C (at subfibril temperature), and when the incubation temperature was lowered to 30°C these cells died, forming filamentous bacteria [4,5]. It is difficult to predict how the properties of the clinical *P. aeruginosa* variants or other bacterial species could change and what effects may result from the interspecies migration of a hybrid plasmid with the transposable phage during a real bacterial infection. With the loss of the RP4 plasmid, *E. coli* clones persistently retain the D3112 genes, as a result of prophage transposition into a bacterial chromosome.

The possibility of interactions of phages of different species and different bacteria, as well as the long-term evolutionary consequences of this, have not yet been investigated. In this regard, it is necessary to be extremely cautious in treating humans with some unusual lytic variants of transposable phages. Thus, the proposed use of phage PA10 [6] – which is closely related to phage D3112 (90% of similarity) and exhibits lytic activity against pathogens of several different species – could lead to unexpected and potentially dangerous results.

From our point of view, ensuring mandatory security in phage therapy remains the most important challenge. Therefore, it is necessary to exercise some caution in the use of multispecies phage mixtures. In phage therapy, we think that it is more secure to use sequential applications of mono-species phage preparations [7].

Work is currently under way in Western Europe to introduce the ‘magistral phage’ system for the therapeutic use of phages in medical practice [8]. One of the obvious and fair objectives of this system is to make phage therapy more accessible for the individual treatment of patients. The technology of the ‘magistral phage’ therapy differs from the phage therapy model used, for example, in Russia, primarily in that an acceptable phage preparation is selected from the existing set of industrial phage samples (see above) – which does not require a long individual selection of phage and thus significantly reduces the costs.

The previously proposed suggestion that phages can be used by introducing them into the blood stream, using modified phage  $\lambda$  (active on *E. coli* strains) [9], also requires further detailed studies – in particular, concerning the need to expand the spectrum of lytic activity in relation to other species of pathogenic bacteria.

Since the efficacy of phage therapy depends on the conditions under which phages are used, and the type(s) of phage(s) used, the acceptability of phages for therapy (from the point of view of patient safety and possible influence on the evolution of pathogenic bacteria) will inevitably involve further research.

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## Forum

### Early-Life Microbiota Perturbations and Behavioral Effects

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**The maternal environment, during the prenatal and postnatal periods, is a determinant of offspring development and health. Perturbations during these periods can affect maternal behaviors and maternal-infant bonding, and also impair transmission of maternal microbiota to the offspring. Impaired microbiota has been associated with alterations of offspring cognitive development and behavior.**

### Microbiota Functions in Human Health

Microbiota refers, collectively, to the microbes that have coevolved with a host; their



collective genomes are known as the microbiome [1]. The microbiota colonizes different body epithelia, and the gut microbiota plays roles in digestion, the production of essential vitamins, the maintenance of epithelial integrity (e.g., with butyrate), and immune system modulation. Numerous factors can impact the composition of the gut microbiota, including host genetics, dietary variations, infection, disease, and drugs. Most recently, it has been demonstrated that gut bacteria cross-communicate with the neuroendocrine and immune systems to effect a wide range of social and affective behaviors, including aggression and anxiety- and depression-like behaviors and brain development [1].

Common practices, such as delivery by cesarean section, early exposure to antibiotics, and lack of breastfeeding, impair maternal microbiota transmission, and early perturbations of the microbiota lead to immune, metabolic, and behavioral disorders [2]. Here, we explore knowledge on the role of the microbiota on maternal behavior and physiology and on offspring neurodevelopment.

### Factors Affecting Maternal Behavior and Physiology

Maternal–infant bonding is universal and is fundamental in mammals. The oxytocin neuroendocrine system plays a key role in the initiation of maternal behavior following birth and also has a role in social bonding and sexual reproduction. Administration of oxytocin antagonists immediately after parturition results in impaired maternal behavior, and it is known that the periventricular nucleus is involved [3]. In addition to maternal recognition of offspring, oxytocin releases aides in parturition and milk production. During the peripartum period, physiological and behavioral changes are under the influence of oxytocin, vasopressin, and prolactin in both the mother and her pups [4].

In rodents, maternal behavior is dependent on stimuli due to the presence of the

pups. Studies suggest that dams identify their pups through scent, which can be produced from microbial metabolic activities (e.g., production of volatile compounds [5]); thus, factors impacting the microbiome might also affect recognition. If new mothers are separated from their pups, and are not permitted to have any form of interaction, their maternal responsiveness declines over the first postpartum week [6]. When pups born to mothers who exhibit high maternal behavior (such as licking, grooming, and arched-back nursing), with high oxytocin levels, were fostered to mothers with low maternal behaviors, the foster mother did not improve her low maternal behavior [7]. Conversely, foster mothers with high maternal behaviors maintain these behaviors when fostering pups born from mothers with low maternal behavior [7]. In subsequent generations cross-fostered offspring exhibited behaviors of their rearing mothers, thus implying that individual differences and behaviors of mothers can be transmitted across generations [7]. It has been demonstrated that antibiotic-treated dams display abnormal behavior, suggesting the involvement of the microbiome. Fostering by normal dams can rescue the behavioral phenotype in offspring born to antibiotic-treated mothers [8], but underlying mechanisms of the rescue and the involvement of the microbiome have not been elucidated.

### Effects of Early Impacts on Offspring Behavior and Physiology

Early interactions during birth and postpartum are critical for neurodevelopment. A recent study by Sgritta *et al.* [9] in autism-spectrum disorder models found that treatment with the bacterial species *Lactobacillus reuteri* reversed social deficits in several mouse models, including vagus-nerve-dependent and germ-free mice. Interestingly, supplementation of *L. reuteri* did not reverse the social deficits in oxytocin-receptor-deficient mouse models, implying the involvement of oxytocin in the response.

Studies have demonstrated that antibiotic-induced changes in early-life microbiota have long-lasting metabolic consequences in body fat distribution and weight gain. Moreover, high-fat diets affect the microbiota [10], which then could affect neurodevelopment. High-fat-diet-induced microbial changes can lead to significant alterations of the offspring exploratory and anxiety-like, cognitive, stereotypical, and compulsive behaviors as well as attention-deficit hyperactivity disorders and autism-spectrum disorders [11]. The behavioral phenotype is rescued by microbes [12].

Although the exact mechanisms are not completely understood, it is clear that the prenatal and perinatal period is a critical window in neurodevelopment and involves the microbiome.

### Concluding Remarks

Maternal factors that are present in the prenatal and postnatal environment are important for offspring development. Perturbations that might impair transmission of maternal microbiota affect behavioral and physiological traits in the offspring. More research is needed to understand the mechanisms of the microbiota's contribution to maternal–infant bonding and the behavioral consequences of impacts in early development. Experiments need to address which individual components of the microbiota (single populations or combinations) exert functions in offspring–maternal bonding and brain development. Investigations are needed to understand the mechanisms by which maternal and early-life perturbations lead to convergence in upregulated pathways of cognitive and physiologic development in the offspring. Furthermore, it would be interesting to understand how the microbiota could be affected by maternal stress and any subsequent effects that this may have on the offspring's neurodevelopment. By understanding how maternal health and stress effects both

the perinatal and postnatal periods, we can better understand the generational effect on the offspring.

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