

## Antimicrobial resistance and phage therapy in the Indian context

Gopika Ranjith, Aditi Ajith Pujar, Ashim Kumar Dubey and Preetham Venkatesh

*The discovery of antibiotics was a turning point in the history of mankind, improving healthcare and increasing life expectancy around the globe. However, the rising number of cases of antibiotic-resistant infections paints a concerning future. Thus, it is essential to understand and explore alternatives and implement policies for their safe usage. This note summarizes the upsurge in antimicrobial resistance in recent years and the feasibility of phage therapy as an alternative in India.*

Antibiotic resistance is an alarming phenomenon that has largely remained out of public discussion ever since the revolutionizing discovery of antibiotics. Despite dire warnings from Alexander Fleming himself, their incredible popularity inevitably led to rampant misuse and a growing threat of bacterial infections which may be resistant to all current medication, aptly titled 'superbugs'. With its high population density and lax regulation on sale of antibiotics, India is especially vulnerable to antimicrobial resistance (AMR) development; alternatives like phage therapy have therefore become matters of national importance.

The transition of AMR from the unknown to capturing the world's attention came with the frightening statistics that WHO's Global Antimicrobial Resistance Surveillance System (GLASS) put out in its report<sup>1</sup>. The report revealed high levels of AMR in the most common bacterial infections globally, focusing especially on seven different bacteria.

Not only is the resistance towards highly potent drugs – especially those used as a last resort for infections, like carbapenems (for multi-drug resistant (MDR) *Klebsiella pneumoniae*) and third-generation cephalosporins (for gonorrhoea) – it is also extremely widespread (up to 51% bacteria worldwide have shown penicillin resistance) and frequent (nearly 100% of the genital *Neisseria gonorrhoeae* isolates obtained from Malawi were resistant to ceftriaxone)<sup>1</sup>.

In 2016, India and the United Kingdom forged a 13 million Euro deal to analyse and help solve this problem. Consequently, the Department of Biotechnology (DBT), Government of India, and the Research Councils United Kingdom (RCUK) conducted an extensive study on AMR and compiled startling statistics<sup>2</sup>.

The situation is incredibly grim in India, with an estimate of more than

50,000 newborns dying from first-line antibiotic resistance-related sepsis annually<sup>3</sup>, and statistics showing that by 2050, at least two million AMR-related deaths are expected to occur<sup>4</sup>. Across antibiotics, infections due to dual carbapenem and colistin-resistant pneumonia have been seen to cause a 69.3% mortality among Indian patients<sup>2</sup>.

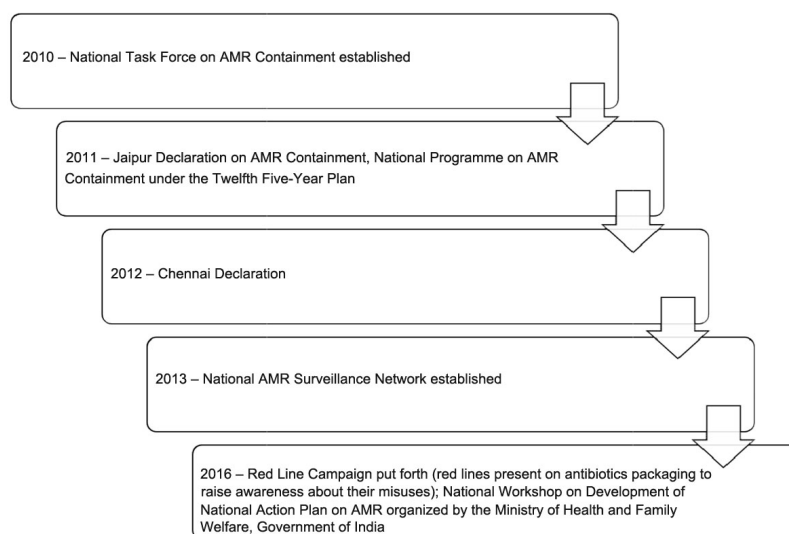
Studies on drug-resistant *Escherichia coli* isolated from the Mula-Mutha river, an important drinking water source for the city of Pune, Maharashtra observed that 28% of these bacteria were resistant to more than six antibiotics, including ceftazidime and ciprofloxacin<sup>5</sup>, while genes resistant to last-resort antibiotics such as *bla*<sub>CTX-M</sub> and *bla*<sub>NDM-1</sub> have been detected in the major rivers of India; sometimes present in more than 60% of the samples studied<sup>6,7</sup>.

Across bacteria, statistics indicates a rising rate in AMR in India<sup>8</sup>, especially among pathogens such as *Salmonella typhi*, *Shigella*, *Pseudomonas* and *Acinetobacter*<sup>9</sup>. Statistics shows that the ubiquity of resistance to broad-spectrum

antibiotics like fluoroquinolones and third-generation cephalosporins amongst target bacteria was more than 70%. In a more recent study on River Kaveri, Karnataka, 100% of *E. coli* isolates were found to be resistant to third-generation cephalosporins<sup>2</sup>.

The prevalence of AMR in India can be related to high consumption of antibiotics – as of 2010, the country was the largest consumer in the world<sup>10</sup>. Self-medication, over-the-counter (OTC) drugs, improper knowledge on when to use antibiotics and the consequent heavy prescription of antibiotics fixed dose combinations (FDCs)<sup>11</sup> are exacerbating AMR.

Administration of antibiotics, especially last resort drugs such as colistin, in poultry and pigs as growth hormones is another matter of concern. The DBT report shows that India is the fifth largest consumer of antibiotics through animals (2010)<sup>2</sup>, and with the rising demand for meat, antibiotics use is expected to increase by 312% by 2030 (ref. 12).



**Figure 1.** A timeline of the National Policy on tackling antimicrobial resistance<sup>2,18</sup>.

**Table 1.** Advantages and disadvantages of phage therapy

Advantages	Disadvantages
As natural predators of bacteria, phages can evolve dynamically to overcome bacterial resistance to antibiotics.	Bacteria may also evolve resistance to the phages; engineering phages to overcome this is challenging.
Phages are extremely specific in their action, thus reducing the chances of side effects.	A single strain of phages may become unviable soon, thus requiring a 'cocktail' of phages to be administered.
Predatory phages are often found alongside bacteria, which reduces developmental costs.	Characterizing each strain in a cocktail, as mandated by medical legislation, is a costly and time-consuming endeavour.
Phages multiply in proportion to the number of bacteria, simplifying dosage.	Phage behaviour inside the human body is not well characterized, making administration possibly risky.

Effluents released from pharmaceutical companies are an often-overlooked source of antibiotic resistance. Studies show that although antibiotic pollution has so far been observed around Hyderabad (Telangana) and Visakhapatnam (Andhra Pradesh), the vast number of antibiotics-producing factories (40 antibiotics active pharmaceutical ingredients (APIs) manufacturers and 250 antibiotics formulation companies) indicates probable environmental pollution due to antibiotics in other regions like Gujarat, Himachal Pradesh (HP) and Punjab<sup>2</sup>.

Festivals and other social gatherings which see large crowds for short periods of time are usually unprepared with respect to proper waste disposal and sanitation, enabling the spread of AMR bacteria. For example, *bla<sub>NDM-1</sub>* was found to be 20 times greater than usual in the Ganges during the pilgrimage season<sup>13</sup> (Figure 1).

Phage therapy is now considered as a viable alternative in light of increasing AMR. It involves the administration of bacteriophages (a group of viruses that affect bacteria), and could enable movement away from antibiotic-central treatment. Table 1 lists the advantages and disadvantages of phage therapy.

With more than 96.1% of their patients responding positively over a decade, Weber-Dabrowska *et al.*<sup>14</sup> established the efficacy of phage therapy in 2000, making it a solid battleground to combat AMR.

India holds a unique position in the history of phage therapy: in 1896, British scientist Ernest Hankin, while studying cholera outbreaks in India, described how despite the presence of cholera-causing bacteria in the water of Ganges and Jumna rivers, this disease was rare in the people who relied on water from these rivers for drinking<sup>15</sup>. He also found that unboiled Ganges water killed the

cholera bacteria in 3 hours, while boiled Ganges water did not have any bactericidal effect.

There is strong evidence that this unknown source may have been bacteriophages, making it one of the first discoveries of these powerful antimicrobial agents. Taking historical inspiration for its name, GangaGen, an Indo-American company was founded in 2000. It worked extensively on phage therapy before moving on to phage-derived lysins.

According to our knowledge, the only public sector venture in phage therapy is the Central Drug Laboratory, Kasauli, HP. Under the Drugs and Cosmetics Act (1940), it has been commissioned to work on phages amongst other products (vaccines, antitoxins and sera).

Moreover, India has no laws that directly address phage therapy. Upon parsing through guidelines for drug administration provided by the Indian Pharmaceutical Association<sup>16</sup>, the lack of juridical structure on phage therapy is starkly evident. The Drugs and Cosmetics Act (1940), requires 'all substances intended for use as components of a drug including empty gelatin capsules' to be tested like drugs. This implies that the phage coat and tail proteins would be subjected to the same stringency as phage's DNA, and that all its effects would have to be well characterized. However, phages undergo random mutations during replication; thus fulfilling either of the stipulations is extremely difficult.

Despite the promise phage therapy has shown, the following have been hindering its progress<sup>17</sup>:

(1) Legal Issues: (a) The continuous adaptation of phage components in a phage cocktail is at odds with the current medical legislation. A juridical system to monitor initial trials of phage therapy is

essential. (b) Intellectual Property Rights need to be upheld through an appropriate legal framework. All knowledge of phages has been public since their discovery; thus a viable alternative is to henceforth patent their downstream processes instead of the phages themselves.

(2) Lack of knowledge (scientific and public awareness): (a) Phages thus far have been improperly documented and incompletely understood; indeed, much of their cons stem from a lack of knowledge (development of bacterial resistance, possible antagonistic effects of phage administration, etc). The field needs to be expounded upon. (b) Lack of public awareness which is to be mitigated by campaigns and drives.

Antibiotic resistance in bacteria is increasing as we speak. Along with efforts to promote awareness on the proper use of antibiotics, we must also focus on developing alternatives. Phage therapy seems to be the most practical solution and India needs to participate in its research more actively, especially as it will be among the countries hit hardest by AMR. This requires the active involvement of the many stakeholders and healthy discussion on the ethical and scientific aspects of phage therapy. The hope remains that misuse of one of mankind's best discoveries is not the cause of its downfall.

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*Gopika Ranjith, Aditi Ajith Pujar, Ashim Kumar Dubey and Preetham Venkatesh\* are with the Undergraduate Department, Indian Institute of Science, Bengaluru 560 012, India.*

*\*e-mail: preethamv@iisc.ac.in*

## Coconut oil – scientific facts

*S. V. Ramesh, Veda Krishnan, Shelly Praveen and K. B. Hebbar*

*Coconut oil and its health benefits have been challenged once again by an US-based professor who has labelled it as ‘a pure poison’. The pertinent question we ask is whether her observations are based on scientific facts. We would like to dispel any negative connotation and arrest the spread of mis-information about the oil of Kalpavriksha. Here we discuss and present scientific facts that support the health benefits of coconut oil.*

### Constituting fatty acids of coconut oil and their health benefits

The negative reputation of coconut oil stems from the assumption that saturated fatty acids (SFAs) are deleterious for the human heart. Like most tropical oils, coconut oil has high (82%) saturated fatty acids, specifically lauric acid (LA) and myristic acid. Hence, it is natural to consider coconut oil as a ‘poison’. From a common man’s perspective, any oil that is rich in SFAs is considered bad for health, whereas the beneficial effects of poly-unsaturated fatty acids (PUFAs), especially  $\omega$ -3 and  $\omega$ -6 fatty acids containing natural oils, are well recognized. According to the guidelines of American Dietetic Association (ADA) and Dietitians of Canada, individual SFAs differ in their effects on blood lipid levels. Also, the effect of SFAs on cardiovascular ailments is still under debate<sup>1</sup>. As early as 1981, Prior *et al.*<sup>2</sup> proved that populations which rely on coconut as a source of edible oil do not develop any harmful health effects. This was further corroborated by Kaunitz and Dayrit<sup>3</sup>,

who proved that high dietary coconut oil did not evince any biomarkers associated with coronary heart disease (CHD). Even though the contributory role of saturated fats in cardiovascular ailments is uncertain, coconut oil chiefly comprises SFAs in the form of medium-chain fatty acids (MCFAs) like caproic acid (C6), caprylic acid (C8), capric acid (C10), LA (C12), etc. Medium-chain triglycerides (MCTs) are easily absorbed in the intestine because of their greater solubility and are transported through portal vein to liver to produce ketones and hence energy. On the contrary, long-chain fatty acids (LCFAs) enter the lymphatic portal system, and are deposited in the body and stored as fat. Also, clinical studies have proven that MCFAs have cardiovascular benefits and play a crucial role in the reduction of cardiovascular diseases (CVDs)/CHD-associated risks<sup>4</sup>. A study by Assunção *et al.*<sup>5</sup> reported that 40 women showed a decrease in abdominal fat when they consumed coconut oil and followed a physical activity routine. Another study<sup>6</sup> categorically substantiated that coconut oil provides a satiated feeling though it

did not affect resting energy expenditure. The effects of coconut oil supplementation on body composition and lipid profile of rats that had undergone physical exercise revealed that such supplementation did not interfere with body mass<sup>7</sup>. Coconut oil is nature’s richest source of LA at about 50% of its composition; and human breast milk comes at a distant second with around 6% LA. MCTs, especially LA in its pure form, have antiviral<sup>8</sup> and antibacterial properties, and studies prove that they may help balance gut bacteria and combat pathogenic bacteria<sup>9</sup>. They also help the digestive system because they are easily utilized by the body. When used with a healthy diet and other ways to support gut bacteria, MCFAs may help improve gut health over time.

### Nutrient richness of virgin coconut oil

Virgin coconut oil (VCO) obtained from fresh coconut endosperm without any chemical process is rich in polyphenols