INNOVATION

Electronic noses and disease diagnostics

Anthony P.F. Turner and Naresh Magan

Rapid developments in sensor technology have facilitated the production of devices — known as electronic noses — that can detect and discriminate the production profiles of volatile compounds from microbial infections *in situ*. Such qualitative and semi-quantitative approaches could have a significant role in the early diagnosis and detection of microbial diseases. Using artificial intelligence and web-based knowledge systems, electronic noses might also have a valuable role in monitoring disease epidemiology.

In today's society, preventative medicine is becoming the accepted therapeutic approach and patients are beginning to demand rapid and early qualitative diagnosis of microbial diseases. Early discrimination between different infections is also important to facilitate rapid treatment as part of a preventative health strategy. It is well known that microbial species produce a range of volatile compounds. Many studies, especially those using analytical tools such as gas chromatography (GC) or GC linked with mass spectrometry (GC–MS) for headspace analysis, have shown that microorganisms produce many volatile organic compounds, including alcohols, aliphatic acids and terpenes, some of which have characteristic odours. Other work has shown that the type of culture media and the age of the culture, as well as the microbial species, all influence the amounts and patterns of the volatile compounds that are produced. As these patterns are characteristic for certain types of infectious microorganisms, they can potentially be used as biomarkers of disease; TABLE 1 summarizes some of the characteristic volatiles that are indicative of microbial infections. Indeed, GC and GC–MS techniques have already been used to monitor the production patterns of volatile compounds as an aid to the clinical diagnosis of aerobic and anaerobic bacterial infections and cardiopulmonary disease, and have been used to analyse several substrates, including human pus, urine, blood plasma and alveolar air. However, so far, the use of GC/GC–MS analysis of these volatile fingerprints has been hampered by the need for expensive analytical equipment, the degree of expertise required to operate such instruments and the length of time required to obtain results.

Early in this research area, the question arose as to whether chemical reactions between volatile markers and various sensors could be amplified and be sensitive enough to enable qualitative differences to be measured between the markers at relatively low concentrations. Attempts were made in the 1970s to study the possibility of using redox reactions of volatile compounds and amplifying volatile conductivity and the contact between volatile molecules and sensor-based materials. This resulted in the first model of an 'electronic nose', reported by Persaud and Dodd in 1982 (REF. 1; see TIMELINE). Their idea was to try to detect different volatile compounds by simulating the different stages of the human olfactory system, including sampling and filtering odours, using biochemical sensors with which volatile compounds can react, amplification and treatment of the sensor signal responses, and using neural networks to evaluate the key useful components of the data, resulting in volatile odour recognition (FIG. 1). Applying this approach has resulted in many patents being filed that relate to the development of appropriate sensor arrays for microbiological, food safety and medical applications[2–7].

Although largely qualitative or semi-quantitative in nature, such approaches are ideal for rapid screening for infectious diseases because the results can be obtained in minutes, rather than the days taken by traditional techniques. The key to the advances in this methodology over the past decade has been combining the rapid development of sensor technology with artificial intelligence approaches[8,9]. This article presents an overview of the most important recent developments, illustrates some applications for the diagnosis of infections and discusses future trends.

Several electronic-nose devices have been developed, all of which comprise three basic building blocks: a volatile gas odour passes over a sensor array, the conductance of the sensors changes owing to the level of binding and results in a set of sensor signals, which are coupled to data-analysis software to produce an output.

Sensor formats

Sensor technology has developed rapidly over the past decade and this has resulted in a range of different sensor formats and the development of complex microarray sensor devices. In the specific area of electronic-nose systems, several different physicochemical techniques have been used to produce sensor arrays for odour characterization. Each of the different sensor formats is described briefly below.

Conducting-polymer sensors. Conducting-polymer sensor arrays consist of unique polymers with different reversible physicochemical properties and sensitivity to groups of volatile compounds. These compounds interact with and attach to the polymer surface, changing the resistance under ambient temperature conditions. This, in turn, changes the signal, which is monitored for

Table 1 | Summary of key volatiles associated with different disease types analysed by GC–MS

Sample	Disorder/Infection	Volatile compounds	References
Microorganism-associated disorders			
Urine	Urinary tract infection	Isovaleric acid, alkanes	28
Intraperitoneal fluid	Aerobic Gram-negative bacteria	Terpenes, ketones	29
Intraperitoneal fluid	Anaerobic bacterial infections	Acetic, butyric acids	30
Human pus	–	Isobutyric, isovaleric, isocaproic acids	31
Other disorders			
Human breath	Breast cancer	Alkanes, monomethylated alkanes	32
Human breath	Lung cancer	Alkanes, monomethylated alkanes	33
Human breath	Acute asthma	Pentane	34
Urine	Metabolic disorders	Isovaleric acid	35
Alveolar air	Hepatic coma	Methyl-mercaptan	36
Alveolar air	Rheumatoid arthritis	Pentane	37
Alveolar air	Schizophrenia	Pentane, carbon disulphide	38
Alveolar air	Ketosis	Acetone	39
Alveolar air	Cardiopulmonary disease	Acetone, ethanol	40
Blood plasma, cerebrospinal fluid	Hepatic encephalopathy	3-methylbutanol	41

each sensor type, enabling an array to be constructed that has overlapping detection ranges for different groups of volatile compounds. Sample presentation is crucial for this type of sensor to avoid humidity and drift problems.

Metal oxide sensors. The oxide materials in these sensors contain chemically adsorbed oxygen species, which can interact with the volatile molecules, thereby altering the conductivity of the oxide. The selectivity of these sensors can be changed by using different amounts of noble metals or by changing the operating temperature. They are very sensitive, robust and resistant to humidity and ageing effects, although they can suffer from drift over time.

Metal oxide silicon field-effect sensors. These sensors are related to metal oxide sensors but the output signal is derived from a change in potential when the volatile molecules react at a catalytic surface. The operating temperature for these sensors is 100–200°C. They are sensitive to many organic compounds.

Piezoelectric crystals. Sensors containing piezoelectric crystals use the radio frequency resonance of quartz materials coated with acetyl cellulose or lecithin membranes. The adsorption of volatile molecules onto the membrane produces a change in the magnitude of the resonance frequency that is related to the mass of the volatile analyte. The selectivity of these sensors is dictated by the thickness of the coatings.

Surface acoustic-wave devices. These devices are an alternative to the above sensors and are based on waves that are emitted along the surface of a crystal by the electric field of surface-deposited aluminium electrodes.

Optical sensors. These sensors are based on a light source that excites the volatile analyte, and the signal can be measured in the resulting absorbance, reflectance, fluorescence or chemiluminescence.

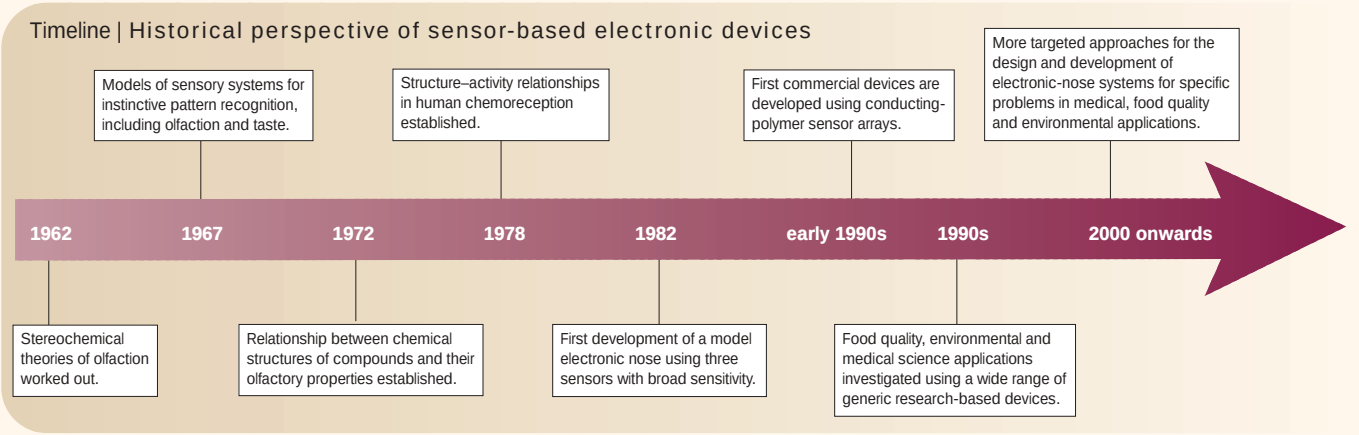
Electrochemical sensors. These sensors contain electrodes and an electrolyte. The responses generated are dependent on the electrochemical characteristics of the volatile molecules that are oxidized or reduced at the working electrode, with the opposite occurring at the counter electrode. The voltage generated by the reactions between the electrodes is measured, and has been used to detect CO, SO_2 and H_2S.

There is a large body of literature on all these sensor technologies[10], but the key to the devices under consideration in this article is the fact that total specificity is not required. Using multifactorial approaches, it is enough for the elements of the array to react differently to various analytes, enabling discrimination to be made between samples.

New sensor technologies. New approaches are being tested to provide better sensor shelf-life, greater sensitivity and improved consistency under variable conditions of temperature and humidity for commercial applications. For example, surface plasmon resonance can be used, in which changes in the optical properties of the polymer materials used in sensors or the resonance of associated cantilevers can be monitored on the basis of changes in the mass of the volatile compound that is being analysed. Discotic liquid crystals, which consist of an aromatic core surrounded by hydrocarbon side chains, are very sensitive to the presence of volatile molecules and insensitive to humidity, and could provide advances. Materials based on metalloporphyrins, which can detect a broader range of fingerprints because of the diversity of metal ions and substituted porphyrins that are available, and semi-selective molecularly imprinted polymers specific to different classes of analytes[11] are also being developed. Optical sensor arrays using fluorescent solvatochromatic dyes have been found to generate responses to organic vapours and, using computational networks, have enabled sensitivity to be increased[12]. Recently, an expanded colorimetric sensor–array system based on metallated tetraphenylporphyrins and chemoresponsive dyes has been developed that has good sensitivity, with thresholds of detection for amines, carboxylic acids and thiols that are better than the human nose. Preliminary data with bacteria using this technique have been promising[13].

Applications in disease diagnostics

Using conventional culture methods, diagnosis of infection can take at least 24–48 hours for bacteria and even longer for fungal infections. For prevention and early treatment strategies to be implemented, it is essential to obtain relevant rapid results in a useable format. Can volatile fingerprinting and electronic-nose



systems detect and discriminate between pathogens? Evidence so far indicates that this is certainly achievable[14] (FIG. 2). TABLE 2 summarizes the *in vitro* and *in situ* results obtained using this technology. Studies *in vitro* have shown that it is possible to discriminate between different aerobic bacteria, such as *Helicobacter pylori*, *Escherichia coli* and *Enterococcus* species that are present in samples, both alone and as a mixture of the three species, on the basis of differences in the amounts of terpenes, trimethylamine and ketones produced[15]. *Mycobacterium tuberculosis*, the causative agent of tuberculosis, has been detected in cultured sputum samples either directly or following treatment with enzymes to enhance *M. tuberculosis* growth and volatile production[16]. Anaerobic bacteria such as *Clostridium* species and *Bacteroides fragilis* have been successfully differentiated in culture on the basis of the discrimination of volatiles, such as isobutylamine, acetic acid and butyric acid[15]. In other work, samples from patients with urinary tract infections (UTIs) and tuberculosis were identified correctly in 90–99% of cases compared with traditional culture techniques[16,17]. Additionally, six different bacterial species responsible for eye infections — *E. coli*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae* — were successfully discriminated into six different classes (98% success) using data that were obtained with a handheld portable electronic nose[18]. A case study on bacterial vaginosis in the United Kingdom has also shown that a conducting-polymer-based sensor array successfully diagnosed 89% of test subjects as being positive or negative for both bacterial and yeast infections[14].

Electronic-nose devices can be used to detect diseases other than infectious diseases. For example, it has been shown that patients with kidney disorders produce characteristic volatile compounds, which can be a useful tool in the diagnosis and control of renal dialysis[19]. Additionally, studies by the same group have shown that lung cancer can be detected by breath analysis using non-selective gas sensors[20]. In this work, all of the patients with the disease and 94% of controls were successfully identified by detecting alkanes and aromatic compounds from breath samples using quartz microbalance gas sensors coated with different metalloporphyrins.

These promising results indicate that, in the future, it might be possible to have electronic-nose devices at the point of medical delivery — in the doctor's surgery — where they could be used as a rapid screen for specific diseases or disorders. Indeed, suggested schemes have included combining visual examination — using techniques such as endoscopy — with instantaneous chemical information to con-

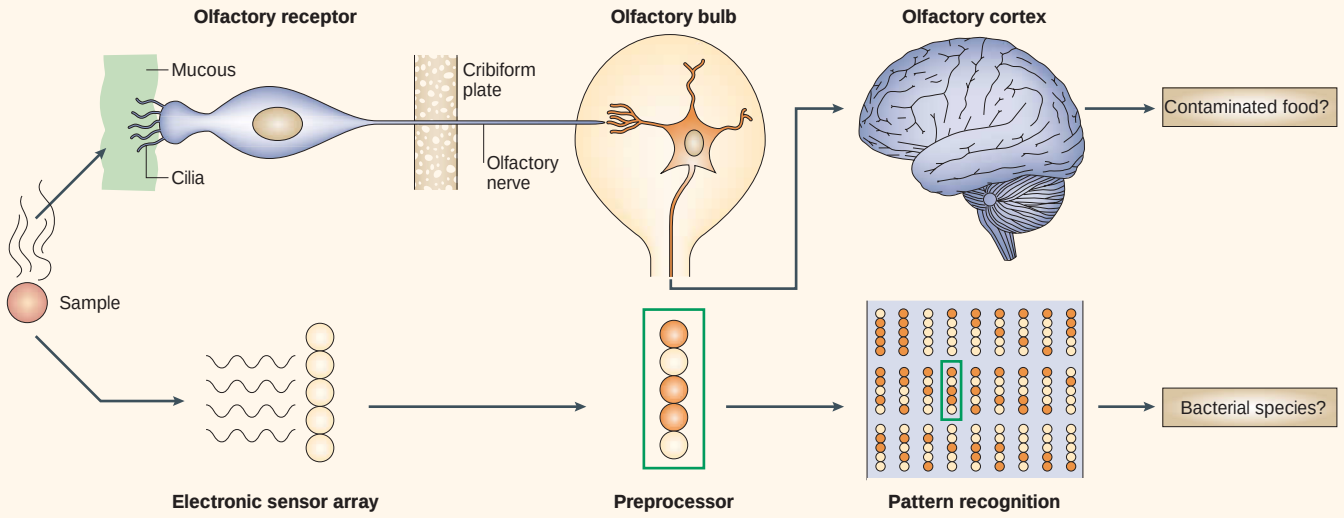


Figure 1 | **Electronic nose devices mimic the human olfactory system.** The first electronic nose device was reported by Persaud and Dodd[1]. The electronic devices simulate the different stages of the human olfactory system, resulting in volatile odour recognition, which can now be used to discriminate between different bacterial infections.

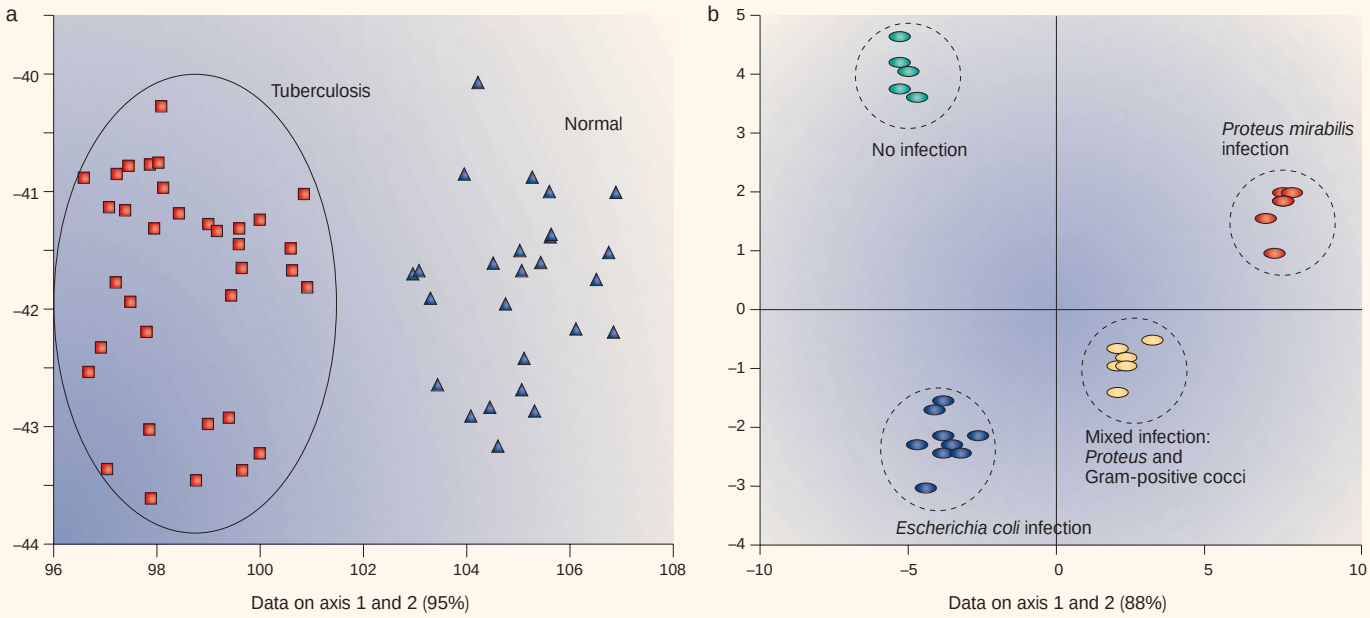


Figure 2 | **Examples of infection discrimination using electronic nose devices. a** | The successful detection of *Mycobacterium tuberculosis* in sputum samples. **b** | The successful discrimination between different bacteria in urinary tract infections. Axes in both **a** and **b** are set to an arbitrary scale.

firm diagnoses. One patent application[2] proposes sampling gas through the biopsy channel of an endoscope to provide confirmation of cancerous growths using online electronic-nose technology. In another approach, wound dressings have been examined to reveal the presence of infection in elderly or infirm patients[21].

Other applications

Recent developments in electronic-nose technology indicate that there are also potential targeted approaches and applications for these devices in the food and drink industry. Within the framework of Hazard Analysis Critical Control Point (HACCP) food-safety systems in food manufacturing, real-time analysis is required as part of quality-assurance schemes. Bacteria and yeast in milk, and mould contamination of bakery products and dairy products, have all been successfully detected using this approach[22]. Interestingly, some studies have shown that a strain of a microorganism producing a particular toxin or secondary metabolite can use different biosynthetic pathways from non-producing strains and, therefore, produce different characteristic volatile patterns. It has been shown that mycotoxin-producing strains of *Fusarium* and *Penicillium* can be rapidly differentiated from non-mycotoxin-producing strains both *in vitro* and *in situ*[23,24]. This could also have implications for medically important pathogenic yeasts such as *Candida albicans*, for which there are a wide range of virulent and non-virulent strains.

Sampling issues and data processing

The usefulness of electronic-nose devices depends on two crucial components. First, the sample must be presented in the correct format to optimize the interaction of volatiles in the headspace with the sensor array. Sample analysis should be consistent and the sample should be presented to the electronic-nose device in the same way. So, humidity, temperature and sample size must be standardized to ensure that data sets can be compared and analysed with confidence. This also minimizes drift problems over time, although these can be overcome by using a set of appropriate standards for calibration. This approach was used in the development of a prototype sampling system for raw food materials before real-time (10 minutes) processing to detect mould spoilage[25].

Second, pattern recognition must enable large data sets to be analysed rapidly to obtain appropriate and useful results[9]. Normally, volatile odour pattern data are received in the form of normalized data sets based on the divergence, area and adsorption or desorption components of the individual sensor responses. This generates a significant amount of data and requires effective data management. The techniques used to analyse such data sets have included simple supervised techniques such as discriminant function analysis (DFA), which can parametrically classify an 'unknown' or 'random' sample from a population or group. DFA has been successfully used to detect bacteria, yeasts and some filamentous fungi[24]. A simple, unsupervised multivariate method, such as cluster analysis, has also been used to identify a volatile odour class without prior information on the nature of the volatile fingerprint[26]. Principal components analysis (PCA) is a popular technique for the visualization of large multivariate data sets that allows the relationships between samples to be identified.

However, for applications in a clinical environment where real-time sensing and results are required, the data analyses must be taken a step further. A neural network (NN) consists of a series of algorithms that are more appropriate for nonlinear sensor systems. This enables a specific system to be developed for a specific disease at the required level of sensitivity. By collecting enough background (control) data and by using so-called back-propagation approaches, sensor drift and nonlinear data sets can be taken into account and effectively used for accurate prediction of the group to which a real sample belongs. As already mentioned, this approach was successfully used to predict the presence of UTIs and *M. tuberculosis* in real samples from patients with >90% success[16,17,27]. Of course, a large number of training sets are often required to develop appropriate NN systems. Where there is still an overlap between groups, the potential exists for the use of 'fuzzy logic' NNs. These are more flexible, and can be trained

Table 2 | Summary of recent work on successful *in vitro* and *in situ* samples

Sample type	References
In vitro *samples*	
Bacterial classes successfully discriminated by growth phase and type	42,43
Helicobacter pylori and gastroeosophageal isolates cross-validation successful	15
Anaerobic bacteria differentiated on agar media using PCA	44
Bacteria, yeasts and filamentous fungi differentiated using PCA and cluster analysis	22,24
Bacteria detected in blood cultures	45
Fungal spoilage detected more rapidly than traditional techniques	46
Toxigenic strains of moulds successfully separated on media and on bakery products	23, 47
Different *Agaricus* species discriminated successfully	48
In situ *clinical samples*	
Contact dressings from leg ulcers: bacterial infection detected	21
Vaginal swabs: bacteria detected	49
Urine: urinary tract infection detected	16
Vaginal swabs: bacterial vaginosis diagnosed	14
Sputum: tuberculosis diagnosed	27
Urine containing blood: haematuria detected	19
Breath samples: respiratory infection detected	50
Breath samples: lung cancer detected	20
Breath samples: diabetes detected	51

PCA, principal components analysis

rapidly with large amounts of sensor array data from samples to provide a foundation of healthy background volatile fingerprints. This subsequently makes differentiation of infected samples easier and more rapid, often in a matter of minutes. However, it is clear that for different microbial diseases, specific NN analysis systems might need to be developed. Some could be qualitative only and useful for screening, whereas others could be semi-quantitative and give more information for the treatment of the disease.

The opportunity now exists to take data gathered remotely at different sites and use advanced information-technology approaches, satellite communication and web-based knowledge systems to analyse this information rapidly and give results from a central point within minutes. This would open up the potential for using such systems for epidemiological studies and the rate of spread of diseases within a country, continent or worldwide. This could have particular implications for the monitoring of the spread of tuberculosis, food-borne pathogens, and perhaps other human and veterinary diseases.

Future trends

The development of robust instrumentation, coupled with remote data acquisition and central processing powered by hybrid intelligence systems, could see electronic-nose technology in common use in the next 5 years. So far, this technology has had limited commercial success, but arguably this is because the instruments have been developed and sold as general analytical instruments and specific applications have been left to the individual user. Given the complex and pragmatic mode of calibration of these instruments, which literally 'learn' to distinguish one sample from another, this has led to an understandable reticence on behalf of users to embrace this technology instead of well-understood chromatographic and spectroscopic methods. The instruments being developed at present can be optimized for use in a specific area and come ready calibrated to tackle a specific problem. This mode of design imposes its own limitations, in that a potential market must be sufficiently large to support the considerable development costs that are associated with such a programme. However, the areas of rapid medical diagnostics and food safety clearly have the potential to warrant such investment and we can expect several exciting commercial developments in the near future.

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Competing interests statement
The authors declare that they have no competing financial interests

Online links

FURTHER INFORMATION
Anthony P. F. Turner's laboratory:
http://www.silsoe.cranfield.ac.uk/staff/apturner.htm
Naresh Magan's laboratory:
http://www.silsoe.cranfield.ac.uk/staff/cv/n-magan.htm
Access to this interactive links box is free online.

OPINION — ANTI-INFECTIVES

Population and evolutionary dynamics of phage therapy

Bruce R. Levin and James J. Bull

Following a sixty-year hiatus in western medicine, bacteriophages (phages) are again being advocated for treating and preventing bacterial infections. Are attempts to use phages for clinical and environmental applications more likely to succeed now than in the past? Will phage therapy and prophylaxis suffer the same fates as antibiotics — treatment failure due to acquired resistance and ever-increasing frequencies of resistant pathogens? Here, the population and evolutionary dynamics of bacterial–phage interactions that are relevant to phage therapy and prophylaxis are reviewed and illustrated with computer simulations.

The history of phage therapy — the use of bacterial viruses to treat bacterial infections — is older than most of the readers of this article (TIMELINE). Prior to the development of antibiotics, research into, and the practice of, phage therapy was a substantial enterprise in Europe, parts of Asia and North and South America, and continues to be a viable, if not thriving, industry in some countries of eastern Europe[1–3] (see phage therapy providers in the Online links). The demise of phage therapy in western medicine in the 1930s and early 1940s can, in part, be attributed to inconsistent therapeutic results and, in part, to its eclipse by effective, broader spectrum antibiotics that became available at that time[4–7]. Ironically, the epitaph of phage therapy was written more than a decade before the genetics of bacteriophage and the mechanisms of bacterial pathogenesis became important subjects of research. Passive immunization, that is, serum therapy for bacterial infections, suffered a similar fate after the advent of antibiotics, despite its demonstrated efficacy for treating *Pneumococcus* bacteraemias and pneumonias[8], diphtheria and other bacterial, as well as viral diseases[9]. The epitaph of serum therapy was written more than 30 years before we knew about T cells and B cells and even longer before the development of monoclonal antibodies.

Now, to paraphrase Victor Hugo, phage therapy is an idea whose time has come again. Fuelled by concerns about antibiotic resistance and lost ground in the antimicrobial chemotherapy 'arms race', the idea of using phages for treating and preventing bacterial infections is experiencing a rebirth. This has taken a number of forms, including the rediscovery of detailed, successful experiments, such as those of H. William Smith and M.B. Huggins[10–12], new experiments[13–19], and mathematical models to facilitate a better understanding of how phage might control bacterial infection[20–24]. Much of this renewed hope for phage therapy also comes from an improved understanding of the genetics and biology of bacteriophage[25–27] and the possibilities offered by genetically engineering bacteriophages for these applications[7,28]. In addition to their use for treating or preventing human infections, phages are being developed for agriculture, to rid environments and domestic animals of the pathogens that could contaminate food supplies[29,30], to control infections in high-density poultry production[31] and for the treatment of fish pathogens in aquaculture[18,32]. Phage have also been proposed as an alternative to antibiotic sprays to control bacterial infections in high value crops, such as citrus canker on oranges[33].

In this perspective, we consider how an understanding of population and evolutionary biology of bacteria–phage interactions will be important to the success and development of the use of phage for therapy and prophylaxis. We first review the elements of the population and evolutionary dynamics of bacteriophage that are necessary to understand how these viruses can prevent or treat bacterial infections, and when their utility for these purposes will be thwarted by resistance. We then consider three different arenas for clinical and epidemiological applications of phages: acute infections — rapidly growing infections by bacteria that, at low densities, can be cleared by the constitutive and/or inducible defences — such as invasive infections caused by *Staphylococcus* or *Pneumococcus*; chronic infections — replicating populations of bacteria that are maintained for extensive periods of time and