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Addressing antibiotic resistance: computational answers to a biological problem?

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The increasing prevalence of infections caused by antibiotic-resistant bacteria is a global healthcare crisis. Understanding the spread of resistance is predicated on the surveillance of antibiotic resistance genes within an environment. Bioinformatics and artificial intelligence (AI) methods applied to metagenomic sequencing data offer the capacity to detect known and infer yet-unknown resistance mechanisms, and predict future outbreaks of antibiotic-resistant infections. Machine learning methods, in particular, could revive the waning antibiotic discovery pipeline by helping to predict the molecular structure and function of antibiotic resistance compounds, and optimising their interactions with target proteins. Consequently, AI has the capacity to play a central role in guiding antibiotic stewardship and future clinical decision-making around antibiotic resistance.

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Introduction

Antibiotic resistance is a product of bacterial evolution, affording bacteria protection against compounds that are detrimental to their survival. It is a subset of antimicrobial resistance, an umbrella term that more broadly describes the evolution of resistance to naturally occurring compounds or targeted drugs in any microbe, including bacteria, fungi, viruses and parasites. Antibiotic resistance is associated with antibiotic use and is exacerbated by the mis- and overuse of antibiotics in medical and agricultural practices, and the ease of public access to antibiotics of varying quality [1]. Increasing resistance in pathogenic bacteria poses a number of serious public health risks, including severe and prolonged illness, increased hospital admissions and complications and higher mortality rates [2], culminating in a substantial economic burden [3].

In some instances, antibiotic resistance can be attributed to intrinsic bacterial mechanisms (e.g. efflux — the transport of compounds out of cells) or acquired through spontaneous mutational events. However, antibiotic resistance is more commonly acquired through the inheritance of mobile genetic elements carrying antibiotic resistance genes (ARGs), via a process known as horizontal gene transfer (HGT) [4]. Despite certain phylogenetic and ecological barriers [5,6], HGT has the potential to generate substantial and rapid evolutionary innovation across greater phylogenetic distances than the parent–offspring constraints of its vertical transmission counterpart [7]. Thus, the aggregate of mobile genetic elements within an environment also represents an adaptive and robust reservoir of ARGs that can be accessed and added to by different bacterial lineages [8].

Efforts to address antibiotic resistance are complicated by its inherent association with antibiotic use. Consequently, antibiotic resistance research must focus on the development of strategies that do not simultaneously exacerbate the current condition. Recently, increased attention has been given to the role of metagenomic profiling (i.e. the untargeted sequencing of bacterial communities), bioinformatics and artificial intelligence (AI) in antibiotic resistance research. AI in particular has shown capacity to infer data patterns beyond the scope of human interpretation, thereby contributing to antibiotic discovery and resistance research.

Here, we discuss the role of AI in antibiotic resistance research, and the ways that metagenomic data can support, or even enhance, those analyses and the decision-making strategies they may inform.

Metagenomics enables culture-independent antibiotic resistance gene surveillance

Metagenomics enables the culture-independent surveillance of microbial communities and by association, the study of all bacteria potentially harbouring ARGs [9]. The identification (ID) of genes in metagenomic data and their subsequent clustering based on sequence similarity can be used to create gene catalogues [10], which can then be mapped against ARG databases to determine the presence and abundance of ARGs within the microbial community [9]. Metagenomics also enables the horizontal transfer of ARGs between bacterial genomes to be explored [11]. In short, these approaches exploit the genetic and phylogenetic disparities that typically exist between vertically and horizontally inherited genomic sequences with distinct evolutionary histories [12]. Given the role of HGT in the spread of antibiotic resistance, the inference of such events can strengthen surveillance data by elucidating how particular ARGs are being disseminated through bacterial communities [13]. Metagenomic approaches may be further strengthened through the incorporation of culture-dependent techniques. For example, long-read metagenomic sequencing of hospital samples following culture-based enrichment has enabled the characterisation of hospital-associated bacterial ARG profiles that included novel combinations of ARGs [14]. Culture-based approaches also enable the differentiation between viable and nonviable sources of ARGs, which has implications for their mode and degree of spread [15].

Recently, metagenomic analyses have been used to profile the reservoir of ARGs (resistome) in the human gut [16] and compare the rates of HGT in different human gut microbiomes [17]. Environmental surveillance has also identified novel ARGs in grassland and forest soil [18], and in freshwater viral metagenomes [19], suggesting that antibiotic resistance is widespread in microbial populations across a range of environments. The influence of human activity on environmental resistomes is exemplified by the effect of wastewater discharge on antibiotic resistance in marine sediments [20]. While the wastewater resistance profile is source-dependent, a core resistome containing an abundance of clinically relevant ARGs can be found across various sources, including healthy populations [21]. Metagenomic analyses have also found that processing wastewater with higher temperatures can reduce the relative abundance of ARGs, limiting their spread within wastewater treatment plants (WWTPs) and to other environments [22]. Therefore, metagenomics can serve a dual role in surveillance, in surveying basal resistance and monitoring the efficacy of mitigation strategies on these profiles.

The interaction between environmental temperature and the prevalence of antibiotic resistance, more generally, is currently unclear. Studies considering the connection between global climate change and antibiotic resistance have suggested that bacterial growth and HGT rates typically show a positive correlation with temperature [23]. However, a recent study that used metagenomics to profile estuary resistomes suggested that although these were influenced by human activities such as antibiotic use, higher temperatures were actually associated with a reduction in ARGs [24]. Such findings warrant further investigation into the possible link between climate change and antibiotic resistance, given the impact both of these crises have on humanity.

Machine learning (ML), a subfield of AI, thrives at identifying complex patterns present in real-world data sets. ML applications to metagenomic data include the inference of ARGs and resistome profiles [25,26]. Such models can also estimate abundances of ARGs in potential environmental reservoirs, such as the ocean [27] and WWTPs [28]. Studies suggest the source-tracking of environmental ARG pollution, the logical next step following the ID of an environmental reservoir, could also benefit from the application of ML models [29]. In agriculture, ML has been used to understand the transfer of ARGs between livestock, their environments and human workers [30], demonstrating the potential of AI to support a One Health approach to antibiotic resistance (i.e. a holistic approach that encompasses the environment, human and animal health) [31]. AI-based surveillance of ARGs and their source is also applicable to infection outbreak monitoring and prediction across populations [32]. Specifically, the implementation of AI forecasting techniques to predict future outbreaks of methicillin-resistant *Staphylococcus aureus* was recently proposed [33]. ML models may enhance outbreak monitoring through the prediction of HGT networks of pathogens harbouring ARGs [34]. Furthermore, the use of real-time metagenomic sequencing to identify outbreak transmission clusters [35] highlights the potential for such data to be used to train AI models for future monitoring of resistance outbreaks. Consequently, ML and AI have the potential to augment the metagenomic surveillance of ARGs, by predicting their presence and spread, within and across populations. However, this approach also presents a number of potential challenges associated with sensitivity, cost, short reads and host resolution [13].

Artificial intelligence predicts antibiotic resistance from gene sequences

The detection of ARGs from metagenomes is generally done through homology searches against ARG databases

such as The Comprehensive Antibiotic Resistance Database (CARD) [36] or ResFinder [37]. While these databases have good coverage of characterised resistance genes, finding genes with low similarity to those in the database is compromised. However, there are AI-based tools that are able to predict novel ARGs with limited sequence similarity to currently characterised ARGs. Fragmented Antibiotic Resistance Gene iENntifiEr (fARGene) [38] is based on hidden Markov models, and DeepARG [25] on a deep learning approach. Both tools have been shown to detect new resistance genes directly from short-read metagenomes with no assembly required.

AlphaFold is a complex ML model that has revolutionised the prediction of three-dimensional protein structure from amino acid sequences, providing atomic-level precision even in instances where homologous protein structures are missing [39]. The prediction of protein structures by AlphaFold has been applied to different facets of antibiotic resistance research, including the successful engineering of antimicrobial peptides (validated by *in vitro* protein synthesis) [40] and the prediction of novel protein structures that are potentially implicated in bacterial antibiotic resistance [41]. Protein sequence and structural information are used by other ML methods such as Deep Functional Residue Identification (DeepFRI), which predicts novel gene functions, including antibiotic resistance, independently of homology-to-database comparisons [42]. Preliminary data suggest that DeepFRI is applicable to metagenomic data [43]. Notably, a recent benchmarking study based on structural data from AlphaFold has suggested that further advances are required before such structural data can be used to predict the interaction between antibiotics and their target bacterial proteins [44]. Understanding molecular interactions is particularly relevant for optimising antibiotic combination therapies, which are often used to treat multidrug-resistant infections [45]. ML models can also help in this regard and have already been used to evaluate the combination of meropenem and polymyxin B for the treatment of *Acinetobacter baumannii in vitro* [46]. Therefore, continual advancement of ML models to predict molecular interactions could have direct clinical relevance by further improving combination treatment regimes for this and other critical resistant bacteria.

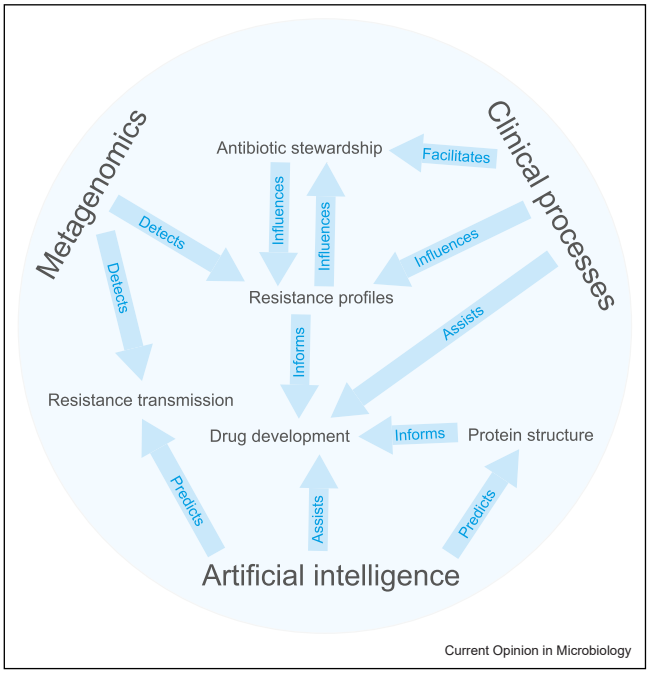
Artificial intelligence facilitates discovery of new antibiotic compounds

An integral aspect of the human response to antibiotic resistance is the discovery and development of antibiotics that are capable of treating bacterial infections, particularly those with resistance to existing antibiotics. Novel drug discovery typically falls under one of two methods: target-based screening, which focuses on drug development against a known molecular target (e.g. gene or protein), or phenotypic-based methods that test chemical compounds for their ability to induce the desired phenotypic change [47]. Antibiotic drug discovery during the resistance era has often relied on target-based screening methods to discover broad-spectrum antibiotics capable of treating a range of potential pathogens [48]. However, broad-spectrum antibiotics can also promote the spread of antibiotic resistance by affecting other, nonpathogenic, bacteria [49]. Moreover, the continued focus on ubiquitous targets is unlikely to counter the increasing prevalence of multidrug-resistant bacteria possessing a combination of resistance mechanisms [50]. One relatively new approach to the discovery of novel antibiotics is drug repurposing screens, which are rapid and inexpensive compared with traditional approaches [51]. The recent discovery of the novel antibacterial compound Halicin demonstrates the capacity for ML models to guide the repurposing of existing drugs as antibiotics, even when the screened drugs are structurally divergent or originally served markedly different purposes [52]. The researchers used algorithms that were trained on a diverse molecular dataset to predict molecular properties, such as antibacterial activity, from the Drug Repurposing Hub. Notably, Halicin demonstrated *in vitro* and *in vivo* efficacy against the high-priority pathogen *A. baumannii* [53], among others [52]. Given the recent increase in ML models to the discovery and design of antibiotics [54,55] and antimicrobial peptides [56–58], the Halicin discovery may not be an isolated event, but rather a sign of how AI methods may continue to assist antibiotic discovery by predicting antibacterial properties from molecular or metagenomic data in future.

Integrated metagenomics and artificial intelligence may support clinical processes

While the factors that contribute towards antibiotic resistance differ between developing and developed countries, one common thread is the increased use of antibiotics [1]. The bidirectional influence, where use drives resistance and resistance shapes use, has prompted the exploration and development of AI as a resource for antibiotic stewardship, giving rise to a new era in medicine and healthcare [59,60] (Figure 1). An important component of antibiotic stewardship is antibiotic susceptibility testing (AST), which can inform treatment options based on the efficacy of different antibiotic dosages against a bacterial pathogen. A number of laboratory methods exist to test antibiotic susceptibility. The gold standard method, disk diffusion, involves exposing bacterial agar plates to differentially dosed antibiotic-infused paper discs. The minimum concentration of antibiotic effective against the infection can be determined by measuring the inhibition of bacterial growth around each disk. Automated disk diffusion interpretation can improve its reliability by mitigating the potential for measurement variability between

Figure 1



Integrated metagenomics and AI methods support different clinical processes associated with antibiotic resistance control.

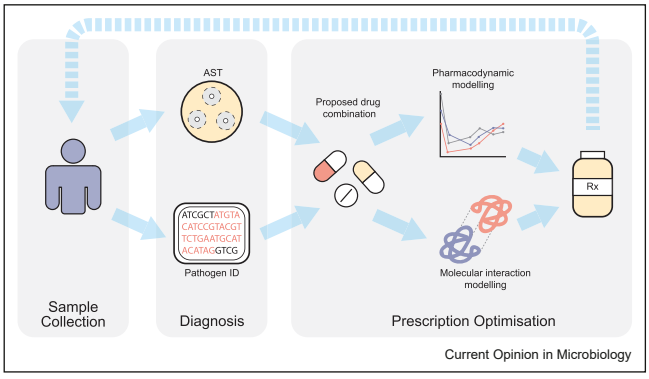
human analysts [61]. In a recently developed mobile app, ML algorithms are used to process images of disk diffusion assays, through the measurement of inhibition zones and the ID of the antibiotic used [62]. Importantly, the app does not require an Internet connection, and is therefore suitable for use in developing countries where clinical misuse is a primary contributing factor to antibiotic resistance [1].

ML models have also been applied to other methods of AST, including flow cytometer-assisted antimicrobial susceptibility testing (FAST), which utilises fluorescent dye uptake to measure the integrity of bacterial cells after antibiotic exposure [63]. This is similar to dynamic laser speckle imaging, where cell viability is determined by detecting changes in cellular motion following antibiotic treatment [64]. In these instances, the primary benefit conveyed by ML is speed, whilst also maintaining accuracy. Diagnosis using AST can take several days [65], which is impractical when treating time-sensitive cases such as severe sepsis. In the aforementioned examples, the FAST method provided same-day predictions of inhibitory antibiotic concentrations [63], while the rapid testing method developed by Zhou and colleagues could predict the minimum inhibitory concentration of two antibiotics for a model *Escherichia coli* strain in one hour [64]. The clinical implementation of such methods could guide, and potentially accelerate, appropriate antibiotic administration, limiting the exacerbation of antibiotic resistance in time-critical settings such as the intensive care unit [66]. Prior small-scale application of ML methods to hospital AST data supports their viability in larger clinical contexts as a tool to potentially improve empiric antibiotic prescription [67].

Another potential benefit of AI-guided antibiotic therapies is a reduction in the clinical reliance on broad-spectrum antibiotics in cases where they are unnecessary [68]. The treatment of urinary tract infections (UTIs), for example, is increasingly reliant on broad-spectrum antibiotics given the growing number of antibiotic-resistant pathogens [69,70]. For this purpose, Kanjilal et al recently developed a ML algorithm that can use electronic health records to predict antibiotic susceptibility profiles and subsequently facilitate appropriate antibiotic prescription for uncomplicated UTIs [70]. While the authors note that further development and testing are required before the algorithm is incorporated into the clinical workflow, it is plausible that a similar concept could also be applied to the treatment of other bacterial infections. Indeed, it has been proposed that the consultation of AI models for this purpose may become a routine aspect of antibiotic stewardship within the next decade [71] (Figure 2).

Metagenomics data and their analysis, too, can support antibiotic stewardship in the clinical setting. Clinical metagenomics concerns the application of sequencing technologies to clinical investigations, which is of particular interest for its potential to bypass the requirement for laboratory culture in infection diagnosis. The emerging field is still largely spoken about in terms of its potential or promise, rather than its *bona fide* impact as yet on the clinical workflow, due to the challenges that must be overcome before its clinical implementation. These include the differentiation between typical microbial colonisation and infection [72], as well as the high level of training needed for sample handling [73]. However, clinical metagenomics could markedly reduce the time taken to process patient samples and determine the putative pathogens causing infection, as well as any ARGs they may be harbouring [65]. For example, the Oxford Nanopore sequencing of plasmids, which are common carriers of ARGs, can generate shallow sequencing reads that can be used to annotate ARGs within 20 minutes [74]. Recent studies have demonstrated the use of Oxford Nanopore-based clinical metagenomics to rapidly and accurately detect ARGs to diagnose lower respiratory tract infection [75,76] and sepsis [77]. As metagenomic analyses are not limited to those bacteria that can be cultured in laboratory settings, diagnosis using clinical metagenomics is not associated with the same level of bias as conventional laboratory culture-based approaches [78], therefore, its integration into AI models may further support their application to clinical decision-making.

Figure 2



Hypothetical infection treatment pipeline assisted by metagenomics and AI methods. Left panel: a patient presents with an infection and samples are collected. Centre panel: AST interpretation is automated with AI, while pathogen ID is supported through metagenomic analysis. Right panel: the proposed drug combination and dosage are optimised through pharmacodynamic modelling, and the interactions between drug and target bacterial protein are verified through three-dimensional modelling.

Barriers hindering integration of artificial intelligence into clinical practice

Various barriers currently prevent the routine incorporation of AI into the clinical workflow. Patient trust, for example, is an important aspect of any treatment, and will not necessarily be given in equal measure to a complex AI algorithm as to the more familiar clinician. Consequently, it will be pertinent to the perception and potential successful integration of AI that the public are informed of AI's role in 'augmented intelligence', as conceptualised by the American Medical Association [79,80], where AI is not viewed as a replacement for human decision-making, but rather as a tool to improve data evaluation. Care must be taken in the application of AI and ML algorithms, given their potential for bias stemming from the limitations of their training dataset. In a clinical setting, these biases could prevent the prediction of novel antibiotic resistance mechanisms. Alternatively, they may limit the generalisability of AI models where the training dataset did not capture appropriately diverse human demographics, compounding inequities in the response to and impact of antibiotic resistance [81]. Novel features of AI-based technologies, such as the ability to function without an Internet connection [62], are an important step towards an equitable antibiotic resistance response, and should be considered in the development of future resources. The cost-effectiveness of AI in clinical settings will depend on the accessibility of clinical data [67]. However, we also suggest that additional barriers associated with cost and the requirement of specialist equipment may be limited to the short term, given the rapid nature of AI development.

For clinicians, appropriate training and technical expertise will be necessary to analyse and interpret AI data. Moreover, the transparency and, by association, explainability of AI algorithms will be important, as well as the extent to which they can be applied across a given population [82]. The clinical application of AI to sepsis management, specifically, has been debated [83,84] due to the leap between research advancements and their genuine capability to handle the complexity of clinical sepsis management. Legal and ethical barriers, including privacy issues [82], as well as accountability issues if a flawed AI model was to mislead a clinician [85], also need to be addressed and regulated. The exact nature of this regulation will be dependent on local jurisdiction. Example AI technologies such as the algorithm that can reduce unnecessary broad-spectrum antibiotic prescription for UTIs [70], the rapid AST methods that could expedite empiric antibiotic prescription [63,64], or the mobile application that can analyse and interpret AST data [62], highlight the promise of real-world AI application to the antibiotic resistance crisis. However, they also illustrate the need for further development and clinical testing before the benefit conferred by AI on both the patients and clinicians can be fully appreciated. Focusing future research efforts to overcome these barriers will determine the true impact that AI can make on antibiotic stewardship and the wider response to antibiotic resistance, an avenue certainly worth pursuing given the potential it has already demonstrated.

Conclusion

It is undeniable that antibiotic resistance is an issue demanding global action to prevent further magnification of its already-present economic and public health burden. The human response to the antibiotic resistance crisis is based around a key question of how to detect and characterise evolution in bacterial systems of interest. The ability of ML, specifically, to improve its own accuracy over time, whilst also functioning independently of continuous human input, may prove an indispensable tool against the rapid evolutionary capacity of bacteria. The implementation of AI methods in real time to predict bacterial antibiotic resistance profiles could complement the decision-making process that underlies antibiotic prescription, reducing unnecessary or unsuitable usage. Moreover, the structural and functional prediction of proteins and other molecules by AI methods has the potential to revolutionise future antibiotic drug discovery through relatively rapid and inexpensive means, by repurposing existing drugs or optimising antibiotic combinations for the treatment of multidrug-resistant bacteria. Future research efforts should focus on developing ML algorithms that can accurately predict molecular interactions to model the interaction of antibiotic treatment combinations, as well as those between antibiotics and their target bacterial

proteins. Further applications of ML to hospital AST data will eventually enable hospital-wide empiric antibiotic prescription. Continued efforts to develop metagenomic sequencing technologies with lower operation costs and that produce longer reads will facilitate its wider application to ARG surveillance. Additional research, employing both metagenomics and AI, is also required to address outstanding questions regarding the possible link between climate change and antibiotic resistance. It is important to conclude by emphasising that despite its increasing application to antibiotic resistance research and mitigation, AI should not be viewed as a replacement for human clinicians, or even for other computational resources such as metagenomics that can provide critical information on the evolution and spread of ARGs. Instead, the two technologies, metagenomics and AI, may function in a complementary, or even synergistic, nature to support human decision-making and minimise the impact of the current antibiotic resistance crisis on humanity.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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