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REVIEW

Antimicrobial resistance in the next 30 years, humankind, bugs and drugs: a visionary approach

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Abstract

Purpose: To describe the current standards of care and major recent advances with regard to antimicrobial resistance (AMR) and to give a prospective overview for the next 30 years in this field.

Methods: Review of medical literature and expert opinion were used in the development of this review.

Results: There is undoubtedly a large clinical and public health burden associated with AMR in ICU, but it is challenging to quantify the associated excess morbidity and mortality. In the last decade, antibiotic stewardship and infection prevention and control have been unable to prevent the rapid spread of resistant Gram-negative bacteria (GNB), in particular carbapenem-resistant *Pseudomonas aeruginosa* (and other non-fermenting GNB), extended-spectrum β-lactamase (ESBL)-producing and carbapenem-resistant Enterobacteriaceae (CRE). The situation appears more optimistic currently for Gram-positive, where *Staphylococcus aureus*, and particularly methicillin-resistant *S. aureus* (MRSA), remains a cardinal cause of healthcare-associated infections worldwide. Recent advancements in laboratory techniques allow for a rapid identification of the infecting pathogen and antibiotic susceptibility testing. Their impact can be particularly relevant in settings with prevalence of MDR, since they may guide fine-tuning of empirically selected regimen, facilitate de-escalation of unnecessary antimicrobials, and support infection control decisions.

Currently, antibiotics are the primary anti-infective solution for patients with known or suspected MDR bacteria in intensive care. Numerous incentives have been provided to encourage researchers to work on alternative strategies to reverse this trend and to provide a means to treat these pathogens. Although some promising antibiotics currently in phase 2 and 3 of development will soon be licensed and utilized in ICU, the continuous development of an alternative generation of compounds is extremely important. There are currently several promising avenues available to fight antibiotic resistance, such as faecal microbiota, and phage therapy.

Keywords: Antimicrobial resistance, Antibiotics, Diagnostic test, Microbiota, Phage therapy, Vaccine

Introduction

Several factors contribute to the high incidence of sepsis in the intensive care unit (ICU) and to the associated poor patient outcomes. One of the most important drivers of the unfavourable outcome is multi-drug resistant (MDR) and increasingly, extensively-drug resistant (XDR) bacterial organisms. The recent identification of new plasmid-mediated genes that confer resistance to colistin emphasises a crisis that is estimated to cause 10 million deaths per annum by 2050, result in huge morbidity and wipe out in excess of USD 100 trillion from the world's economy [1] (Fig. 1).

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Antimicrobial resistance in 2050

In the last decade, antibiotic stewardship (AS) and infection prevention and control (IPC) measures have been unable to prevent the rapid spread of resistant Gram-negative bacteria (GNB), in particular carbapenem-resistant *Pseudomonas aeruginosa* (and other non-fermenting GNB), extended-spectrum β-lactamase (ESBL)-producing and carbapenem-resistant *Enterobacteriaceae* (CRE), notably due to carbapenemases. For example, in the south of Europe, approximately 25–50% of *P. aeruginosa* isolates are carbapenem resistant, with up to 10–50% of strains classified as MDR [2]. In non-fermenting GNB, MDR may emerge following sequential chromosomal mutations; however, of more concern is their ability to acquire mobile genetic elements that encode ESBL and carbapenemase genes [2]. Equally worrying are colistin-resistant isolates of *Acinetobacter baumannii* which are now increasingly reported worldwide, especially in patients previously exposed to this agent [3].

A recent meta-analysis that included 66 papers found a pooled prevalence of colonisation of healthy individuals in the community by ESBL-producing *Enterobacteriaceae* to be 14% (95% confidence interval 9–20%), and the authors estimated that this was likely to increase by 5.38% annually [4]. Thus, in 2050, although it remains to be seen what percentage of patients in the ICU will be colonised by ESBL-producing pathogens on admission and what impact this will have on patient outcome, it is likely to be substantial. It is also predicted that without improved and/or alternative or novel infection control strategies CRE would become an even more important cause of healthcare-associated infections (HCAIs) [5].

Currently, the relative efficacy of different IPC strategies for the prevention of MDR-GNB in adult ICUs suggest that compared to standard care alone, a four-component strategy consisting of standard care, stewardship, environmental cleaning and source control was the most effective intervention; however, no evidence of an association between IPC interventions and ICU mortality was found, and neither was an association observed between the number of intervention components and ICU mortality rate [6]. Whereas rapid genotypical detection of CRE and subsequent implementation of IPC precautions may prevent transmission of isolates, these steps would not impact on horizontal genetic transfer within and between the species that colonise patients. In light of this, prevention would require a paradigm shift, including the establishment of novel IPC and novel management strategies that are responsive to specific gene-based transmission [7]. Finally, it is likely that novel mechanisms of resistance that occur in response to selection by existing or new antibiotic classes are likely to emerge and even disseminate globally, perhaps in a manner similar to plasmid-mediated colistin resistance (provided by *MCR-1* gene) [8].

Staphylococcus aureus, and particularly methicillin-resistant *S. aureus* (MRSA), remains a cardinal cause of HCAIs worldwide. However, the current situation appears to be more optimistic compared to that for GNB. Several factors drive this optimism, including the availability of active and new antimicrobials and shifting

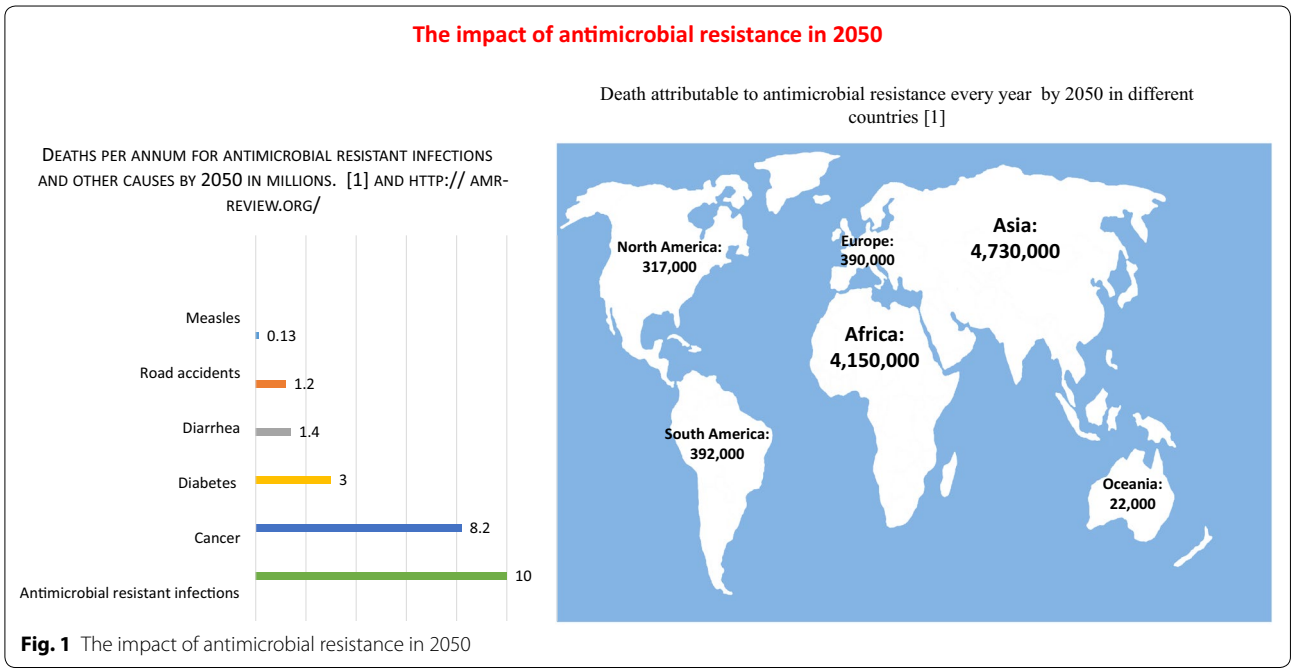

Fig. 1 The impact of antimicrobial resistance in 2050

epidemiology, especially with regards to declining rates of MRSA bloodstream infections across countries [9].

Irrespective, perhaps of greater concern is the alarming loss of distinction between community- and healthcare-derived infections. In this regard, there has been a steep worldwide increase of community-acquired MRSA infections and a concurrent transfer of healthcare-associated resistant clones to the community with unpredictable but probably negative consequences by 2050 [10].

Resistance to newer agents (daptomycin and linezolid) remains rare based on data from several large surveillance studies. Ceftaroline is the exception, with marked regional differences of resistance up to 24 and 47% of MRSA isolates recently documented in Europe and China, respectively [11]. However, the picture in terms of vancomycin-resistant *Enterococcus faecium* (VRE) infections is less favorable with ever increasing rates of infections and rising resistance. Unlike MRSA, for which resistance remains uncommon, linezolid resistance or daptomycin non-susceptibility is becoming an important consideration when treating patients. How to effectively control this organism remains unclear.

Future perspectives to limit the spread of antimicrobial resistance from now to 2050

The evolution of the numerous and diverse micro- and macro-level resistance determinants makes it very challenging to predict what the situation will be in 2050 [12]. Another confounder will be the success of potential control measures and interventions designed to influence the emergence and dissemination of AMR (Fig. 2).

In this regard, curtailing the volume of antibiotics consumed by food animals, as a standalone measure, is likely to have little impact on the level of resistance in humans [13]. Thus, the conflicting objectives of upholding animal welfare and food security on the one hand and keeping the interests of human health in mind on the other will necessitate instigation of One Health (including not only food animals but both agricultural and aquacultural industries) stewardship involving governance with widespread stakeholder engagement, surveillance and interventions at the country level for shared-use antibiotics.

The organisation and effectiveness of quality improvement proGrams to combat AMR requires participation and commitment from a multi-disciplinary team and that includes the hospital administration. Addressing HCAIs and AMR from an organisational perspective has seen tremendous progress in the application of organisational factors as a critical component of IPC and stewardship, particularly with regard to the breaking down of the "silo mentality" that so often plagues institutions [14]. These tasks, so often the responsibility of small, discreet groups of infectious diseases, microbiology and infection control teams, are increasingly being recognised to be a group

Pathogen and microbial ecology

Determinant	Potential control measures and interventions
Evolution	Evolutionary engineering
Survival fitness	Inhibition of gene expression
Virulence	Anti- virulence strategies - Targeting toxins and secretion systems - Targeting signalling and regulation - Targeting biofilms and adherence - Biological response modifiers
Constitution of microbiome	Prebiotics Probiotics Faecal microbiota transplantation
Laboratory detection, identification and antimicrobial susceptibility testing	Improved rapid microbial diagnostic tests Real-time whole (meta)genome sequencing

Clinician prescribing practices

Determinant	Potential control measures and interventions
Training and knowledge	Under-and post graduate training Targeted educational interventions
Antimicrobial prescribing patterns	Multi-modal stewardship interventions
Prescribing heterogeneity	Decision support tools
Accountability	Individualized audit and feedback with comparative benchmarking Institutional regulations
Behaviour change	Targeted behaviour change techniques and interventions
Diagnostic uncertainty	Novel biomarkers and diagnostic tools Rapid, bedside molecular diagnostics

Population characteristics

Determinant	Potential control measures and interventions
Migration, travel and globalization	Screening and improved global surveillance from a "One health" perspective
Case mix and host susceptibility	Improved management of chronic comorbid diseases Vaccination
Antimicrobial demand and beliefs	School education and public information campaigns
Transmission and infection rates	Sustainable hand and food hygiene behaviour change Improved environmental cleaning and auditing

Politics and health-care policy

Determinant	Potential control measures and interventions
Healthcare policy	Change in reimbursement practices
Promotional industry activities	Regulation Novel antimicrobial discovery, development and marketing models
Antimicrobial use in food production	Novel agents for growth promotion and meta-prophylaxis
Technological research and development	Novel treatment and prevention approaches

Fig. 2 Potential determinants influencing future dissemination and control of antibiotic resistance. Reproduced and adapted with permission from Harbarth and Samore [12]

responsibility. For AMR activities to be embedded into routine clinical practice they ideally need to be formalised in legislative, regulatory and organisational frameworks, which will potentially result in shifting the legal risk or emotional drivers for prescriptions away from the individual, thus allowing for a "zero antibiotic" approach to be considered and to be feasible [15].

The unprecedented volume of information derived from whole-genome sequencing (WGS) is a powerful tool in the identification of and response to infectious diseases that pose a threat to public health. WGS enables the analysis and identification of all resistance genes and their precursors (the "resistome"), the genetic determinants of toxin production (the "toxome") and virulence factors (the "virulome"). Accurate and comprehensive understanding of the transmission dynamics and knowledge of clonality could prevent dissemination of more lethal strains in an ICU. In fact, advances in WGS have been made: core genome multi-locus sequencing typing (cgMLST) analysis for real-time surveillance of resistant HCAIs and the practical aspects of implementing this type of intervention in a large, tertiary care facility have recently been described [16].

Critically ill patients are likely to have a unique "microbial footprint" which could lead to infection and dissemination and further to environmental contamination. Thus, the composition of the gastro-intestinal (GIT) microbiome (GiMb) has specific relevance with regard to acquisition, colonisation and subsequent infection with MDR bacteria in ICU patients. The composition of the GiMb is associated to the degree of "colonisation resistance", which is altered after an antibiotic exposure, thereby increasing susceptibility to colonisation by pathogenic bacteria [17].

New diagnostic tests?

Broad antimicrobial coverage to ensure adequate treatment and optimal survival in critically ill patients is acknowledged as a major argument for antibiotic overuse in the hospital setting, resulting in continuous pressure towards selection of resistance [18, 19].

Rapidity and acuity of microbiological diagnosis may impact significantly on AS and antibiotic consumption. Recent advancements in laboratory techniques allow for a rapid identification of the infecting pathogen and antibiotic susceptibility testing (AST). Their impact can be particularly relevant in settings with a prevalence of MDR microorganisms, since they may guide fine-tuning of an empirically selected regimen, facilitate de-escalation of unnecessary antimicrobials and support infection control decisions [20]. New diagnostic technologies with the potential of reduced ID and/or AST compared to conventional microbiology include matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS), rapid immunochromatography, molecular biology and automated time-lapse microscopy [21–24]. These new technologies are currently quite expensive in terms of acquisition and reagent cost and cannot replace standard culture approaches; furthermore, they require considerable manpower of qualified staff for a 24/7 readiness.

MALDI-TOF MS has already proven efficacious in rapid bacterial identification from isolated colonies or monomicrobial blood cultures, whereas detection of certain resistance genes can be provided with add-on software [25]. Mass spectrometric beta-lactamase assays censoring enzymatic activity of the beta-lactamases can be applied to freshly tagged positive blood cultures after a 1- to 4-h incubation period [25]. However, there is still way to go until identification of the type of beta-lactamases by this method [26]. Currently available molecular biology-based diagnostic platforms can detect several genes mediating antibiotic resistance from bacterial cultures or rectal swabs (e.g. Xpert Carba-R, Cepheid, Sunnyvale, CA or Check-Direct CPE for carbapenemases, Check-Points, Wageningen, The Netherlands). Other diagnostic platforms can detect resistance genes together with the identification of the pathogen from positive blood culture bottles within 1 h (e.g. FilmArray BC-ID or Verigene) [27].

A PCR/electrospray ionisation-mass spectrometry platform, the IRIDICA infectious disease diagnostics platform (Abbott Laboratories, Chicago, IL), represents a significant advancement in the field. This tool is able to detect in approximately 6 h more than 750 bloodstream infection-relevant pathogens and identify antibiotic resistance genes. The IRIDICA BAC LRT assay performed in bronchoalveolar lavage (BAL) samples demonstrated equal or superior sensitivity to conventional methods.

An important technical aspect of these methods would be that they can be performed directly on clinical samples, which would reduce time to pathogen identification and AST to a minimum; based on recent publications such methods will probably be routine by 2050. The application of new sequencing methods to clinical samples is especially promising as it potentially identifies the genomes of all putative microorganisms in a sample and their antibiotic resistance determinants. The latest generation of sequencers (Oxford Nanopore Technologies, Oxford, UK) are smartphone-sized and can provide results in <6 h. However, apart from rapidity, which has already been gained with molecular diagnostics, the most important challenge is the ability to include these technologies in the context of point-of care (POC) tests. Furthermore, multiple tests can be combined into a POC platform, facilitating pathogen identification and AST.

POC tests would alleviate the need of laboratory readiness 24/7 for PCR testing; however, the necessity for human interaction in the form of multi-disciplinary AS teams will still be indispensable, as mentioned above. Costs will probably be reduced as well due to a lower required workload [28].

Successful paradigms of application of rapid tests in the nosocomial setting is MRSA detection from clinical samples using PCR and POC tests [28, 29]. In a recent multicentre randomised controlled trial, molecular detection of pathogens in addition to standard blood culturing increased the rates of microbial diagnosis and shortened the time to onset of a species-specific regimen [30].

An additional ability of POC tests is the potential to include inflammatory parameters. The addition of inflammatory panels in multiplex POC tests to identify pathogens and/or resistance mechanisms would be a revolution in the holistic assessment of the critically ill patient; this is an important challenge to be implemented by 2050. Although a biomarker-driven diagnostic approach has not been proven as a gold standard, POC tests measuring procalcitonin and C-reactive protein and/or other parameters may provide important guidance on antibiotic discontinuation [31]. Finally, in the setting of critically ill ICU patients with a suspicion of lower respiratory tract infections, breath volatile analysis requires more attention and research. The detection of patterns of volatile organic compounds in breath, the so-called "e-nose", has been adopted to identify patients with malignancies or lung diseases (colorectal cancer, head and neck cancer, tuberculosis, cystic fibrosis etc.) [32]. Early data comparing the predictive value of e-nose to the clinical pulmonary infection score or to chest computer tomography in the prediction of ventilator-associated pneumonia (VAP) have been promising [33, 34]. However, more recent data comparing breath volatile analysis to BAL failed to prove a distinctive ability between VAP and ventilator-associated tracheobronchitis or colonisation, but this diagnostic modality may be further developed by 2050 [35].

There is a long way to go before POC tests are routinely applied in clinical practice. However it seems to be a promising diagnostic approach to reduce antimicrobial resistance by 2050.

How to fight antimicrobial resistance in the future?

At the present time, antibiotics are the primary anti-infective solution for patients with known or suspected MDR bacteria in the ICU. Numerous incentives have been provided to encourage researchers to work on alternative strategies to reverse the resistance trend and to provide a means to treat these pathogens. There are currently several promising avenues available to fight antibiotic resistance (Table 1).

The intestinal microbiota is the battlefield of the war against MDR

In our battle against MDR bacteria, our intestinal microbiota could be our best ally provided that it remains unaltered. Interestingly, our intestinal microbiota is resistant to the sustained colonisation by exogenous bacteria (including the MDR ones), which is referred to as colonisation resistance (CR) [36, 37]. CR is mainly exerted by anaerobes [38], yet the precise bacterial species underlying CR are still to be identified [39]. As a consequence, antibiotic exposure has potentially a dual side effect on the microbiota: alteration of CR and selection of resistant bacteria over the susceptible ones, which together pave the way for the intestinal acquisition and expansion of MDR bacteria [40–43]. Of note, the higher the intestinal relative abundance of MDR bacteria, the higher the risk that they would be involved in infections [43, 44], disseminated in the environment [40] and carried in the long term [45]. Accordingly, preserving the microbiota against antibiotics and/or restoring when it is altered are promising strategies against MDR bacteria (Fig. 2).

Indeed, the precise effect of antibiotics on the intestinal microbiota with respect to the acquisition and expansion of MDR bacteria remain to be studied in order to help clinicians to choose for the more ecologically friendly drug [46]. One way to preserve the microbiota from antibiotics is to remove the antibiotic residues active in the colonic space where the highest concentrations of intestinal bacteria are found. Two options are currently being investigated. The first is the use of an engineered, broad-spectrum beta-lactamase that aims at decaying any beta-lactam residues in the gut [47]. Currently in phase II testing, SYN-004 (an orally delivered beta-lactamase developed by Synthetic Biologics, Rockville, MD) was shown to efficiently remove ceftriaxone residues in dog and pig models [47]. The second option is DAV-132 (also in phase II), a colon-delivered active charcoal which aims at adsorbing free colonic compounds (including antibiotics) (developed by DaVolterra Co., Paris, France) [48]. In mice, a specific formulation of DAV-132 was interestingly found to lower the intestinal concentrations of ESBL-producing *Klebsiella pneumoniae* [49].

Once altered, the most efficient way to restore the integrity of the intestinal microbiota is currently fecal microbiota transplantation (FMT). A recent paper showing its potent activity against recurrent *Clostridium difficile* infections (CDI) [35] has renewed clinical interest in FMT for this indication and others. Indeed, a common "collateral damage" observed after FMT is the intestinal clearance of MDR bacteria likely via the restoration of CR [50]. To date, only case reports have been published, but six trials using FMT to decolonise the microbiota from MDR bacteria are currently registered

Table 1 Major types of new antimicrobial compounds with their mechanisms of action [69]

Future therapy	Mechanism of action	Current research status
Antimicrobial peptides	Mainly cellular membrane damage	A good number of synthetic AMPs and at least 15 peptides or mimetics are undergoing advanced clinical trials or have completed trials as antimicrobial or immunomodulatory agents
Phage therapy	Use of lytic phages to kill bacteria	Few clinical trials (regulatory issues), for topical applications
Eligobiotics	CRISPR-Cas9 system injected by a phage	Pre-clinical
Phage endolysins	Use of a phage endolysin instead of the whole phage	Phase II
Anti-virulence factors	Adjuvants or adjunct therapies to complement the use of antibiotics. They target specific virulence factors or pathways in selected pathogens (mostly investigated as anti-toxins for *Staphylococcus aureus*)	Pre-clinical
Phytochemicals	Multiple actions	Novel antimicrobial structures have been created synthetically. Synthetic analogs and modification of the structure may lead to the discovery of novel structures with a broader spectrum of activity
Metalloantibiotic	Increased spectrum of conventional antibiotic action	Although interactions with essential metal ions make more controllable conditions for bacterial infections, the unwanted side effects like toxicity and hemolytic activity sometimes increase simultaneously. So the potential for oral administration or internal use may be hampered
Efflux pump inhibitor	Molecules to inhibit the active protein pump in the bacterial cell	The major advantage of efflux pump inhibitors is the possibility for slower development of resistance by the target bacteria. Several disadvantages have also been documented, including their chemical synthesis due to bulky structure, solubility or permeability problems, requirement at higher concentration and chance of decreased activity at one or both target sites for steric or electronic configurations, unless these molecules are carefully designed
LPS inhibitors	Inhibitor of an enzyme important in LPS pathway	

AMPs, Antimicrobial peptides; LPS, lipopolysaccharide; CRISPR-Cas9 system, genome editing tool

[51–55], and these should provide further insights in the efficacy of FMT in clearing MDR bacteria from the gut while maintaining safety. Importantly, FMT comes with a risk of pathogen transmission even though an extensive screening is performed and donors are carefully selected. These risks could be overcome by autologous FMT, i.e. the administration of the patient's own feces that would have been preserved before a scheduled alteration of the microbiota (such as an antibiotic exposure), but also by the use of semi-synthetic [56] or synthetic microbiota [57]. Nonetheless, further studies aimed at identifying the precise bacterial strains and specific functions associated to the clearance of MDR bacteria will be the prerequisite to designing a bacterial consortium capable of eradicating MDR bacteria from the GIT. The potential role and benefit or otherwise of selective decontamination for MDR GNB carriage in light of novel interventions need to be clarified as current evidence suggests a transient rather than sustained effect on MDR carriage [58].

Phage therapy

Phages are viruses that specifically target bacteria. They can either stay in the chromosome of their host, their DNA being replicated along with that of the bacteria (lysogenic cycle), or they can replicate in the cytoplasm of the bacteria, make dozens of virions and lyse the bacterial cell in order to disseminate and infect other surrounding hosts in an exponential fashion (lytic cycle) [59]. Nowadays, the antibiotic dead-end caused by MDR has put phages back into clinicians' scope of interest [60]. Strikingly, phage therapy remains poorly characterised in terms of efficacy, pharmacokinetics, immunisation, safety, tolerance and selection of resistance, partly because the publications from the East could not reach the West. Recently, they have been successfully used in otitis mediated by *P. aeruginosa* [61], and they appeared to be well-tolerated when orally administered [62]. Also unexpected is the limited number of registered clinical trials involving phage therapy in bacterial infections, as only two multi-centre clinical trials of phage therapy for human infections are currently running, one on diabetic foot ulcer [63] and one on burns [64].

A serious advantage of phages over antibiotics is that they are highly specific. Indeed, there are no "broad-spectrum" phages that can infect several bacterial species, rather they infect a given species at best, and most of time a sub-population of strains within a species. Hence, one possibility in the case of infections is that a broad-range cocktail of phages (empirical phage therapy) could be administered, before the pathogen is cultured, followed by susceptibility testing (like an antibioGram) to ensure that the right phage for the pathogens causing the infection has been chosen. Clinicians then would have a deadly, highly specific phage preparation towards a specific pathogen that would leave other surrounding bacteria unharmed. Conceptually speaking, this would be the perfect weapon to decontaminate MDR bacteria from the GIT, as only MDR strains would be targeted while commensal strains would be spared.

Phages will likely not replace antibiotics, but phage therapy shall nicely complement them. Indeed, phage therapy has intrinsic disadvantages that limit the extent of their use, one of which is that they cannot be administered intravenously since they are destroyed by the immune system. Hence, bacteria located at locations deep in the body and intracellular bacteria may barely be accessible to phages, even though a precise injection of phage may be possible in some cases. Accordingly, phage therapy will be focusing on infections where bacteria are easily accessible, such as wounds and possibly pneumonia (phages could be administered via aerosols). In addition, phages raise regulatory issues that need to be resolved as there is currently no framework for phage therapy [65], and interventions are performed under the Helsinki convention (that applies for situations where no other available therapeutic options are available). Last but not least, phages as natural products lack the attraction for investors since they are not patentable.

In vitro experiments have indeed shown that such engineered phages could selectively kill MRSA (bearing the *mecA* gene against which the RNA guide was targeted) over the methicillin-susceptible counterparts [66]. Moreover, eligobiotics could also be used to "vaccinate" susceptible bacteria against resistance genes, thereby teaching the former how to recognise and degrade bacteria when they meet. Eligobiotics are currently being developed in pre-clinical stages by the Eligo Biosciences hosted at the Institut Pasteur of Paris (France).

Instead of using living phages as antibacterial agents, it has been proposed to use the endolysin itself. Hence, a recombinant form of a phage endolysin, named N-Rephasin SAL200, which targets staphylococcal infections is being developed by iNtRON Biotechnology, Seoul, South Korea (currently in phase II) [67, 68]. Likewise, lysin CF-301 was found to be active against *S. aureus* (including MRSA) in murine models and is now being developed by ContraFect (Yonkers, NY; phase I trials completed).

Other alternatives to antibiotics

A dedicated, exhaustive review on alternatives to antibiotics has recently been published [69]. In the following sections we summarise the various solutions that are being developed in parallel to antibiotics.

Antibodies: passive immunisation

Antibodies have long be designated potential weapons against bacteria, with the first applications using sera. To overcome the issue of immune reaction against monoclonal antibodies (mAb), they are now humanised. Like phages, the advantage of antibodies over antibiotics is their specificity as they are selected for their specific binding to targets of clinical interest. Hence, mAb that are being developed in this context target virulence factors: alpha-toxin of *S. aureus* [AR-301 (Aridis Pharmaceuticals LLC, San Jose, CA); MEDI4893 (Medimmune LLG, Gaithersburg, MD); ASN100 (Arsanis Biosciences GmbH, Vienna, Austria)], the type III secretion system of *P. aeruginosa* [KB001 (Kalobios, Brisbane, CA); MEDI3902 (MedImmune LLG)], the lipopolysaccharide of *P. aeruginosa* [AR-101 (Aridis Pharmaceuticals LLC)], the toxin B of *Clostridium difficile* [bezlotoxumab (Merck KGaA, Darmstadt, Germany)], extra-intestinal pathogenic *E. coli* (ExPEC) virulence factors [70], and the poly-beta-1,6-*N*-acetylglucosamine (PNAG) that is found in the biofilm of several bacterial (including *S. aureus*, *Enterobacteriaceae*, *Acinetobacter baumannii* and *Burkholderia cepacia* which are among the most frequent MDR bacteria), fungal and eukaryotic pathogens [71]) targeted by Alopexx F598 (Alopexx Enterprises, LLC, Concord, MA). Taken individually, KB001 has failed to meet efficacy criteria in a phase 2 trial of cystic fibrosis patients after showing promising results in animal models and has now been withdrawn. Still, this target is still being explored by Medimmune, with the product MEDI3902 currently in phase 2 testing and shown to be superior to KB001 in murine model [72]. The same company is also developing a MEDI4893 targeting *S. aureus* alpha-toxin that was showed to reduce the risk of pneumonia in a murine model [73]. Antibodies targeting PNAG (Alopexx F598) have been shown to protect mice against PNAG-producing MDR Enterobacteriaceae and *P. aeruginosa* [74]. Bezlotoxumab has also been shown to be efficient in preventing the recurrence of CDI when compared to placebo (7 vs. 25%) [75] and will be the first mAb to reach the market as an antibacterial mAb.

Vaccines: active immunisation

In addition to the antibodies targeting toxins A and B of *C. difficile*, a vaccine is being developed by Sanofi-Pasteur (Lyon, France) (currently in phase 3) and Valneva (Lyon, France) (phase II completed in August 2016) [76]. Together with bezlotoxumab and FMT/synthetic microbiota, we shall soon have several options to prevent and cure CDI by 2050. Likewise, the same PNAG that is targeted by mAb (Alopexx F598) is also being developed as a vaccine (AV0328, in phase I/II, trial no NCT02853617). Of note, unlike natural antibodies to PNAG, the deacetylated glycoform conjugated to carrier proteins is effective in conferring immunity [77]. In addition, a vaccine against MDR *A. baumannii* is also under investigation at the preclinical stage [78]. Eventually, one should keep in mind that the overuse of antibiotics can partly be attributed to the difficulty of diagnosing viral infections from bacterial ones. Hence, vaccination against viral agents, such as influenza, shall indirectly limit the use of antibiotics [79].

Anti-virulence therapy

The concept of anti-virulence therapy aims at attenuating bacterial infections without decreasing the growth of pathogens. Quorum sensing (QS) is used by bacteria to coordinate the expression of virulence factors when their density is high. Hence, the inhibition of QS has been pointed out as a potential target to design anti-QS therapies using quorum quenchers or even an antibiotic, azithromycin, which is a potent inhibitor of QS in *P. aeruginosa*. Still, the analysis of *P. aeruginosa* populations under azithromycin exposure showed that, unexpectedly, the inhibition of QS promoted the increase of virulent strains over less virulent ones [80]. Nonetheless, in a randomised control trial, the same group observed that azithromycin could reduce the risk of VAP in patients colonised with *P. aeruginosa* strains with a high level of QS expression [81].

Antimicrobial peptides

Antimicrobial peptides, also referred to as defense peptides, are the soul of innate immunity in invertebrates. AMPs are specific and rapidly kill their target. They are structurally diverse and include ribosomal (proteins, usually <100 amino acids) or non-ribosomal (made by enzymes) compounds. They are usually cationic (to bind the outer membrane of bacteria), amphiphiles, easy to produce (but expensive) yet unstable due to their susceptibility to proteases (especially the linear AMPs) and have a short half-life. To date, magainin is the only AMP which has be submitted for approval to the Federal Drug Administration, but it was rejected because it was assessed to be no more active than other compounds. Still, it was not toxic to humans in topical application. This rejection sent a bad signal to investors and introduced a pause in the AMP market. Nonetheless, the increase in MDR has triggered a renewal of interest in AMPs, and several companies are now developing AMPs: Ardea (AstraZeneca, Cambridge, UK) Agennix (but stopped in phase II/III due to a higher mortality rate in the talactoferrin arm), Polyphor (Roche Diagnostics, Indianapolis, IN; with POL7080 targeting *P. aeruginosa*, in phase II), NVB302 (Novacta Biosystems, Welwyn Garden City, UK; targeting *C. difficile*] [82]), and (AA139 against GNB, and AP138 against *S. aureus*; Adenium Biotech, Copenhagen, Denmark; both at preclinical stages).

Humankind, bugs and drugs by 2050: a visionary and optimistic approach

Provided that the pace of growth of scientific and social progress is maintained for the next 33 years, up to 2050, and with the wishful thinking that mankind will not again participate in the stupid disease called "generalised war", in our opinion there will be in 2050 a totally different scenario for infectious diseases and the use of anti-infective therapy. First, the concept of "empirical therapy" in 2050 will sound as an obsolete denomination used by physicians in the past. Diagnosis of most infectious processes will be made with precise, easily available techniques, practiced at the patient's bedside for critically ill patients, at the point-of-care by the primary care physicians or in the laboratories. Isolation and culture of pathogenic microorganisms will be anecdotal, if ever used, whereas applications of metagenomics will provide easy, rapid and low-cost diagnostic access. Techniques based on physiopathogenic responses of the hosts will be, in our opinion, be used much more than today, along with the development of better and more precise multiple biomarkers. Techniques based mainly on physics and chemistry with results obtained and transmitted to the personal mobile phone or "mobile communicator of health" will be a routine way of practicing medicine. As imaginative examples, can we really envision 2050 with millions of cases of a disease called influenza that is not widely preventable with an inhaled effective vaccine? Can we imagine a diagnosis that is not immediate, made bedside and based on a saliva sample? Do we really believe that antiviral treatment against influenza and other respiratory viruses is going to remain in its present status in 2050? Can we imagine that malaria is going to remain what it is today? Is it difficult to understand that bacterial sepsis would be detected in an efficient, instant or almost instant way and its resistance mechanism depicted within minutes? At the same time, the point of host response to the sepsis episode will be easy to determine and will provide a proper guide for action.

In our opinion, a great achievement of the next three decades will be the clarification of the mysteries of the human microbiota and microbiome, thus providing immense therapeutic possibilities. Antibiotic treatments leading to new antimicrobial treatments, antibacterial therapy leading to antifungal therapy, among others will also be, at least to a large degree, a reminiscence of the past.

Antimicrobial agents in 2050 for human use, in our view, will have less importance than at the present time, in terms of net amounts, even considering the anticipated expansion of human life on earth if the dreadful plague of war does not emerge. This will probably tackle resistance in human pathogens, along with other pivotal interventions related to the use of antimicrobials for animal growth and health. Important and substantial improvements in veterinary medicine, parallel to those in humans, have to be anticipated.

Another area of particular concern is the one related to the better knowledge of water and marine microbiology with the potential risks for massive interventions in that immense ecosystem. The oceans, in our opinion, will be areas of progressive human control and human interventions, and wealth but also danger (e.g. emergence of new resistance genes) is going to come from water.

Returning to individual human treatment, prevention will represent a much higher proportion in the control of infectious diseases, and treatment, when required, is going to rely less and less on antimicrobial agents and interventions against the structure and physiology of microorganisms but in the reinforcement of defense mechanisms and immunotherapy.

This optimistic vision is far from naïve. There is no question that new microorganisms and new threats are going to challenge the new generation, but predictions made in the framework that only accept our present state are not open to the appearance of new technologies, and new solutions may be too simplistic.

Abbreviations

AMR: Antimicrobial resistant; AS: Antibiotic stewardship; AST: Antibiotic susceptibility testing; CR: Colonisation resistance; CRE: Carbapenem-resistant Enterobacteriaceae; ESBL: Extended-spectrum β-lactamase; GNB: Gram-negative bacilli; GPC: Gram-positive cocci; HAI: Health-care associated infections; ICU: Intensive care unit; ID: Infecting pathogen; LNZR: Linezolid resistant; MDR: Multi-drug resistant; MRSA: Methicillin-resistant *Staphylococcus aureus*; PPC: Infection prevention and control; VISA: Vancomycin-intermediate *Staphylococcus aureus*; VRE: Vancomycin-resistant enterococci; WGS: Whole genome sequencing; XDR: Extensively-drug resistant.

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Compliance with ethical standards

Conflicts of interest

MB serves on scientific advisory boards for AstraZeneca, Bayer, Cubist, Pfizer Inc, MSD, Tetraphase and Astellas Pharma Inc.; has received funding for travel or speaker honoraria from Algorithm, Angelini, Astellas Pharma Inc., AstraZeneca, Cubist, Pfizer MSD, Gilead Sciences, Novartis, Ranbaxy, Teva. ER serves on the scientific board of MaaT Pharma, and is consultant for DaVolterra. AB

serves on advisory board for MSD and Takeda pharmaceuticals and speaker's bureau for Pfizer, MSD and Sanofi-aventis. The other authors declare that they have no conflict of interest.

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