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Review

Antimicrobial Resistance – The Silent Pandemic

Claudia Gabriele Werner*, Wolf Sittner and Frank-Dietrich Wagner

Charité Research Organisation, Berlin, Germany

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***Corresponding author:** Claudia Gabriele Werner, Charité Research Organisation, Berlin, Germany

ABSTRACT

The potential risk that bacteria, might become resistant to antibacterial medicines, has been already acknowledged by the pioneers of antibiotic research. In the 21st century, antimicrobial resistance has become a global public health problem - a silent pandemic that threatens mankind. In 2019, bacterial infections were associated with 7.7 million deaths, of which almost 1.27 million were directly attributable to antimicrobial drug resistance. In the same year, the WHO declared Antimicrobial Resistance as one of ten threats to global health.

Factors contributing to the antibiotic crisis are a low outcome of new antibiotic principles, infeasible trial conduct standards and economic limitations. As a consequence, big pharma divested own research and development over the last 20 years. Efforts to identify new active ingredients and mechanisms to overcome antimicrobial resistance have since been driven primarily by small and medium-sized companies. Recent R&D incentives in Europe and national efforts of scientists and regulatory bodies seek to tackle this big challenge.

The recent update (2024) of the WHO Bacterial Priority Pathogens includes 15 families of antibiotic resistant pathogens. Future development strategies should focus on antibiotics that are active against multidrug-resistant (especially carbapenem resistant) Gram-negative bacteria and tuberculosis. Particularly important antibiotic classes for the treatment of bacterial infections, whose efficacy must be maintained at all costs include 3rd to 5th generation cephalosporins and macrolides.

The current antibacterial clinical drug pipeline continues to be dominated by combinations of β-lactam antibiotics with ß-lactamase inhibitors and bacteriophages targeting WHO priority pathogens. Among the innovative agents there are two novel boronate-based serine ß-lactamase inhibitors (taniborbactam and xeruborbactam) which target also metallo-ß-lactamases which continue to grow in prevalence. As part of the national 'Phage4Cure' project, the clinical development of a bacteriophage-cocktail consisting of three phages directed against multidrug-resistant Pseudomonas aeruginosa was recently started at the Charité Research Organisation (Berlin, Germany).

Keywords: Antimicrobial resistance, WHO bacterial priority pathogens, antibacterial drug pipeline, metallo-ß-lactamases, microbiome modulators, bacteriophages.

1. Introduction

1.1. Historical Background

It is now around 100 years ago since the mysterious death of Lord Carnarvon during his expedition that led to the discovery of Pharaoh Tutankhamun's gold treasure in Egypt. His death within days was rumoured around the world as the "curse of the pharaoh"; in fact, the wound was infected with streptococci.

The lord was bitten by a mosquito and cut himself in the small swelling while shaving. In those times, just a small cut could cause a serious infection which depending on the hygienic conditions could result in "blood poisoning" and death.

At the same time, Gerhard Domagk in Wuppertal discovered the antibiotic effect of a dye from the sulphonamide group. Domagk's discovery of the new agent Prontosil saved not only the life of his six-year-old daughter, but also the lives of thousands of soldiers combating for Hitler's Germany. The discovery earned Domagk the Nobel Prize for Medicine in 1939, but the Nazis forbid the acceptance of the Nobel Prize. The triumph of the sulphonamides seemed unstoppable: puerperal fever was conquered in barely five years, pneumonia and meningitis lost their threat and gonorrhoea was cured within ten days in most cases. But the spectrum of sulphonamide sensitive microbes remained limited and even more alarming was the observation that previously sensitive bacteria became resistant.

According to the Scottish bacteriologist Alexander Fleming, the era of antimicrobials was however just starting. He was convinced to hold an even better antibacterial substance in his hand. It was in September 1928 that he noticed a mold on his research samples that kept a bacterial culture suppressed **(Figure 1)**. Fleming assumed that an antibacterial substance was produced by this mold and called the substance penicillin, Latin for the brush, after which the tubular mold was named. About five years later, Ernst Chain and Howard Florey succeeded to produce concentrated penicillin from a mold in their laboratory in Oxford. In 1941, the first successfully treatments with injectable penicillin were reported and penicillin became the wonder weapon in the fight against deadly microbes. The USA made the mass production of the drug - one of its most important projects alongside the development of the atomic bomb. Penicillin saved the lives of thousands of soldiers of the Allied Forces against Hitler's Germany.

Figure 1: Alexander Fleming in his Lab.

In 1945, Alexander Fleming, Ernst Chain and Howard Florey received the Nobel Prize for Medicine for the discovery and production of penicillin. In his speech in Stockholm, Fleming was prophetic: "The time will come," he said, "when penicillin can be bought by anyone, anywhere. Then there is a danger that the uninformed will administer the drug in too low a dose and make the microbes resistant rather than killing them."

Thus, the development of antimicrobial resistance, i.e. the ability of bacteria, parasites, viruses and fungi to resist these medicines, has been already acknowledged by the pioneers of antibiotic research. Any use of antimicrobials, however appropriate and conservative, contributes to the development of resistance, but widespread unnecessary and excessive use makes it worse.

The loss of effective antimicrobials might send us back to a time when we were unable to easily treat infections such as pneumonia, tuberculosis, gonorrhoea and salmonellosis. The inability to prevent infections could seriously compromise surgery and other procedures such as chemotherapy. Antimicrobial resistance (AMR) has now become a global public health problem and also a social issue.

"At the current rate of emergence and spread of antimicrobial resistance, annual loss of life is expected to reach 10 million deaths by 2050 with an estimated economic cost of $100 trillion. This is a looming global crisis, yet one which the world can avert if we take action soon."[1] **(Figure 2)**.

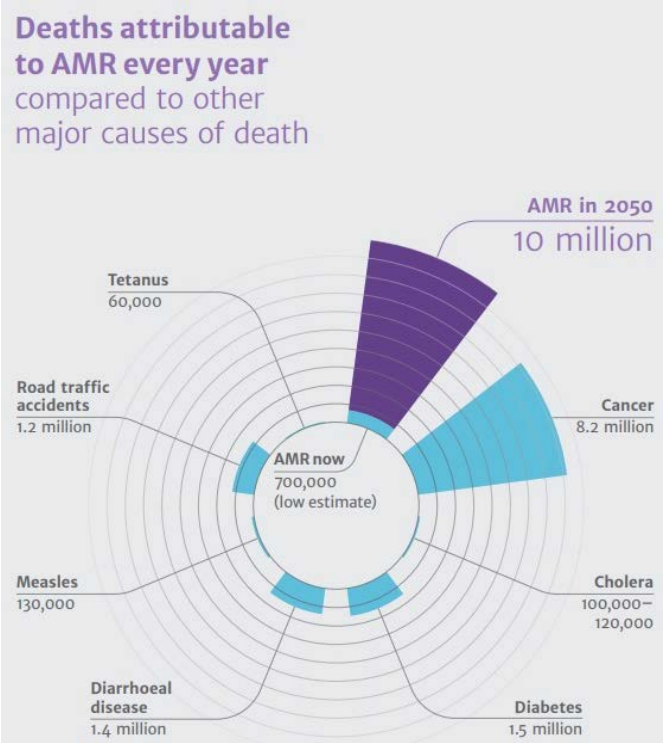

Figure 2: Annual deaths attributable to Antimicrobial Resistance (AMR).

Compared to other major causes of death

Source: Figure adopted from[1] The Review on Antimicrobial Resistance

In 2019, bacterial infections were associated with 7.7 million deaths, of which almost 1.27 million were directly attributable to antimicrobial drug resistance[2]. In the same year, the WHO declared AMR as one of ten threats to global health [WHO - Ten threats to global health in 2019]. In the WHO European region in 2019, about 541.000 deaths (95% UI 370.000–763.000) were associated with bacterial AMR (i.e., preventable by reducing infections in general) and 133.000 deaths (90.100–188.000) attributable to bacterial AMR (i.e., preventable by reducing AMR)[3].

The seven leading pathogens responsible for about 457.000 deaths associated with AMR in 53 countries in the European region were:

- Escherichia coli (E. coli)
- Staphylococcus aureus (S. aureus)
- Klebsiella pneumoniae (K. pneumoniae)
- Pseudomonas aeruginosa (P. aeruginosa)
- Enterococcus faecium (E. faecium)
- Streptococcus pneumoniae (S. pneumoniae)
- Acinetobacter baumannii (A. baumannii)

Methicillin-resistant S. aureus (MRSA) was shown to be the leading pathogen-drug combination in 27 countries for deaths attributable to AMR, while aminopenicillin-resistant E. coli predominated in 47 countries. The increase in the spread of MRSA has been halted worldwide thanks to numerous antibiotic resistance surveillance systems, infection prevention and hospital hygiene measures. In addition, the obligation to adhere to the 5 "D"s of antimicrobial stewardship in the clinics[4,5] **(Table 1)**, led to a more responsible use of antimicrobials.

Table 1: Principles of antibiotic stewardship in the clinics.

5 "D"s of antimicrobial stewardship				
Dose correct	Drug choice correct	Drug-route correct	Duration suitable	De-escalation timely to pathogen-directed therapy

Source: Doron & Davidson, 2011, DYAR et al. on behalf of ESGAP, 2017[5,6]

1.2. Surveillance systems for Antimicrobial Resistance (AMR)

National surveillance system: In Germany, the Antibiotic Resistance Surveillance (ARS) collects and analyses data from national laboratories microbiologically analysing samples from medical care facilities and medical practices **(Figure 3)**. The AMR situation was reported to be largely stable in 2022 compared to the previous year[7]:

Figure 3: Antimicrobial susceptibility testing.

The vancomycin resistance rate in E. faecium from blood cultures was 18.1% (below 20% for the first time since 2018). Also the MRSA rate declined (resistance rate was 7.1% in all samples from inpatient care and 3.8% in outpatient care anThe vancomycin resistance rate in E. faecium from blood cultures was 18.1% (below 20% for the first time since 2018), also the MRSA rate declined (resistance rate was 7.1% in all samples from inpatient care and 3.8% in outpatient care and 4.0% in isolates from blood cultures). Among gram-negative rod bacteria, the increased resistance rate to carbapenems was almost unchanged compared to the previous year in P. aeruginosa (5.4 %) and in the most frequent Enterobacterales - Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter cloacae, Citrobacter freundii (<1%), but was almost doubled in A. baumannii complex compared to the previous year (5.9% versus 3 %). Escherichia coli was found only in few isolatesd 4.0% in isolates from blood cultures). Among gram-negative rod bacteria, the increased resistance rate to carbapenems was almost unchanged compared to the previous year in P. aeruginosa (5.4 %) and in the most frequent Enterobacterales - Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter cloacae, Citrobacter freundii (<1%), but was almost doubled in A. baumannii complex compared to the previous year (5.9% versus 3 %). Escherichia coli was found only in few isolates.

International surveillance systems for antibiotic resistance: The WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) was launched in 2015 to foster AMR surveillance and inform strategies to contain AMR. The system started with surveillance of AMR in bacteria causing common human infections and has expanded its scope to include surveillance of antimicrobial consumption (AMC), invasive fungal infections and a One Health surveillance model relevant to human health. As of the end of 2022, 127 countries, territories and areas (CTAs) participate in GLASS. WHO also received data from the Central Asian and European Surveillance of Antimicrobial Resistance network (CAESAR), the European Centre for Disease Prevention and Control including the European Antimicrobial Resistance Surveillance Network (EARS-Net).

Regarding the AMR indicators monitored under the Sustainable Development Goals (SDG) [WHO - Ten threats to global health in 2019] framework, the median rates of third-generation cephalosporin resistant E. coli and methicillin-resistant S. aureus (MRSA) causing bloodstream infections reported by 76 CTAs were 42% and 35%, respectively. These rates were much lower in 19 CTAs with better testing coverage (11% for third-generation cephalosporin-resistant E. coli and 7% for MRSA). Most low- and middle-income countries presented lower testing coverage compared to high-income CTAs for both SDG indicators [GLASS Report 2022] (**Figure 4**).

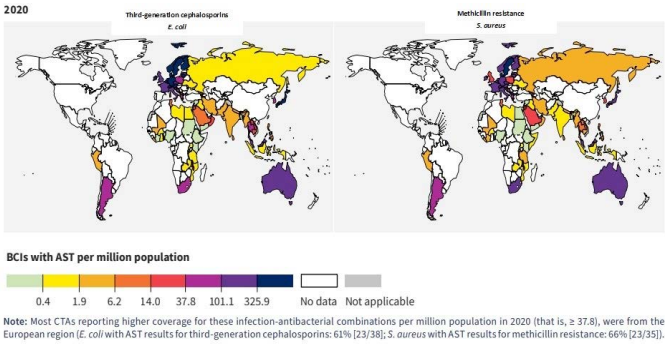

Figure 4: Bloodstream infections with third-generation cephalosporins (E. coli) or methicillin resistance (S. aureus) susceptibility test results reported to GLASS-AMR, per one million population (2020).

Abbreviations: AST = antimicrobial susceptibility test/testing, BCIs = bacteriologically confirmed infection/s

Source: Figure adopted from GLASS Report 2022

1.3. Factors contributing to the antibiotic crisis

1.3.1. Science Low outcome of new antibiotic principles: In the last 20 years half state-owned research institutes, small

and medium-sized enterprises (SMEs)/ research spin-offs tried to identify new mechanisms to overcome microbial resistance mechanisms. Experienced drug discovery experts have identified several scientific and technical shortcomings, such as lack of understanding of toxicology standards, insufficient antibacterial activity data and weak scientific rationale for potential antimicrobial compounds as the main reasons for failure in antibacterial drug development[8]. Central features of the drug discovery process, such as the definition of criteria for the further development of a compound (go/no-go decisions) including the 'compound properties' and 'proof-of-concept studies', which are decisive for success, were often missing.

1.3.2. Regulatory Infeasible trial conduct standards: Most new antibiotics are approved on the basis of clinical trials designed to establish non-inferiority, which means that the new antibiotic is not inferior to a comparable existing antibiotic. In the absence of evidence of superiority, factors like prices, potential side-effects and ease of administration get more influence on clinical decisions. For example, linezolid and tedizolid are the only oxazolidinones that have been approved in the last 20 years for the treatment of skin and soft tissue infections by Gram-positive bacteria including vancomycin-resistant Enterococci (VRE) and methicillin-resistant S. aureus (MRSA). Tedizolid (maximum price: USD 1778 per package) received marketing authorization in 2015, primarily on the basis of clinical trials showing non-inferiority to linezolid (maximum price: USD 707 per package). Therefore, tedizolid has been rarely used in Europe in the last decade **[Arda et al., 2017)**. Nevertheless, the particular benefits that tedizolid offers over current antibiotics (good safety profile and a low risk of developing resistance) are further driving its market's expansion. Its oral formulation and once-daily dosage schedule further improve patient compliance and adherence to therapy.

1.3.3. Economics Antibiotics are short-course therapies, have no competitive prices and small market sizes: The essential obligation to adhere to the principles of antimicrobial stewardship permitting only short-course therapies led to economic limitations, no competitive prices and small market sizes. As a consequence, big pharma companies divested own research and development over the last 20 years. Efforts to identify new active ingredients and mechanisms to overcome AMR, which have since been driven primarily by small and medium-sized companies, have been hampered by increasing demands on testing and production standards as well as rising labour costs in the face of increasing price competition and cost constraints on healthcare systems. It soon became clear that antimicrobials are not sufficiently value generating for company's shareholder relative to projects in other disease areas. The Net Present Value of an antibiotic development project has been estimated at minus 100 Mill USD compared to plus 720 Mill USD for a neurological drug[9].

In Germany, many of the antibiotics used are generics, i.e. medicines whose patent protection has expired and which are (much) cheaper than the originals. For example, one tablet of penicillin V often costs just about seven cents from the manufacturer. Furthermore, recently approved antibiotics often have short shelve lives and if they are kept in stock they can 'die' in the storage facilities if not used within this time window. Pricing in disposal costs and compensating for the loss of revenue from the destroyed goods is impossible given the low margins typical of generics.

As a consequence, the generic companies off-shored the production to manage the profitability requirements. Europe is now heavily dependent on China, especially when it comes to antibiotics. More than one third of the manufacturers of the 15 most important antibiotics are based in China - more than in any other country. India follows in second place with a share of just under 30% and Europe in third place with around 25%. Manufacturers in the Far East often concentrate on just a few active ingredients and can therefore produce huge quantities at unbeatable low prices for the whole world. At current, the active ingredients of the 15 most important antibiotics (e.g. amoxicillin, doxycycline or ciprofloxacin) are produced by only 60 manufacturers in Europe. As an analysis by the Munich-based big data company QYOBO shows, there are still companies producing antibiotic active ingredients in Spain and Italy in particular [QYBO platform] **(Figure 5)**.

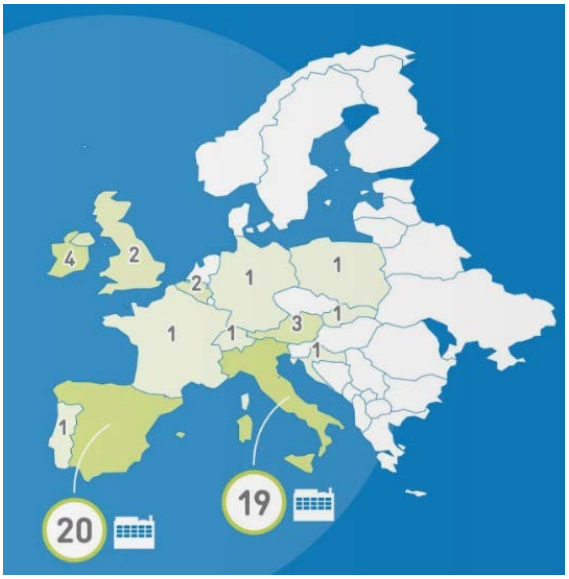

Figure 5: Production sites for antibiotics in Europe.
Source: Adopted from QYBO platform

As a result of cost competition German pharmacies can only dispense medication from insurance-specific manufacturer lists with low reimbursement prices (so called reference prices). In 2023, the National Association of Statutory Health Insurance Funds (GKV-SV) proposed to reduce these reference prices for some active ingredients that are critical to care such as the antibiotic amoxicillin/clavulanic acid. However, this antibiotic combination and therapeutic alternatives are currently affected by shortages. In addition, the daily therapeutic doses of generic antibiotics are usually the subject of discount agreements (so called "rebate contracts") between a health insurance company and just one pharmaceutical manufacturer. Discount agreements should generally be awarded to more than one company, because if the manufacturer drops out during the course of the contract, no one else can usually step in so quickly. And for important antibiotics, manufacturer should be located in Europe as a secure source of active ingredients to assure diversity and stability of important supply chains.

Taken together, the successful development of novel antimicrobials requires scientific, regulatory, economic expertise and innovative concepts in all research areas associated with AMR. The aim is now to strengthen all relevant research areas in a One Health context - from basic research to clinical research, health services research, environmental and climate research,

logistics through to research in collaboration with the health and food industry, agriculture, the construction sector and the healthcare sector and communication[10].

1.3.4. AMR R&D Incentives in Europe: To incentivize research and development of new antibiotics in Europe, a combination of several pull incentive options may be required, including but not limited to subscription payments, market entry rewards, transferable exclusivity extensions and milestone payments[11]**.** In addition, continued investment is needed by the EU in push incentives, such as direct funding and grants, to incentivize drug discovery and preclinical stages of development. The EU Global Health Strategy (2022) recognizes the increasingly global nature of antibiotic resistance as a priority. Access to new and existing antibiotics should also be sustainably secured in low and middleincome countries (LMICs). Proposals for joint procurement mechanisms, license and technology transfer agreements and the strengthening of production capacities in LMICs are currently being evaluated. The WHO should have strong leadership in coordinating international initiatives between Europe and LMICs and promote the European and Developing Countries Clinical Trials Partnership (EDCTP) for the clinical development of new antibiotics.

1.3.4. National efforts to stimulate Science and Regulatory: In Germany, the Federal Institute for Drugs and Medical Devices (BfArM) and the Paul Ehrlich Institute (PEI) have been supporting the German Centre for Infection Research (DZIF) for years in clarifying regulatory and technical issues. Current developments explore natural substance-based antibiotics or antimicrobial peptides with novel mechanisms of action. In addition, new alternative or immunomodulatory therapeutic methods (e.g. antibodies, bacteriophages, non-coding RNAs) may represent important strategic approaches to the treatment of bacterial infections and sepsis. Many alternatives are already being investigated in basic research, but clinical proof of efficacy is often not yet available.

One of the national bacteriophage-projects, namely 'Phage4Cure', is discussed in some more detail at the end of the section Non-traditional antibacterial agents in clinical development. The Charité Research Organisation GmbH is conducting the First Time in Human (FTIH) trial to initiate the clinical development of a bacteriophage-cocktail directed against P. aeruginosa as part of the 'Phage4Cure' project.

2. Antimicrobial Targets in Bacteria

Traditional antibacterial agents are direct-acting small molecules classified according to their mode of action and specific targets such as inhibition of microbial cell membrane synthesis, protein synthesis as well as DNA functionality or bacterial metabolism **(Table 2)**.

The most important antibiotics for critical care patients are still the ß-lactam antibiotics (penicillins, cephalosporins, monobactams and carbapenems) and macrolides (erythromycin, roxithromycin, clarithromycin and azithromycin). However, problematic bacteria such as E. coli and K. pneumoniae are becoming increasingly insensitive to the 3rd-generation cephalosporins ceftriaxone, ceftazidime, cefotaxime and cefixime as well as to the carbapenems doripenem, ertapenem, imipenem and meropenem. Ceftazidime and the 4th-generation cephalosporin cefepime are also effective against P. aeruginosa. Ceftaroline is a 5th-generation cephalosporin and is also effective against MRSA. However, all cephalosporins are ineffective against enterococci and listeria.

Table 2: Traditional antibacterial agents and their targets.

Target(s)	Antibacterial class
Cell wall synthesis	ß-lactam antibiotics, glycopeptides, fosfomycin
Structure and function of cell membrane	Colistin, polymyxin B, daptomycin
Structure and function of DNA	Quinolones, nitrofurane, nitroimidazole
DNA-dependent RNA polymerase	Rifampicin
Protein synthesis: Blocking the 50S subunit; inhibition of transpeptidation	Chloramphenicol; streptogramine
Protein synthesis: Blocking the 50S subunit; inhibition of translocation	Macrolides, azolides, ketolides (50S+30S)
Protein synthesis: Blocking the 30S subunit	Tetracyclines, glcylcyclines
Protein synthesis: Incorrect control of synthesis	Aminoglycosides
Protein synthesis: Inhibition of the formation of the initiation complex	Oxazolidinones
Folic acid metabolism	Trimethoprim, sulphonamides

In the last 6 years (2017-2023), only 16 new antibacterial agents have been approved worldwide; most of them (13) are traditional antibacterial agents and only 3 are non-traditional agents [WHO (2024) - 2023 Antibacterial agents in clinical and preclinical development: an overview and analysis]: Approximately 77% (10/13) of the traditional agents approved since July 2017 belong to existing antibiotic classes for which resistance mechanisms are well known. Only two antimicrobial agents with activity against carbapenem-resistant A. baumannii (CRAB)- cefiderocol and sulbactam-durlobactam - have been approved since July 2017.

Importantly, several novel β-lactamase inhibitors (BLIs) have been developed, either belonging to the diazabicyclooctane class of molecules (avibactam, relebactam, zidebactam, nacubactam and durlobactam) or to the boronate class of molecules (vaborbactam and ledaborbactam). Those BLIs inhibit a wide range of serine-based β-lactamases including classes A, C and D, but lack significant inhibitory activity against class B enzymes, namely metallo-ß-lactamases (MBLs). At current, two novel boronate inhibitors with an additional inhibitory action against MBLs are in clinical development, namely, taniborbactam and xeruborbactam[12] (for more details, see section Drug Pipeline Antimicrobials, **Table 4**).

Non-traditional antibacterial agents aim to prevent or treat bacterial infections by directly or indirectly inhibiting bacterial growth, inhibiting virulence, ameliorating antibacterial resistance, boosting the human immune system and positively altering and/or restoring a healthy microbiome. The first microbiome-modulating agents (three products against C. difficile) were authorized in 2022 and 2023 **(Table 3)**.

3. Types of Antimicrobial Resistance

The main AMR mechanisms are well known:

- Natural resistance may occur in all strains of a species against certain antibiotics (gaps in the spectrum of activity).
- Mutation-induced resistance
- Primary - some of the strains of a species become spontaneously resistant without contact with the antibiotic

- Secondary - resistance acquired under the selection pressure of an antibiotic therapy

3. R-plasmid or transposon-induced resistance

Plasmids or prophages multiply independently of the division of the bacterium and can be exchanged between the same or between the same or different strains.

Table 3: Non-traditional antibacterial agents that gained market authorization between 1st July 2017 and 31st December 2023.

Name (trade name)	Marketing authorization Holder(s)	Approved by (date)	Antibacterial class	Route of administration	Approved indication/s	Pathogen	Reference (product information)
SER-109 (VOWST (faecal microbiota spores, live-brpk))	Seres Therapeutics	FDA 04/2023	Live biotherapeutic product	PO	Recurrent/ refractory diarrhoea prevention[a]	*C. difficile*	Vowst
BB128 (Biomictra faecal microbiota)	BiomeBank	TGA (Aus) 11/2022	Live biotherapeutic product	Endoscopic delivery or enema	Recurrent/ refractory diarrhoea prevention[a]	*C. difficile*	Biomictra
RBX2660 (Rebyota (faecal microbiota, live-jslm))	Ferring Pharmaceuticals	FDA 11/2022	Live biotherapeutic product	Enema	Recurrent/ refractory diarrhoea prevention[a]	*C. difficile*	Rebyota

Source: Table adopted from WHO (2024)- 2023 Antibacterial agents in clinical and preclinical development: an overview and analysis.

Transposons are specific DNA elements that can jump from one plasmid to another or to the chromosome.

Examples of successful acquisition of specific resistance genes by globally dominant clones of a species have been identified, for example, acquisition of the extended-spectrum β-lactamase CTX-M-15 by E. coli ST131, or the carbapenemase KPC by K. pneumoniae ST258, both of which spread globally[13,14].

More specifically, there are several escape mechanisms for bacteria to resist antibiotics as reviewed in De Oliveira et al. (2020). Escape mechanisms are increasingly used by E faecium, S aureus, K pneumoniae, A baumannii, P aeruginosa and Enterobacterales and those are therefore so called ESKAPE pathogens. Mechanisms facilitating antimicrobial resistance in ESKAPE pathogens can be broadly categorized into four groups **(Figure 6)**:

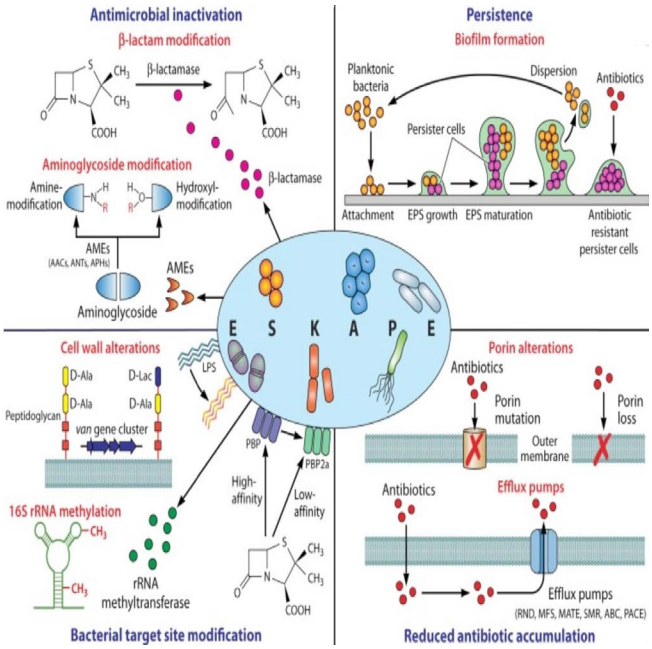

Figure 6: Mediators of ESKAPE pathogen antimicrobial resistance.

AMEs, aminoglycoside-modifying enzymes; AACs, aminoglycoside acetyltransferases; ANTs, aminoglycoside nucleotidyltransferases; APHs, aminoglycoside phosphotransferases; LPS, lipopolysaccharide; PBP, penicillin-binding protein; RND, resistance-nodulation division; MFS, major facilitator superfamily; MATE, multidrug and toxic compound extrusion; SMR, small multidrug resistance; ABC, ATP-binding cassette; PACE, proteobacterial antimicrobial compound efflux; EPS, extracellular polymeric substance.

Source: Figure adopted from[15].

Enzyme-mediated antimicrobial inactivation, which either irreversibly destroys the active antibiotic site (e.g., hydrolytic cleavage of the ß-lactam ring by ß-lactamases) or covalently modifies key structural elements of the drug to hinder the bacterial target site interaction (e.g., aminoglycoside-modifying enzymes that catalyse hydroxyl/amino group modifications);

Bacterial target site modification, which prevents the binding or which reduces the affinity of the antibiotic molecule at the cell surface (e.g., LPS modification, PBP2a expression with reduced ß-lactam affinity and van gene cluster-mediated peptidoglycan modification) or intracellularly (e.g., 16S RNA methylation);

Reduced antibiotic accumulation through the mutation or loss of outer membrane channels (e.g., OprD in P. aeruginosa, CarO in A. baumannii and OmpK36 in K. pneumoniae) and expression of efflux systems to actively extrude drugs out of the cell (e.g., RND, MFS, MATE, SMR, ABC and PACE); and

Persistence through biofilm-embedded cells which demonstrate a markedly higher tolerance to antimicrobial agents than planktonic bacteria.

3.1. Multidrug-resistant (MDR) bacteria

Bacterial resistance provides the bacteria with flexible ability to adapt and survive. Every use of antibiotics also promotes the selection of resistant bacteria, because sensitive bacteria are killed off (selection pressure). This allows the resistant

bacteria to multiply unhindered during antibiotic treatment. The accumulation of highly resistant bacterial populations in hospitals leads to an increase in nosocomial infections with multi-resistant bacteria such as P aeruginosa or Enterobacteriacea, vancomycin-resistant enterococci (VRE) or methicillin-resistant S aureus (MRSA).

3.2. Methicillin resistant Staphylococcus aureus – MRSA

Due to the inclusion of the methicillin resistance-encoding gene (mecA), MRSA are resistant to beta-lactam antibiotics and often also to other classes of antibiotics. MRSA can colonize the skin and mucous membranes of humans and animals **(Figure 7)**. Depending on their occurrence, the bacteria are categorized as LA-MRSA (livestock-associated), HA-MRSA (hospital-associated) and CA-MRSA (community-associated).

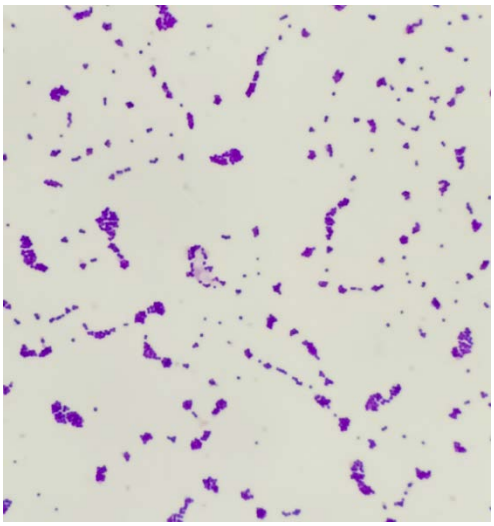

Figure 7: MRSA on surface of skin. (Microscopic zooming image).

3.3. MRGN - Multiresistant gram-negative bacteria

This group includes, for example, A baumannii, P aeruginosa, E coli, K pneumoniae and E cloacae. The terms 3MRGN and 4MRGN describe pathogens which, due to the simultaneous presence of resistance to three or four defined antibiotic classes, respectively, require increased attention in terms of infection prevention measures.

4. Regulatory Aspects on the Evaluation of Medicinal Products Indicated for Treatment of Bacterial Infections

4.1. Recommended Study Design - Non-inferiority trials

Non-inferiority trials must demonstrate non-inferiority (NI) of the test regimen to an appropriate reference regimen to support infection type-specific indications. NI trials allow acceptable deviations of prechosen worse efficacy with new antimicrobials compared with older, effective interventions, termed the NI margin, for patients with effective therapeutic options. In any case, NI margins at a post-therapy test of cure (TOC) visit should be acceptable to patients. For most indications, EMA allows up to 10% worse effectiveness for new antimicrobials, increasing to 12.5% worse only for hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP)[16]. The US-FDA also allows up to 10% worse effectiveness for new antimicrobials and even increasing it to 20% in recent studies[17]. However, a recent survey study in US adults found that 50% of respondents would not participate in NI trials where efficacy may be up to 10% worse than existing treatments[18]. Thus, the currently allowed NI margins seem to limit the feasibility of NI-trials. Smaller NI margins and/or more adaptive designs that allow a switch to the established comparator drug may be required to facilitate NI trials. In contrast, Superiority trials must demonstrate superiority of the test regimen over placebo or over an active comparator to support infection type-specific indications[16]. The proof of superiority may be required when there is either no licensed treatment or standard of care treatment available or the treatment effect is unknown. The measurable outcome of interest is resolution of clinical signs and symptoms in the microbiologic intention-to-treat population at a TOC visit. Suitable indications are self-limiting infections of short duration and with low sequelae such as bacterial sinusitis or superficial skin infections. But certain indications/infections may require surrogate endpoints for demonstration of superiority such as time to specific clinical response measures or improvements in clinical parameters.

4.2. Antimicrobial resistance testing in drug development and post-marketing

The EMA recommends to investigate the mechanisms of AMR in drug development or post market authorisation based on the minimum inhibitory concentrations (MIC) of the antibiotics [EMA (2022) Guideline]:

- If MICs are unusually high (e.g. at or above the upper end of the MIC distribution curve)
- If MICs are above the interpretive criterion for susceptibility testing (if this has been established for the species being tested).

Antibacterial agents of a new class should exclude cross-resistance in in-vitro susceptibility studies comparing test compound with licenced agents of other classes including tests on strains demonstrating multi-drug and/ or multi-class resistance challenging mechanisms mediated via impermeability or efflux pumps.

Antibacterial agents of existing classes require in-vitro susceptibility for cross-resistance within the same class.

Antibacterial agents in previously unlicensed compounds or of their combinations resp. co-administration require an initial estimation of the frequency of selection of resistance in species relevant for the indication. The pharmacodynamic in-vitro model should mimic the drug concentration profile probably achievable in patients.

After approval, the regulators should be notified about all information about resistance by the Marketing Authorization Holder including discussion of possible implications (e.g. via SmPC part 5.1, PSUR, direct communication).

5. WHO Bacterial Priority Pathogens List 2024

In 2017, the WHO published the first Bacterial Priority Pathogens List (BPPL) to guide investment in R&D and that serves as reference for regional surveillance programs and control of antibacterial resistance. A main requirement for future development strategies was to focus on antibiotics that are active against multidrug-resistant Gram-negative bacteria and tuberculosis. Especially, Carbapenem-resistant (CR) Gram-negative bacteria are difficult to treat due to high

levels of antibiotic resistance and are associated with high mortality. The global strategy included antibiotic-resistant bacteria responsible for community-acquired infections such as Salmonella spp, Campylobacter spp, Neisseria gonorrhoeae and Helicobacter pylori[19]. In addition, the WHO has identified particularly important antibiotic classes for the treatment of bacterial infections in human medicine, whose efficacy must be maintained at all costs. These critically important antibiotics include 3rd to 5th generation cephalosporins and macrolides.

The recent update of the WHO BPPL in 2024 builds on the 2017 list to address current challenges and provides essential guidance for policymakers, national health authorities and others involved in decisions about R&D and investment[20]. The 2024 BPPL includes 15 families of antibioticresistant pathogens, grouped into critical, high and medium categories of priority for R&D and for public health measures (**Figure 8**).

Figure 8: WHO Bacterial Pathogens Priority List 2024. Source: WHO Bacterial Priority Pathogens List, 2024[20]

In this update, Gram-negative bacteria that are resistant to last-resort antibiotics, such as A. baumannii and various pathogens in the Enterobacterales order, as well as rifampicin-resistant Mycobacterium tuberculosis, are listed as of critical priority because of their ability to transfer resistance genes, the severity of the infections and disease they cause and/or their significant global burden, particularly in LMIC.

The inclusion of Salmonella and Shigella as of high priority reflects their increasing resistance to existing treatments and their high disease burden, particularly in LMIC. Especially, stewardship across the One Health spectrum is crucial to combat AMR. For example, AMR in non-typhoidal Salmonella mainly results from antibiotic usage in animal husbandry. Therefore, control of unjustified use of fluoroquinolones both in humans and animals is key in directly mitigating AMR non-typhoidal Salmonella. Other high-priority pathogens in the 2024 BPPL are antibiotic-resistant P aeruginosa and S aureus, due to their global threat, especially in health-care settings. Also included in the high-priority category are pathogens that present distinct public health challenges, such as N gonorrhoeae, of which multidrug-resistant (MDR) strains have emerged, limiting treatment options. Another pathogen of public health importance is antibiotic resistant E faecium, a bacterium that is able to transmit resistance elements across the One Health spectrum. The 2024 BPPL includes Group A and B Streptococci, S pneumoniae and H influenzae in the medium-priority category, indicating an urgent need to address their public health impacts, particularly in vulnerable populations in resource-limited settings.

6. Drug Pipeline Antimicrobials

In 2024, WHO published the sixth annual review of the antibacterial clinical drug pipeline consisting of 57 traditional (direct-acting small molecules) and 40 non-traditional antibacterial agents in phase 1 to 3 of clinical development (from 1 July 2017 to 31 December 2023), which had not, at that date, received market authorization for human use anywhere in the world[21]. The current antibacterial clinical drug pipeline continues to be dominated by combinations of β-lactam antibiotics with ß-lactamase inhibitors targeting WHO priority pathogens.

6.1. Traditional antibacterial agents being developed against WHO priority pathogens

The current antibacterial clinical drug pipeline includes 57 traditional antibacterial agents, 32 (56%) of them are intended against WHO 2024 bacterial priority pathogens (WHO BPP); most of them are β-lactam or β-lactam/β-lactamase inhibitor (β-lactam/BLI) combinations with a major gap in activity against metallo-β-lactamase (MBL) producers[21]. Of the 32 traditional antibiotics in development against WHO BPP (excluding TB drugs), 12 meet at least one of the four WHO innovation criteria (no cross resistance, new chemical class, new target or new mode of action) (**Table 4**). Among these innovative agents there are two novel boronate-based serine BLIs, taniborbactam and xeruborbactam, which in combination with ß-lactams such as ceftazidime, cefepime or meropenem show also a significant activity against a wide range of MBL producing E. coli and P. aeruginosa[12].

Furthermore, 19 (33%) of the traditional antibacterial agents are targeting drug-resistant M. tuberculosis, 5 (9%) are being developed against C. difficile and one (2%) against H. pylori[21].

Table 4: Traditional antibiotics under development against WHO BPPs (excluding TB drugs) meeting at least one of the four WHO innovation criteria.

Product (Sponsor)	Antibacterial Class/ Mode of Action	Indication/ Route of administration	Activity against priority pathogens					Development Phase/ NCT
			CRE	3GCRE	CRAB	CRPA	MRSA	
Taniborbactam in combination with Cefepime (Venatorx/ GARDP)	Taniborbactam is a boronate-based serine and metallo-ß-lactamase inhibitor including activity against NDM, VIM, GIM-1 and DIM-1 producing E. coli or P. aeruginosa. Cefepim is a fourth-generation Cephalosporin.	Complicated UTI, HABP and VABP due to P. aeruginosa and other Gram-negative bacteria Intravenously administration	A	A	NO	A	NT	NDA February 2024
Gepotidacin (GSK)	Triazaacenaphthylene; novel bacterial topoisomerase II inhibitor (NBTI), Gyrase A binding site distinct from fluoroquinolones	Targeting 3GCRE responsible for UTI and N. gonorrhoeae Intravenously/Oral administration	A	NT	NT	NT	NT	Phase 3/ Completed: NCT04010539 NCT04020341 NCT04187144 Phase 3/Completed: NCT05630833
Zoliflodacin (Innoviva, former Entasis Therapeutics/ GARDP)	Spiropyrimidene-trione; NBTI, Gyrase B binding site distinct from fluoroquinolones	Uncomplicated genital gonorrhoea and active against MRSA Oral administration	NT	NT	NT	NT	A	Phase 3/Completed: NCT03959527
BWC0977 (Bugworks Research Inc.)	Pyrazino-oxazinone; NBTI (equipotent against gyrase and topoisomerase IV, stabilizing single strand breaks in the DNA unlike ciprofloxacin which stabilizes double strand breaks)	Critical care infections Intravenously or oral administration	PA	PA	PA	PA	PA	*Phase 1/Terminated* (CMC challenges): NCT05942820
Afabicin (Debiopharm)	Pyrido-enamide FABI Inhibitor; FAB = Enoyl-Acyl-Carrier-Protein Reductase (the rate-limiting enzyme in the final step of bacterial fatty acid biosynthesis)	Bone or joint infection due to S. aureus Intravenous or oral administration	NT	NT	NT	NT	A	Phase 2/Recruiting: NCT03723551
Murepavadin (Spexis)	Macrocyclic peptidomimetic compound; Disruption of cell membrane integrity	Chronic Cystic Fibrosis and non-CF bronchiectasis infections specifically targeting P. aeruginosa Inhalative administration	NT	NT	NT	A	NT	*Phase 3/Terminated* (Safety Data Review) NCT03582007 NCT03409679 Phase 2/ Completed: NCT02096328
OMN6 (Omnix Medical)	Antimicrobial peptide; Inhibitor of serine protease LepB, a key enzyme involved in protein export across the inner membrane	HABP and VABP due to A.baumannii and other Gram-negative bacteria Intravenous administration	PA	PA	A	PA	PA	Phase 2/ Recruiting: NCT06087536
TXA-709 (TAXIS Pharmac.)	Difluorobenzamide; Inhibitor of filamenting temperature-sensitive mutant Z bacterial cell division protein (FtsZ inhibitor)	Anti-resistance drug candidate that might enable the re-use of generic antibiotics against antibiotic-resistant ESKAPE pathogens Oral administration	NT	NT	NT	NT	A	Phase 1/ Completed: First Time in Human trial
Xeruborbactam in combination with ß-lactams (Qpex Biopharma Inc.)	Xeruborbactam is a boronate-based serine and metallo-ß-lactamase inhibitor including activity against NDM, VIM, IMP, GIM-1 and DIM-1 producing E. coli and P. aeruginosa	Infections due to Enterobacterales producing ESBL and CRE; ORAvance™: oral delivery of Xeruborbactam prodrug + oral ß-lactam Serious infections due to A.baumannii, P. aeruginosa, Enterobacterales; OMNIvance™: IV delivery of Xeruborbactam + IV ß-lactam	A	A	A	A	NT	Phase 1/Completed: NCT04380207 Phase 1/ Recruiting: NCT06079775

Product (Sponsor)	Antibacterial Class/ Mode of Action	Indication/ Route of administration	Activity against priority pathogens					Development Phase/ NCT
			CRE	3GCRE	CRAB	CRPA	MRSA	
KSP1007 in fixed combination with meropenem (Sumituvant Biopharma)	KSP1007 is a boronate-based ß-lactamase inhibitor Meropenem is an approved carbapenem.	Serious, life-threatening infections due to carbapenem-resistant Gram-negative bacteria IV administration	PA	PA	PA	PA	NT	Phase 1/Completed: NCT05226923

Abbreviations: Activity grades: A=active, NO=no activity, NT=Not tested, PA=possibly active, VSR=susceptibility rates with wide geographic variability depending on the resistance mechanism; Resistances of priority pathogens: CRAB=carbapenem resistant A. baumannii, CRPA=carbapenem resistant P. aeruginosa, CRE=carbapenem resistant Enterobacterales, 3GCRE=third-generation cephalosporin-resistant Enterobacterales, ESBL = extended spectrum ß-lactamase, MRSA=methicillin resistant S. aureus; Other: HABP=hospital acquired bacterial pneumonia, GARDP=Global Antibiotic Research and Development Partnership (WHO), NDA=New Drug Application, UTI=Urinary Tract Infection, VABP=Ventilator Associated Bacterial Pneumonia, ESKAPE pathogens=E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa and Enterobacter species, NMD=New Dehli metallo ß-lactamase, VIM=Verona Integron-Borne metallo-ß-lactamase

Source: Data adopted from WHO (2024) - 2023 Antibacterial agents in clinical and preclinical development (updated according (to clinicaltrials.gov, accessed on 02 October 2024

6.2. Non-traditional antibacterial agents in clinical development

Non-traditional antibacterial agents use different treatment approaches than traditional antibacterial small molecules. Most of them do not by themselves kill bacteria or affect bacterial growth. Traditional measurements of the Minimal Inhibitory Concentration as correlate of clinical outcomes cannot be used as diagnostic in vitro tools. New product-specific tests that may predict safety and clinical outcomes are therefore required.

The current clinical antibacterial drug pipeline lists 40 non-traditional antibacterial agents including 30 agents that are intended against WHO BPP, nine that are directed against C. difficile and one agent against Helicobacter pylori[21].

The 30 non-traditional agents targeting WHO BPP can be sub-grouped according to the following treatment strategies:

- Seven antibodies inactivating or neutralizing a pathogen, a virulence factor or a toxin or binders. Two products, Tosatoxumab (AR-301, Aridis Pharmaceuticals) and Suvratoxumab (AR-320, Arides Pharmaceuticals), have already reached phase 3 of clinical development; both of them are targeting S. aureus.
- Thirteen bacteriophages and phage-derived enzymes causing direct lysis of a target bacteria by phages or recombinant enzymes and/or phages that have been engineered as nanodelivery vehicles. Only one phage, the CRISPR-Cas3 enhanced phage LBP-EC01 (Locus Biosciences), has currently reached phase 3 of clinical development. LBP-EC01 is targeting multi-drug resistant E coli in patients with recurrent uncomplicated urinary tract infections.
- Three anti-virulence agents interfering with bacterial virulence factors but are neither bacteriostatic nor bactericidal. All three agents are still in phase 1 or 2 of clinical development targeting either P aeruginosa, E coli or S aureus.
- Two immuno-modulating agents stimulating or suppressing host immune responses that modify the course of infection. One product has reached NDA stage, namely, Reltecimod (AB103, Atox Bio), that targets S. aureus in patients with necrotizing soft tissue infections.
- One microbiome-modulating agent (SER-155, Seres Therapeutics) modifying the microbiome to eliminate or prevent carriage of resistant or pathogenic Gram-positive and Gram-negative bacteria, has entered the pipeline since the last WHO 2021 Report. SER-155 is aimed at reducing breakthrough bacteraemia in certain high-risk populations, such as hematopoietic stem-cell transplant patients.
- Four miscellaneous agents that inhibit the production or activity of virulence factors – toxin production and virulence factor secretion, impeding bacterial adhesion to host cells and biofilm formation, interrupting or inhibiting bacterial communication and downregulating virulence. All four agents are still in phase 1 or 2 of clinical development targeting either P. aeruginosa, S. pneumoniae or Gram-positive and Gram-negative pathogens.

Only three new non-traditional products entered the clinical pipeline since the last WHO report in 2021; all are microbiome-modulating agents **(Table 5)**.

8. Phage4 Cure Project

The **Charité Research Organisation GmbH** has experience in the conduction of early phase projects for new antimicrobial drugs (**Figure 9**). At current, the Charité Research Organisation GmbH is conducting the *First Time in Human* (FTIH) trial to initiate the clinical development of a bacteriophage-cocktail consisting of three phages directed against P. aeruginosa that are part of the academic Phage4Cure project[22].

A mixture of three different phages was chosen to provide a broader spectrum of antibacterial potential and to mitigate the emergence of phage-resistant bacterial variants[22,23]. The first two parts of this *FTIH* trial (randomized, double-blind, placebo-controlled) have already been completed and evaluated the safety and tolerability of one day ascending doses and multiple doses of the inhaled (nebulized) phage cocktail in healthy volunteers. The third part is now investigating the preliminary

efficacy of multiple doses of the phage-mixture in patients with chronic colonization of the lung with P. aeruginosa, who have shown susceptibility to the phages. Patients have to pause their antibiotic therapy three weeks prior to randomization and until study completion. The long-term aim of the project is to establish bacteriophages as drugs in the fight against bacterial infections and as such gain legal authorization as medicinal products in a variety of dosage forms for different indications.

Table 5: New non-traditional antibiotics since last WHO 2021 Report.

Product (Sponsor)	Mode of Action/ Route of Administration	Sought Therapeutic Indication	Targeted pathogen(s)	Development Phase/ NCT
MBK-01 (Mikrobiomik Healthcare Company)	Live biotherapeutic product (heterologous lyophilized faecal microbiota) Oral administration	Recurrent C. difficile infection and Non-alcoholic steatohepatitis	C. difficile	1) Phase3/Completed: NCT05201079 2) Phase 2/ Not yet Recruiting: NCT05622526
NTCD-M3 formerly VP20621 (Shire/Takeda) Fast Track status by the FDA. Destiny Pharma acquired global rights to the NTCD-M3 program in November 2020.	Live biotherapeutic product (naturally occurring non-toxigenic strain of C. difficile bacteria, which lacks the genes that can express C. difficile toxins) Oral administration	Recurrent C. difficile infection	C. difficile	Phase 2/Completed with Results: NCT01259726 NTCD-M3 Phase 3 Design – Agreed with FDA and EMA
SER-155 (Seres Therapeutics)	Microbiome modulator (fermented microbiome, commensal bacteria) Oral administration	Reduction of breakthrough bacteraemia and GvHD in allo-HSCT recipients by decolonizing potential pathogens and restoring gastrointestinal colonization resistance	WHO BPP: Gram-positive and Gram-negative pathogens	Phase 1b/Completed: NCT04995653

Abbreviations: GvHD = graft vs host disease, HSCT = hematopoietic stem cell transplant

Source: **WHO (2024) - 2023 Antibacterial agents in clinical and preclinical development** (updated according to clinicaltrials.gov, accessed on 02 October 2024)

Figure 9: Location of Charité Research Organisation GmbH at Charité Campus in the center of Berlin

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8. Concluding Remarks

- Overall, antibacterial agents in the current clinical pipeline combined with those approved in the last six years are still insufficient to resolve the ever growing threat of the emergence and spread of drug-resistant bacteria.
- Experienced drug discovery experts have identified several scientific and technical shortcomings, such as lack of understanding of toxicology standards, insufficient antibacterial activity data and weak scientific rationale for potential antimicrobial compounds as the main reasons for failure in antibacterial drug development.
- Continued investment is needed by the EU in push incentives, such as direct funding and grants, to incentivize drug discovery and preclinical stages of development. Economic incentives are also needed to encourage use of diagnostics to inform treatment and thereby improve antimicrobial stewardship.
- And for important antibiotics, manufacturer should be located in Europe as a secure source of active ingredients to assure diversity and stability of important supply chains.
- Antibiotics should be ring-fenced the same way as we protect a military defence which will also be hopefully never be used. Nevertheless the chain of people involved needs to be considered as acknowledged and beneficiary but the tax payers/ states need to pay for the own defence remembering the Corona crisis, the next pandemic is certain.

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Drafting the article: Claudia Gabriele Werner

Critical revision of the article: Frank-Dietrich Wagner

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