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REVIEW

Reviving the past for a healthier future: ancient molecules and remedies as a solution to the antibiotic crisis

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ABSTRACT

Options to combat bacterial infections are becoming scarce. We require innovative approaches to enhance the discovery of effective antimicrobials capable of combating bacteria resistant to multiple or all antibiotics. These methods should either directly eliminate resistant bacteria or indirectly influence their viability by inhibiting their virulence or reducing their resistance to antibiotics. One interesting approach is to analyze ancient remedies used to treat bacterial infections, formulate them, and test them against modern microbes. This field has recently been named "ancientbiotics." This approach allows us to leverage centuries of empirical knowledge accumulated, from traditional medicines across various ancient cultures worldwide. The strategy has already yielded promising formulations to combat the ESKAPE group of nosocomial pathogens. Additionally, molecular de-extinction, which involves genome analysis of extinct species to search for useful antimicrobials, such as peptides, offers another avenue. In this review, we compile the antimicrobial effects of ancient remedies and de-extinct molecules known to modern science and discuss possible new strategies to further harness the potential of past remedies and molecules to fight the rise of superbugs.

PLAIN LANGUAGE SUMMARY

Resistant bacteria that cannot be treated with medicine are making people sick. In this article, we talk about ideas to find new antibiotics by looking at the past. One way is to look at old medicines from around the world to see if they can beat these resistant bacteria. These medicines are called "ancientbiotics." Another way is to decode the sequences of small proteins or viruses that attack bacteria from organisms that are now extinct. Then, they can be made to target modern resistant bacteria. We think that some of these old medicines and extinct molecules will soon help doctors fight resistant bacteria.

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1. Introduction

Antimicrobial resistance (AMR) is a growing global challenge, resulting in 1.27 million direct deaths worldwide from infectious diseases resistant to available antibiotics and 3.7 million additional associated deaths in 2019 [1]. The situation is deeply concerning, with projections estimating 10 million deaths globally by 2050. AMR has been accelerated by factors such as the excessive or inappropriate use of antibiotics and the contamination of natural water resources with antimicrobial agents. The COVID-19 pandemic has further exacerbated AMR, as heightened antibiotic use was observed in efforts to prevent bacterial co-infections among patients [2]. Unfortunately, the development of new antibiotics is stagnating, and the scientific community's efforts appear insufficient to address the growing threat of AMR [3]. Pathogenic bacteria acquire resistance through several distinct mechanisms, including decreased antibiotic permeability, modification of antibiotic targets, antibiotic inactivation, and active efflux outside bacterial cells (Figure 1).

Hence, a current priority is the search for therapeutic agents that offer new opportunities in treating bacterial multidrug-resistant (MDR) infections without favoring the development of new resistance in pathogens [4]. Although a few promising novel molecules are under development [5], complementary approaches, such as machine learning and other artificial intelligence algorithms, have identified interesting antimicrobial candidates [6]. However, options for novel

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Article highlights

- The current increase in antibiotic resistance poses a significant threat to human health, necessitating diverse and innovative approaches to combat bacterial infections.
- Ancientbiotics are ancient remedies able to eliminate bacterial infections, found in the traditional medicine of various cultures and traditions worldwide.
- Molecular de-extinction employs modern technologies, such as AI-driven algorithms, to identify peptides and other small molecules encoded in the genomes of extinct organisms that exhibit potent antibacterial effects against current bacterial infections.
- Simple microorganisms, such as extinct bacteriophages, are also candidates to de-extinction, although this is more challenging than reviving molecules.

antimicrobials may remain limited, making drug repurposing a useful strategy [7]. Furthermore, the development of powerful sequencing technologies and data analysis is intensifying, enabling the identification of sequences of extinct organisms of diverse origins. Algorithms designed to identify useful molecules with antimicrobial properties from extinct species are under development [8], opening new avenues for the discovery of novel antimicrobials by looking into the past.

It is worth noting that traditional medicine has accumulated wisdom over centuries from various civilizations, with remarkable examples of ancient antimicrobials throughout history. For instance, the ancient Egyptians used moldy bread to treat infections, which likely had antibacterial properties due to the production of penicillin or other fungus-derived antimicrobials [9]. This knowledge could be valuable in the search for new antimicrobials. To ensure an extensive literature analysis, a database of relevant articles on the activity of ancientbiotics and the de-extinction of antimicrobial molecules was established by searching the following online databases: Medline (PubMed), Science Direct (http://sciencedirect.com) database, Web of Science, Scopus, and Google Scholar system. The following keywords were used: ancientbiotic, folk remedy antibacterial, anti-virulence remedy, antibacterial plants, bacterial infection, traditional medicine, as well as Boolean operators, such as "AND" and "OR."

2. Ancientbiotics and ancient antivirulence compounds

2.1. *Ancientbiotics from medieval medicine*

Many modern societies with advanced science and technology tend to underestimate the knowledge and traditional medicine of ancient civilizations. However, some past cultures developed complex and effective medications to treat several diseases through trial and error. These treatments were potent despite lacking a deep understanding of their underlying mechanisms. Although we possess advanced technology and abundant resources, recent pandemics caused by viruses with low mortality rates, such as Sars-Cov-2, have demonstrated the vulnerability of our health services and economies. We struggled to contain the spread of the virus or provide adequate treatment globally [10]. Leading to the collapse of

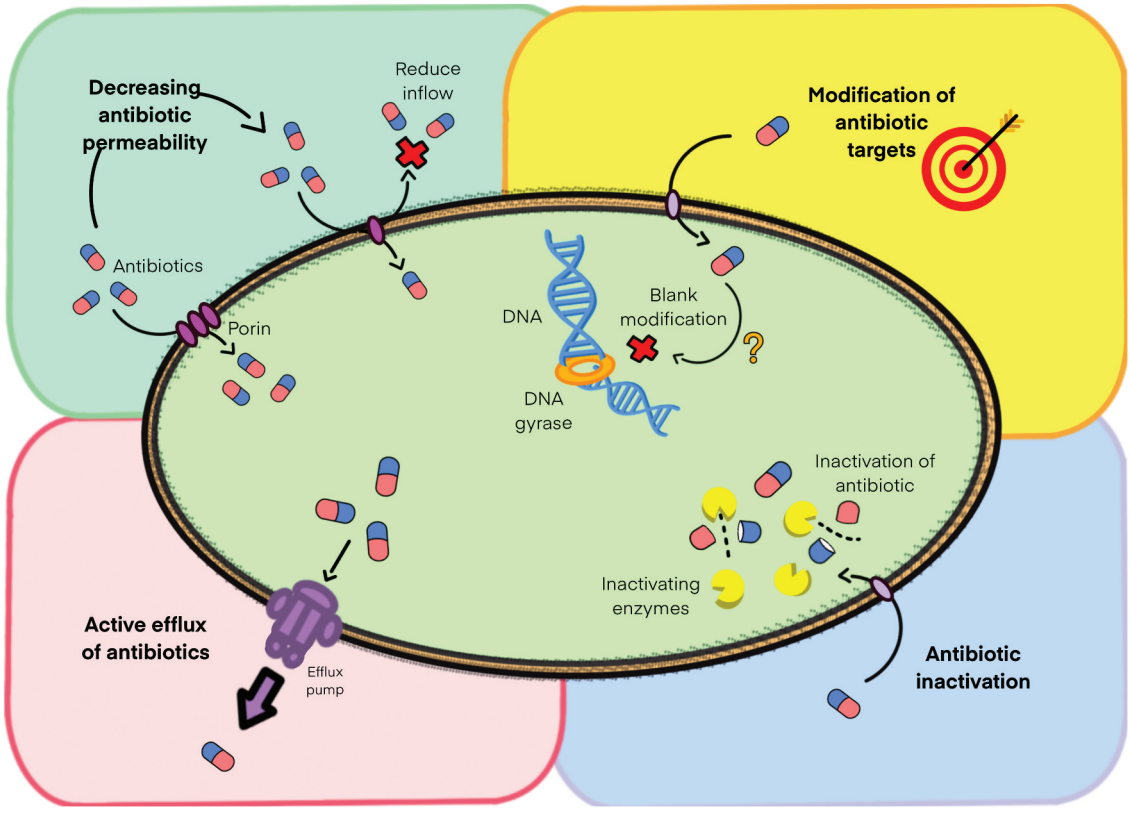

Figure 1. Action mechanisms of the most common antibiotics.

health services and devastating economic impacts worldwide. Furthermore, if the predictions regarding the consequences of antimicrobial resistance hold true, bacterial infections are expected to become the leading cause of death by 2050 [4], with potentially catastrophic consequences for humanity if preventive measures are not taken.

Therefore, it is crucial to learn from the traditional medicine of ancient civilizations, which may offer insights for developing new treatments and solutions to today's challenges [9]. We must also invest in health services and support ongoing research to prevent future pandemics and tackle antimicrobial resistance. The term "ancientbiotics" was coined in 2015 through an interdisciplinary collaboration between microbiologists and historians in the UK [11]. The consortium´s first published research identified a remedy for eye infections from the tenth century Saxon Bald's Leechbook. The formulation was relatively simple, consisting of garlic (*Allium sativum*), wine, either onion (*Allium cepa*) or leek (*Allium porrum*), and oxgall, originally prepared in copper vessels and marinated for nine days. The recipe was replicated with slight modifications, such as adding brass sheet pieces instead of using copper vessels and incubating it in plastic bottles for nine days at 4°C. Remarkably, the preparation killed planktonic and biofilm *Staphylococcus aureus* and performed well in an *in vitro* soft tissue infection model and *in vivo* against methicillin resistant *S. aureus* (MRSA) chronic wound infections in mice. The remedy's potency was maximal when all ingredients were combined, except brass, indicating synergy among them [12]. Notably, Bald's eyesalve highlights the efficacy of this synergy, whereas modern science often focuses on single compounds in drug development, overlooking how combinations with other substances could reveal novel mechanisms of action and potentially lead to new antibacterial treatments [12].

Later, the same research group demonstrated that the antibacterial effect extended to several other Gram-positive and Gram-negative species, including A*cinetobacter baumannii, Stenotrophomonas maltophilia, S. aureus*, *Staphylococcus epidermidis,* and *Streptococcus pyogenes* [13].

Bald's original eyesalve recipe includes ingredients with antibacterial properties, such as garlic or onion, while the alcohol in wine acts as an extraction agent, facilitating the release of active compounds from other ingredients [12]. Bile salts may disrupt bacterial cell membranes or biofilms [12]. In a recent study, Bald's eyesalve was fractionated to identify its main antibacterial agents [14]. This study pinpointed allicin as the principal antibacterial compound, suggesting that combining wine and bile salts in Bald's eyesalve promotes the extraction of allicin from minced garlic. Previous reports indicated that allicin exhibits antagonistic antibacterial activity with other compounds when used against *S. aureus*, but synergistic activity against *Pseudomonas aeruginosa* [14]. The authors suggest that the efficacy of Bald's eyesalve against resistant bacteria stems from the unique combination of ingredients working synergistically to produce potent antibacterial effects. However, further studies are needed to unravel the remedy's mechanism of action.

Results from *in vivo* models, such as bovine corneal permeability and opacity (BCOP) test, slug mucosal irritation (SMI) assay, and wound models in mice, showed that Bald's eyesalve exhibits low levels of cytotoxicity and irritation and does not inhibit skin wound closure. This suggests that this ancient remedy may be a strong candidate for topical use in humans and could serve as a future treatment for bacterial infections, particularly in conditions like diabetic ulcers and neonatal conjunctivitis [15].

A step forward in applying novel antimicrobials based on ancient remedies is to confirm their safety in humans through phase 1 clinical trials. In 2022, Harrison and her group evaluated Bald's eyesalve in 109 healthy volunteers [16]. The administration involved applying a patch containing the remedy's ingredients (garlic, onion, white wine, and Ox gall), sterilized by filtration, to the upper arm, covered with a dressing. It was left in place for 48 hours, and skin-related adverse effects were recorded. As expected, no significant adverse events were identified; only about 13% of participants experienced minor symptoms such as mild irritation, itchiness, and garlic odor, validating the crude remedy safety [16] and paving the way for further trials evaluating its antibacterial properties in treating skin infections.

Obtaining practical ancient recipes for antimicrobial mixtures may be laborious. Thus, a systematic automatization approach aimed at identifying ingredients with putative or known antimicrobial properties that frequently co-occur in remedies may uncover useful combinations whose effectiveness can be tested *in vitro* and *in vivo*. To this end, Connelly and collaborators created an electronic database of ingredients from recipes of putative antimicrobial remedies used to treat cutaneous and oral infections in the fifteenth-century *Lylye of Medicynes* book [17]. Notably, of 360 recipes for treating 124 diseases, 62 may have targeted infections, of which the authors identified around 41. The complexity of the task was evident from the identification of nearly 3600 different ingredients. Their network analysis algorithm allowed them to identify four core ingredients and three core combinations; some core ingredients, such as acetic acid, and one recipe were selected for the study of antibacterial properties. The chosen recipe, a gargle intended to alleviate mouth and throat infections, comprised ox gall, rind, and pomegranate blossoms, mastic (resin from *Pistacia lentiscus*), frankincense, honey, and vinegar. The remedy and its components were tested against two Gram-positive and two Gram-negative bacteria that infect soft tissue: *S. aureus, Enterococcus faecalis, P. aeruginosa,* and *Escherichia coli*. Remarkably, the reconstituted remedy killed 100% of all bacteria, as did frankincense alone [17]. Interestingly, some combinations, such as ox gall with honey or frankincense-sumac with honey, exhibited synergistic effects in killing *E. faecalis*, while other combinations were antagonistic. A similar approach could be applied to analyze various ancient texts for undiscovered antimicrobial ingredients or combinations.

Vinegar and honey frequently combine to treat infections in medieval Europe. Both have validated antimicrobial properties and are used individually to treat infected wounds. Their combination has its own name: *oxymel*.

Recently, Harrison and coworkers found that these ingredients appear in about 36% of 418 recipes for treating skin, eye,

mouth, and throat infections, an infected wounds in four medieval manuscripts from the nineth to the fifteenth centuries [18]. They tested different types of vinegar for antibacterial effects against *P. aeruginosa* and *S. aureus* ESKAPE bacteria in both planktonic and biofilm cultures, beyond the effects of their acetic acid content. They also analyzed the metabolic profiles of the vinegars using HPLC. Based on HPLC, two groups of vinegar with similar chromatography patterns were identified. One from wine, and the other from pomegranate. Not surprisingly, some vinegars had weaker antimicrobial effects than their acetic acid content for either planktonic or biofilm cultures, while others had stronger effects, indicating complex interactions among metabolites and synergic effects from some ingredients. They also demonstrated that specific vinegars had synergistic effects with medical-grade honey against biofilm cultures, validating the ancient use of *oxymel* [18]. A limitation of this study was its use of only reference bacterial strains, so the effects of vinegar or honey, alone or combined, against clinical strains remain untested.

Other studies by the same research consortium showed that, unlike vinegar and honey, other natural ingredients commonly used in medieval recipes for topical infections, such as nettle, lack significant antibacterial properties [19]. These were likely included for other functions, such as acting as an absorbent medium, allowing the persistence of antibacterial and emollient substances at the infection site.

2.2. Ancientbiotics from North and Central America

Given the current crisis caused by the rise of MDR strains, the study of ancientbiotics is highly valuable [17]. Recently, ancestral formulations or folk remedies with antivirulence capacity ("ancient antivirulence") have also begun to be investigated. Antivirulence-type antimicrobials do not directly inhibit microorganism growth; their effects rely on blocking the factors that enable pathogens to establish themselves and cause damage [20]. Various antivirulence targets have been described, with biofilms, quorum sensing (QS), and bacterial secretion systems being the most studied [21].

Notably, among natural products, those of microbial origin stand out for their bactericidal properties [22]. In recent years, plants have emerged as a relevant source of antivirulence products [21]. Likewise, the antibacterial and anti-pathogenic activity of formulations or folk remedies containing plant species with antivirulence capacity has been documented [23,24].

Native American cultures thrived in regions with great plant diversity and other elements, which they used to design remedies for various diseases [25]. Most of this knowledge was lost during European colonization of America; however, in some historical texts and ancestral communities, it preserves information about these medical practices.

During the American Civil War (1861–1865), many soldiers died from complications due to microbial infections. As this conflict occurred a century before modern antibiotics, Confederate doctors turned to medicinal plants used by North American natives to save lives [23]. Botanist Francis Porcher was tasked with documenting plants Native Americans used to treat wounds and infections [26]. His work formed the basis for Confederate doctor Samuel Moore's manual detailing the collection, preparation, and use of medicinal plants [27].

Some of the plant species documented by Porcher (*Liriodendron tulipifera*, *Aralia spinosa,* and *Quercus alba*) have been analyzed and shown to inhibit the growth of MDR bacteria associated with wounds, such as *S. aureus*, *Klebsiella pneumoniae,* and *A. baumannii* [23]. They also exhibit antivirulence activity by inhibiting *S. aureus* biofilm formation, while *A. spinosa* reduces transcription of *agr* gene operons necessary for QS [23]. The authors propose that these plants' effectiveness in controlling infections stems from an adjuvant effect of antivirulence metabolites with bactericides [23]. However, none of the plant extracts showed bactericidal activity against *P. aeruginosa* AH7, although their anti-virulence properties were not analyzed.

A recent study explored the antibacterial potential of traditional remedies based on native plants from Canadian indigenous populations, specifically against MRSA planktonic and biofilm cells in regular media and a wound infection-simulating medium. Remarkably, some plants exhibited antibacterial activity only in the wound infection medium, not in conventional ones, highlighting the importance of the specific infection conditions for identifying extracts and molecules with antimicrobial activity and underscoring the value of ancient Canadian indigenous knowledge [28].

The World Health Organization lists *P. aeruginosa* as a "critical priority" for developing new antimicrobials due to the rise of resistant strains [29]. This opportunistic pathogen causes hospital-acquired infections, mainly in immunocompromised patients, those on mechanical respiratory assistance or those with major burns [30].

A characteristic of *P. aeruginosa* infection in burn patients is its ability to establish itself in the lesion, spread to the bloodstream, and cause septicemia [31]. Thus, preventive treatments in the first hours after a burn are essential to prevent patient death [32].

Recently, in a thermal injury model in mice, the antipathogenic activity of a pre-Hispanic remedy using cuachalalate stem bark (*Amphipterygium adstringens*) was validated for treating lesions infected with *P. aeruginosa* [24]. Previous studies documented that the organic extracts from the bark lacked bactericidal capacity against *P. aeruginosa* but reduced its virulence [33]. Specifically, nonpolar extracts like hexane and one of its major constituents, anacardic acids, reduced QS-regulated phenotypes, and the phospholipase ExoU secreted by the type 3 secretion system (T3SS) [33]. However, although *in vitro* results were promising, topical application of the hexane extract or individual constituents was inactive in counteracting *P. aeruginosa* infection in mice and failed to prevent death [24]. Remarkably, the outcome differed when applied following a folk method: washing wounds with an aqueous bark extract and then applying it as a powder [24].

This folk method reduced animal mortality by preventing systemic dispersion and septicemia. It also stimulated granulation tissue and blood vessel formation, aiding damaged tissue restoration [24]. Chemical analysis of the aqueous bark extract revealed glycosylated flavonoids and catechins. The lyophilized aqueous extract exhibited antivirulence capacity by

inhibiting caseinolytic activity and pyocyanin production in *P. aeruginosa* [24].

Notably, this ancient method outperformed silver sulfadiazine, a broad-spectrum synthetic sulfonamide used in burn patients to prevent infections [34].

The folk method with cuachalalate aligns with historical texts. The sixteenth century work *History of the Plants of New Spain* mentions that "chalalactli" bark should be applied to "reduce tumors" [35]. An 1832 essay on Mexican Materia Medica states that "cuauchalalá" bark must be cooked in water to cure animal skin sores [36]. The Mexican Pharmacopeia of 1846, compiled by the Pharmaceutical Academy of the Capital of the Republic, indicates that "cuanchalalate" or "cuanchalalá" barks heals wounds [35]. Although further studies are required, recent results suggest that cuachalalate bark's antimicrobial effectiveness depends on its application form, the synergistic action of several antivirulence compounds, and mechanisms aiding tissue healing.

The Mayan civilization, one of the most important pre-Hispanic cultures, has descendants who safeguard ancestral knowledge [37]. A pioneering study in Mayan communities of Mexico's Yucatan Peninsula identified antivirulence properties in plants traditionally used to treat infectious diseases [38]. Notable are the anti-biofilm properties of *Ceiba aesculifolia* and *Colubrina yucatanensis*, and the ability of *Bonellia flammea*, *Capraria biflora*, *Cissampelos pareira,* and *Bursera simaruba* to reduce *P. aeruginosa* pyocyanin and exoprotease activity.

In a subsequent investigation, Castillo-Juarez and colleagues tested three popular formulations used by residents of the Yucatan peninsula to treat injuries and infections [38]. These formulations, made with local plant species, were prepared using fresh plant structures under the supervision of traditional Mayan doctors [39]. In a thermal damage injury model in mice, the antipathogenic activity of a formulation called "herbal soap" (HS) was identified. HS consists of an aqueous extract from seven plant species (*Astronium graveolens*, *Parthenium hysterophorus*, *Hamelia patens*, *Momordica charantia*, *Psidium guajava*, *Tradescantia spathacea*, and *Kalanchoe laciniata*) incorporated into a bar of commercial soap for local use [39].

Washing the burns with HS one day before inoculation with *P. aeruginosa* and applying it for ten days reduced the establishment and bloodstream dispersion, preventing animal death [39]. Analysis of HS's herbal component revealed antivirulence properties related to swarming inhibition and phospholipase ExoU secretion. These results align with prior studies showing swarming and T3SS (especially ExoU) as pathogenicity determinants in murine infection models, making them promising targets for new antibacterial compounds [40].

2.3. Ancientbiotics from South America

Zuccagnia punctata is a plant used in Argentine traditional medicine. Its infusions and decoctions in water, and ethanolic maceration extracts exhibit antiseptic activity [41–43]. A hydroethanolic extract of *Z. punctata* aerial parts was active against several Gram-negative bacteria isolated from human lesions, including *E. coli*, *K. pneumoniae*, *S. marcescens*, *A. baumannii*, and *P. aeruginosa* [44]. Three major compounds isolated from *Z. punctata* extract were 7-hydroxyflavanone (HF), 2,'4'-dihydroxychalcone (DHC) and 3,7-dihydroxyflavone (DHF). Antibacterial effects of *Z. punctata* extract, DHC, 3,7-DHF and 7-HF were assessed using a mouse model infected with *S. pneumoniae*. After infection, the products were orally administered the next day. Treatment with *Z. punctata* extract and 7-HF significantly decreased viable *S. pneumoniae* in the lungs, while DHC and 3,7-DHF showed no discernible effect *in vivo* [45]. *Z. punctata* has also been tested against growth and virulence factors of *Candida* species [46]. The extract inhibited planktonic cells of all tested *Candida* species, with the main active compounds identified as chalcones (2',4'-dihydroxy-3'-methoxychalcone, 2',4'- dihydroxychalcone), flavones (galangin, 3,7-dihydroxyflavone, and chrysin), and flavanones (naringenin, 7-hydroxyflavanone, and pinocembrine).

Numerous reports document the antimicrobial activity of plants from the Argentinean Puna, a highland region of the central Andes stretching from southern-central Peru to northern Argentina and Chile. Characterized by high altitude (3,000–5,000 masl), intense ultraviolet radiation, low oxygen levels, significant daily temperature fluctuations, and annual rainfall of 100–200 mm, plants in this ecosystem have adapted by synthesizing secondary metabolites with notable pharmacological properties [47].

Zampini and coworkers [48] tested the antimicrobial activity of 11 Puna plants used in traditional medicine to treat skin and soft tissue infections in humans and animals. Ethanolic extracts of aerial parts of *Baccharis boliviensis, Fabiana bryoides, Fabiana densa, Fabiana punensis, Fabiana triandra, Parastrephia lucida, Parastrephia lepidophylla,* and *Parastrephia phyliciformis* showed antibacterial activity against methicillin, oxacillin and gentamicin resistant *Staphylococcus*. *Chuquiraga atacamensis* exhibited antibacterial activity against multi-resistant Gram-negative strains, while *Parastrephia* species were active against *Enterobacter cloacae, P. aeruginos*a and *Proteus mirabilis*. Ethanolic extracts outperformed aqueous extracts against sensitive and multi-resistant clinical isolates [48].

Later, D'Almeida and coworkers [49] identified the main antimicrobial constituents of *P. lucida*, partially explaining its traditional use in the Andes highlands medicine. The main antimicrobial constituents were phenylpropanoid esters yielding prenyl or phenethyl alcohol, effective against *E. faecalis, S. aureus,* and *C. albicans* [49].

Otero and coworkers reviewed the effect of 11 plants native to Argentina and Chile [50]. Though often unexamined scientifically, some are consumed by native indigenous or rural populations for their well-known health benefits. Endemic species like *Peumus boldus* and *Quillaja saponaria* have been extensively studied for their components and biological properties, though less so scientifically [51]. *P. boldus*, endemic to central and south-central Chile [52], has leaves traditionally used in infusions for gastrointestinal and liver issues [16]. Its main components are phenolic compounds like catechin, epicatechin, and rutin. The extracts were evaluated against different members of the ESKAPE group. The aporphine alkaloid boldine, abundant in the leaves, was evaluated in a murine macrophage model with reduced

Leishmania amazonensis infection. Tietjen and coworkers showed boldine inhibited the replication of HIV-1NL4-3 and hepatitis C virus (HCV) [53]. *Quillaja saponaria* has a high content of saponins, with triterpenic aglycone quillaic acid as the prevalent molecule [54]. Its commercial aqueous bark extract, rich in saponins, polyphenols, and tannins, demonstrated antimicrobial activity against Enterohemorrhagic *E. coli*, causing severe membrane damage after short-term exposure [55]. A saponin-rich extract was effective against *S. aureus* ATCC 49,525, *S. Typhimurium* NCIM 2719, and *E. coli* ATCC 933 [56].

2.4. Ancientbiotics from Asian traditional medicine

An ancient Chinese herbal mixture, Xiao Cheng Qi (XCQ), has been used for years to treat slow-transit constipation (STC) by incorporating antivirulence therapies [57]. Recent research suggests a link between constipation and gut microbiota, particularly through microbial metabolites, such as short-chain fatty acids (SCFAs) impacting serotonin (5-HT) production via GPR43 receptor activation to maintain metabolic homeostasis. To assess XCQ's effectiveness, a study induced STC in mice with loperamide and administered varying doses of the herbal concoction. Results showed XCQ relieved constipation symptoms, accompanied by significant gut microbiota composition changes, higher SCFA levels, increased plasma 5-HT, and enhanced colonic expression of GPR43 and 5-HT4 receptors [57]. These findings suggest that XCQ's constipation relief stems from influencing gut microbiota, stimulating SCFA production, elevating plasma 5-HT, and boost colonic receptor expression. Thus, Xiao Cheng Qi emerged as a promising natural remedy for managing constipation, positioning it as a promising natural ancientbiotic [57].

The method of consumption can also be significant. Ayurveda, an ancient Indian medical system, focuses on balance and harmony through diet, herbal remedies, yoga, and lifestyle practices. It emphasizes individualized treatments tailored to a person's unique constitution, known as doshas, to promote overall health and prevent illness. A recurring theme is the consumption of fermented products like Panchagavya (PG), a multicomponent formulation of cow-derived substances: urine, dung, milk, curd, and ghee [58]. The fermented dishes were often served in copper vessels, a common Ayurvedic practice. Studies tested PG's potential by infecting *Caenorhabditis elegans*, with highly drug-resistant *S. aureus*, *Chromobacterium violaceum*, *Serratia marcescens*, *P. aeruginosa*, *S. pyogenes,* and *E. coli* [58]. PG was administrated with varying copper exposure rest periods. Samples fermented and rested in copper vessels for 30–60 minutes significantly combated infections, possibly due to increased Planctomycetes, Gammaproteobacteria, and Verrumicrobiota in the gut microbiome [58]. These bacteria aid against pathogens via free metal absorption, secondary metabolite production, and T immune cell regulation. Combined with holistic Ayurvedic approaches, PG emerges as a potent ancientbiotic with multiple infectious disease applications.

A study analyzing 10 Ayurvedic herbal complex preparations found that the extracts of Dashmula, Hareetaki, and Triphala churna exhibited antibacterial activities against Gram-positive and Gram-negative bacteria, including *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli,* and Salmonella typhi [59], warranting further studies to elucidate mechanisms and test *in vivo* efficacy.

Other regions of the world, such as Asia Minor and Asia Central, hold vast ancient knowledge suitable for antimicrobial research. Endemic medicinal plants and minerals were central to traditional medicine during the Middle Ages, as evidenced by manuscripts from the fifth century onward. Armenia's pharmacognostic encyclopedias list thousands of plants, with over 14,000 manuscripts in the "Mesrop Mashtots Matenadaran" available for analysis. In Georgia, scientists are developing the National Formulary of Georgian Medicine project, based on 500 ethnomedicine-related manuscripts [60]. The Uzbek Academic Collection holds 181 manuscripts from the thirteenth century onward, detailing local medicinal herbs in "The Canon of Medicine" [61,62]. Endemic plants in Kazakhstan, Kyrgyz Republic, Tajikistan, and Turkmenistan, though less documented, are now studied for modern pharmacology [61,63–65]. Leaves, fruits, berries, rhizomes, seeds, and barks were used to treat leprosy, for wound healing, and respiratory and intestinal infections.

Hypericum perforatum, (St. John´s Wort), rich in lipophilic phloroglucinol (a hyperforin), hypericin and flavonoids [66], inhibits Gram-positive bacteria, especially MRSA and penicillin-resistant *S. aureus*, but not of Gram-negative bacteria [67]. Also, components, such as pseudohypericin, quercetin, kaempferol, and 22 essential oils likely contributed to its ancient antiseptic use [63].

Endemic plants used in antiquity for wound healing and as antiseptics in Tajikistan [65], Kyrgyzstan, and bordering Turkestan [63,64] showed that essential oils obtained from local *Achillea filipendulina and A. arabica* L.: Asteraceae, *Galagania fragrantissima*, and *Origanum tyttanthum* exhibit high antimicrobial and antioxidant activity against MRSA.

Flowers and berries from *Sambucus nigra* L.: Adoxaceae were used in medieval medicine in Europe and Armenia (with their own endemic species: *Sambucus tigranii*) [68]. *Sambucus* spp. is rich in polyphenols, quercetin, anthocyanins, kaempferol, and other compounds, and is extensively reviewed by Mlynarczyk and coworkers [69]. Ethanol extract from berries and elder flowers inhibits *Staphylococcus*, *Salmonella*, *Pseudomonas,* and other nosocomial pathogens [70]. An aqueous extract from leaves decreases the growth of Enterobacteria *S. marcescens, S. pyogenes, Streptococcus* Group C, and G, *and Moraxella catarrhalis* [71].

Imported species like *Phyllanthus emblica* (used in Armenian gerontology and Ayurveda) exhibit antioxidant, antiviral, and antibiotic properties against Gram-negative bacteria [72]. Manuscripts in the Matenadaran repository mention *Laserpitium L*. Apiaceae for ulcers and abscesses; physicians from Asiatic countries treated gastrointestinal disorders with *Laserpitium* as well [64]. Ethanolic and water extracts of essential oils (very rich in terpenes) from *Laserpitium ochridanum* were able to interrupt QS activity of *P. aeruginosa*, inhibiting pigment production and reducing twitching and swimming motility [73].

Many other ethnomedicinal plants are mentioned in the manuscripts or in folk medicine: *Plantago psyllium* L., *P. mayor*

L., and *Inula helenium* for antiseptic purposes and treatment of intestinal and respiratory infections, as well as *Bryonia dioica*, *Nigella sativa*, *Ficus carica L.*, *Allium* sp., *Apiaceae,* and several others, await indexing for potential MDR bacteria applications.

Japan's Kampo medicine offers a rich remedy repertoire. Hochuekkito, a mixture of several different roots, rhizomes, seeds, and mandarin epicarp, eliminates vancomycin-resistant enterococci from the human intestine, significatively decreasing mortality [74]. Choreito, comprising minerals, rhizomes, the sclerotium of two fungal species, and donkey-hide gelatin, traditionally used for acute cystitis inflammation, shorten antibiotics courses in female patients as shown in a retrospective cohort study of over 8,000 patients in Japan, validating its traditional utilization [75].

Japan's raw food traditions, like sushi and sashimi, heighten foodborne illness risks. To mitigate this risk, wasabi (Japanese horseradish, *Eutrema japonicum*) has long been utilized to prevent food poisoning. Historically, wasabi holds a significant place in Japan, as evidenced by the discovery of the word "wasabi" on excavated artifacts dating back approximately 1,400 years. Moreover, culinary texts from the twelfth to the fourteenth centuries mention its uses in cooking. Its traditional role as a preservative has been passed down through generations. Wasabi is endemic to Japan and is the sole cultivated species in the *Eutrema* genus [76,77]. However, cultivating Japanese wasabi is challenging. As a result, a wasabi-like paste made from horseradish (*Armoracia rusticana*) is commonly sold. While this paste shares similarities with Japanese wasabi in composition, it differs significantly in its botanical classification. The primary pungent component of wasabi is allyl isothiocyanate [78], and its aromatic, pungent taste and tear-inducing smell play a crucial role in defense against pathogens [79]. Recently, it has been discovered that the 60% ethanol extract of Japanese wasabi exerts the strongest anti-inflammatory, antibacterial, and cytotoxic activity [80]. However, numerous mysteries still surround Japanese wasabi. Thanks to recent advancements in sequencing technology and bioinformatics, a high-quality reference genome was recently obtained [81]. It is hoped that this will lead to further advancement in wasabi research, not only in terms of understanding its ingredients and antibacterial activity but also its cultivation.

2.5. Ancientbiotics from Africa

Around 5,000 medicinal plants are used in its diverse, ancient traditional medicine, yet much remains undocumented, warranting efforts to catalog their uses [82]. African plants contain antibacterials. Tannins and saponins present in barks from *Acacia senegal L.: Mimosoideae* (Arabic gum), native of the Northern and Western coasts of Africa, have antimicrobial properties against *E. coli*, *S. aureus*, *S. pneumoniae*, *K. pneumoniae*, *Salmonella typhi*, *Shigella dysenteriae*, *S. pyogenes*, *P. aeruginosa*, and *Proteus vulgaris* [83]. Root extract from *Pelargonium sidoides* or Geraniaceae, (Umckaloabo RM), endemic to South Africa, is one of the most widely used remedies against respiratory infections. Its main component, umkalicin, absent in ornamental Pelargonium cultivars, alongside catechin and gallic acid, inhibits *P. aeruginosa* biofilms and quorum sensing [84]. Aqueous and methanol extracts of *Lippia adoensis*, *Polysphaeria aethiopica*, *Rumex abyssinicus,* and *Cirsium englerianum* (Ethiopia and Madagascar); *Cucumis pustulatus* (Mali and Arabian Peninsula); *Discopodium penninervium* and *Euphorbia depauperate* (Tropical Africa) showed appreciable activity against MRSA, *S. pyogenes*, MDR *E. coli* and *K. pneumoniae* [85]. Isolated anthraquinones from *Aloe pulcherrima* of the Asphodelaceae family, one of the fifteen endemic Aloe species from Ethiopia, inhibited the growth of *P. aeruginosa*. The endemic South African plant *Aloe ferox* Mill has 130 medicinal agents [82,86]. Notably, *A. ferox* grows in association with endophytic actinobacterium adapted to the interior leaf pulp. An extract from the endosymbiotic *Streptomyces olivaceus* had no anthraquinones but exerted bactericidal action against Gram-positive bacteria [87]. Otherwise, plant endosymbionts are part of a novel field of discovery called bioprospection, which, together with ancientbiotics, may provide safe and effective solutions for modern medicine.

2.6. Ancientbiotics from Oceania

Oceania's ancient Aboriginal societies preserve traditions offering medicinal plants with antibacterial activity. In 2001, Palombo and Semple tested 39 Australian Aboriginal plants, finding that twelve inhibited at least one bacterial species, with five, especially Eremophila species, showing broad-spectrum activity against Gram-positive bacteria [88]. Later, the molecules responsible for bacterial inhibition in extracts of *Eremophila alternifolia* were identified (three flavanones and a diterpene), validating the traditional use of this plant by aboriginal Australians to treat different kinds of infections, including those of the eyes, skin, throat, and wounds [89]. Another remarkable example of ancientbiotics identified in the Australian traditional medicine used by the Dharawal people was the discovery of several endophytic microorganisms (bacterial and fungal species living in healthy plants) found in association with Australian medicinal plants that produce antimicrobials with broad-spectrum activity against both Gram-positive and Gram-negative bacteria [90], thus being a potential source for the isolation of effective new antimicrobials. Similarly, several plants with antimicrobial compounds used in New Zealand by traditional Maori medicine have been identified [91,92] and the antibacterial properties of Manuka Honey are well-documented [93].

Different historical cultures developed unique ancientbiotics due to geography, resources, cultural practices, and medical knowledge. Geographical diversity has led to the availability of different plants, minerals, and animals, influencing the selection of materials used for medicinal purposes. These factors may have contributed to differences in the physiological components of microbiota, affecting the medicine's potency throughout civilizations. Cultural practices and beliefs have additionally shaped the development and transmission of medical knowledge, resulting in unique approaches to healthcare and the use of specific remedies. Furthermore, varying levels of scientific understanding and technological advancements in different civilizations influenced their ability to isolate and identify effective antimicrobials.

Ancient cultures developed diverse ancientbiotic treatments based on their respective contexts, resources, and understanding of disease. Regardless of their individual components, these ancientbiotics converge in their effectiveness in treating several infections. Figure 2 maps the global distribution of the ancientbiotics and ancient anti-virulence preparations discussed in the text.

3. De-extinction of molecules and bacteriophages

Over 90% of the species that once existed are extinct, suggesting a vast untapped repertoire of antibacterial molecules; however, reviving entire species is complex and may raise ethical concerns. We had access to genomic data regarding several ancient organisms, and to computational methods suitable for the identification of antibacterial molecules. De la Fuente and colleagues surveyed Neanderthals and Denisovans proteomes and identified several potential antimicrobial peptides absent in the *Homo sapiens* genome using machine learning [8]. Beyond membrane disruption and promotion of the production of active oxygen species, antimicrobial peptides also have immunomodulatory properties [94], combat biofilm formation, and inhibit the production of virulence factors [95]; therefore, molecular de-extinction of peptides may also identify those with immunostimulant and antivirulence properties. Beyond molecules, bacteriophages (viruses that infect and kill bacteria) may be resurrected and used as antimicrobials. Bacteriophages are and are the most abundant biological entity on earth and were discovered in the early twentieth century by Felix d'Herelle, who pioneered phage therapy; which was developed in Soviet countries like Georgia, but overshadowed by antibiotics in the West [96]. Today, phage therapy is becoming a therapeutic option for recalcitrant infections untreatable with antibiotics, often curing patients where antibiotics fail [97,98]; moreover, the first promising trials for curing a large number of patients with standardized phages alone or in combination (phage cocktails) are under development [99]. Importantly, phages can also be used to treat animal and plant infections, offering an eco-friendly alternative to antibiotics in agriculture, reducing selective pressure for antibiotic resistance [100]. Some phages can also reduce antibiotic resistance and virulence, since bacteria often acquire bacteriophage resistance by losing virulence factors or proteins directly involved in antibiotic tolerance and resistance, such as lipopolysaccharide, pili, and components of antibiotic efflux pumps [101–103]. Moreover, other phages interfere directly with the horizontal transfer of genetic information, including plasmids encoding antibiotic resistance genes, by blocking bacterial conjugation [104].

Historical Indian folklore suggests that the waters of some rivers, such as the Ganges, had curative effects against cholerae and the antibacterial activity of those waters was

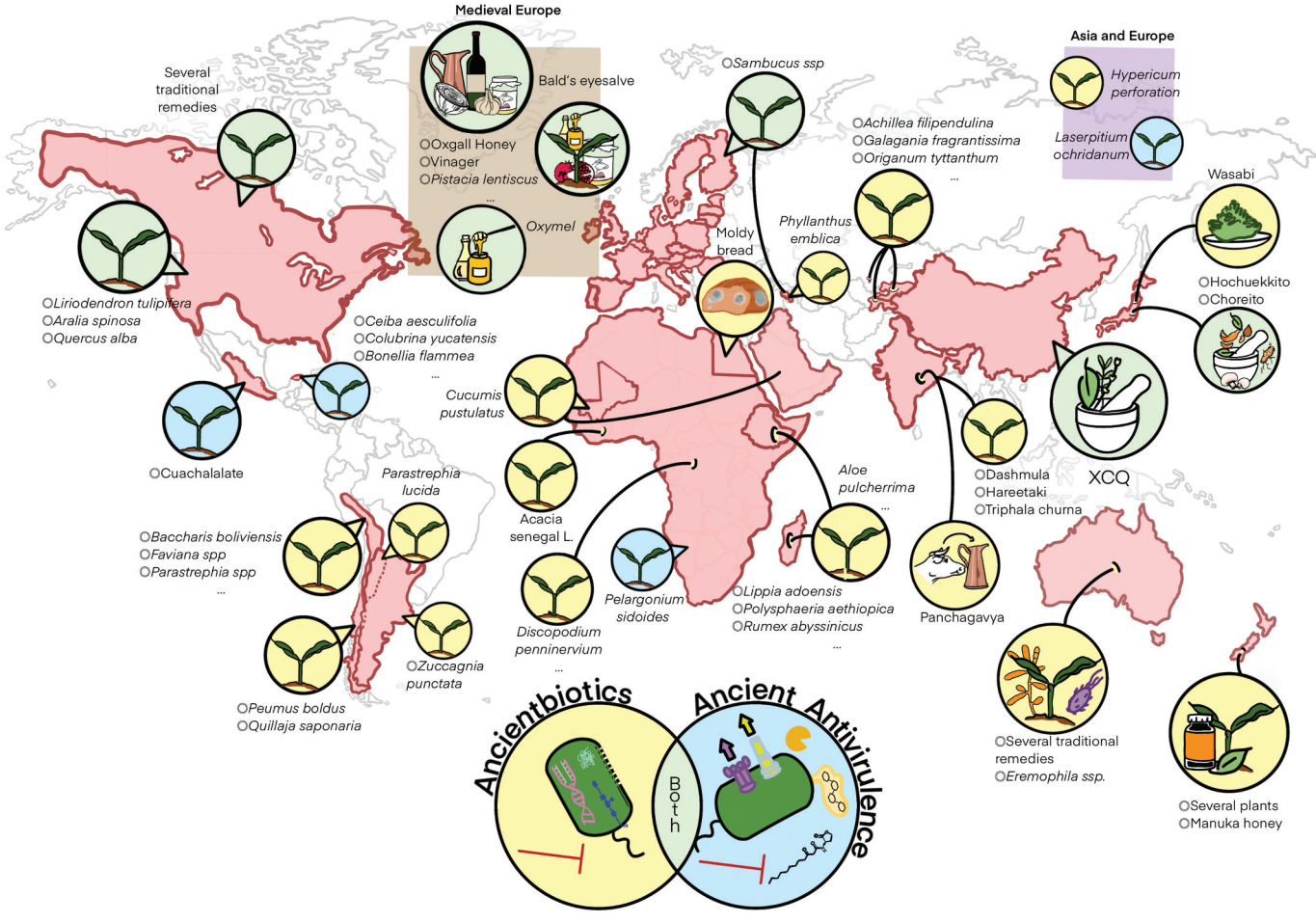

Figure 2. Global distribution of the ancientbiotics discussed in the text.

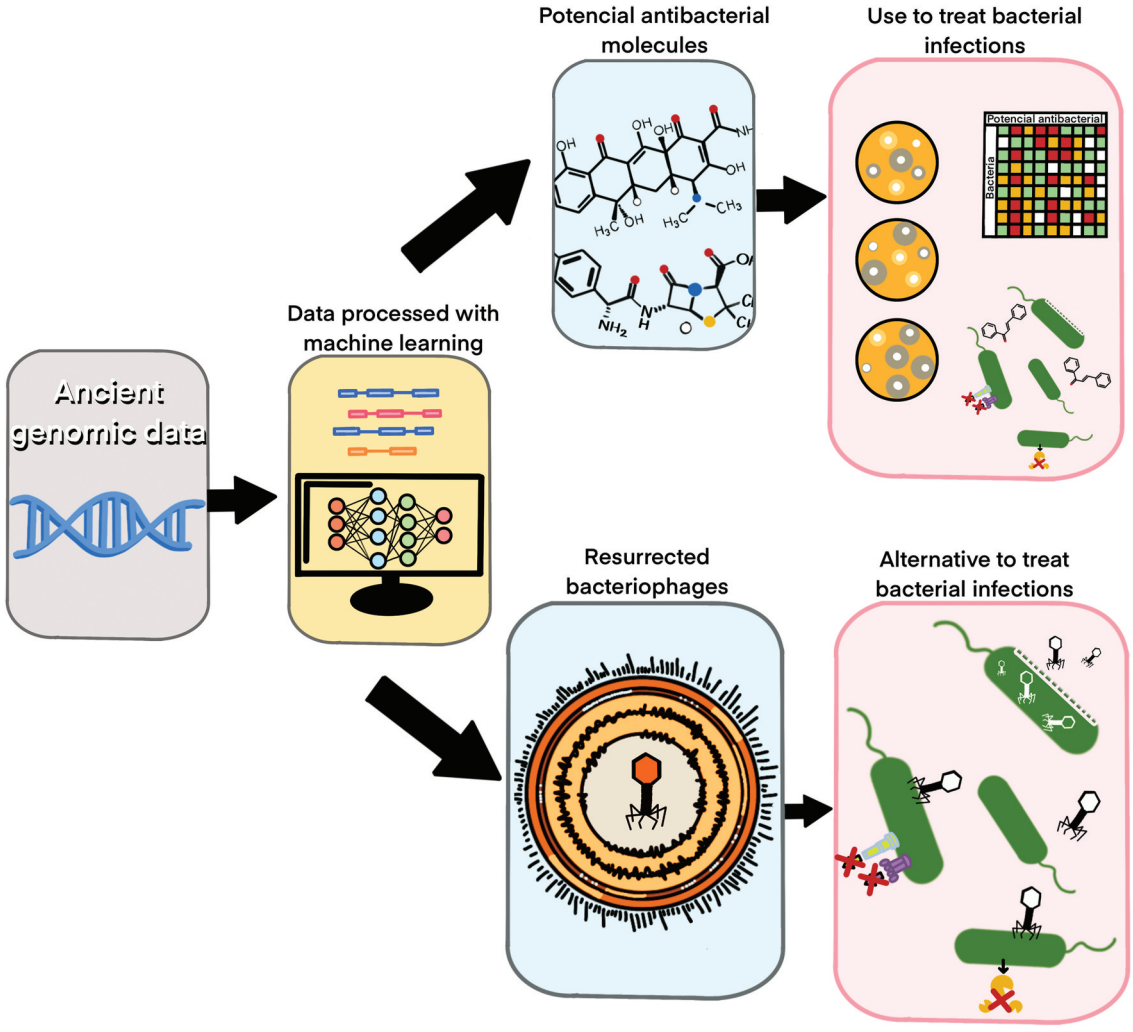

Figure 3. Graphical explanation of the molecular de-extinction approach.

confirmed by investigations in the late nineteenth century. Nevertheless, some researchers argue that it is unlikely that phages were the antibacterial agents since high phage densities may have been needed and, more importantly, since the activity of the waters remains after heating in sealed containers, a treatment that presumably would inactivate phages [105]. However, some phages resist temperatures near the boiling point of water for prolonged periods [106].

Although our current phage repertoire seems to be enough to constitute a robust collection and design cocktails suitable to combat several MDR bacteria, it is conceivable that in the future bacterial resistance against phages may limit our available phage arsenal, which has already happened with antibiotics [107]. Therefore, a diversified approach consists in analyzing metagenomes of ancient samples to unravel ancient extinct bacteriophages whose genomes could be synthesized *de novo*, or modifying modern-day related phages and testing their potential against the bacteria of interest. Remarkably, some ancient eukaryotic viruses have been resurrected [108]. Regarding phages, recently the genomes of around 300 ancient bacteriophages were recovered from palaeofaeces (150–5,300 years old) from Europe and North America. Interestingly, around half had no similarity to modern-day phages [109], being, therefore, potential candidates for de-extinction. Another potential source for the recovery of ancient dormant bacteriophages is permafrost. Already, several eukaryotic viruses trapped there have been resuscitated [110,111]. Besides yielding bacteriophages, permafrost can also be used as a source of ancient microorganisms, which can produce antimicrobial compounds and/or predate pathogenic bacteria. However, permafrost may present a risk for releasing ancient pathogenic organisms [112], hence samples should be handled carefully. Figure 3 illustrates the de-extinction approach for the identification of suitable antimicrobials.

4. Conclusions

Discovering and testing of ancient antibacterial recipes is challenging, requiring interdisciplinary teams of humanities and microbiology experts. Such collaborations have identified promising remedies outperforming modern antibiotics [11]. Given the multi-resistance crisis, modern formulations of ancient remedies may soon enter clinical practice. However, these remedies' complex ingredient mixtures can have synergistic or antagonistic effects, necessitating identification of the main antimicrobial components and their

interactions. Ingredient properties may differ from ancient versions due to natural variability (e.g., plant location, age, environment), but this complexity may reduce resistance compared to conventional antibiotics – a hypothesis needing exploration.

Analyzing bactericidal or antivirulence properties in ancestral formulations relies on constituent synergy, not single-target compounds. Likewise, damaged tissue healing promotion and the effects on the immune system must also be considered. Thus, one of the critical challenges in using complex ancestral formulations is to identify the bioactive molecules and interpret the synergies involved. Currently, technologies that allow analyzing and interpreting the results of the interactions of several compounds that act on various targets are still evolving. Overcoming these challenges and others will be essential in designing effective and safe antimicrobial formulations.

Also, searching for antimicrobial molecules using sequences of extinct organisms has its complications, such as getting samples of high enough quality to recover intact gene sequences that allow searching for peptides or other kind of molecules with antimicrobial properties and the development of robust and accurate algorithms to maximize their identification. However, they may strengthen the potential of expanding the repertoire of antibacterial molecules that may be helpful in our fight against superbugs. We encourage further research into these fields and innovative strategies for beneficial antibacterials.

5. Future perspective

Since it is anticipated that pathogenic bacteria will continue to increase their resistance to current antibiotics in the near future, we believe the approaches presented here enable the identification of more suitable antibacterial preparations and small molecules. Additionally, as the field of ancientbiotics emerges, many important aspects, such as their potential synergy or antagonism with antibiotics remain unexplored. Once these are investigated, they could facilitate the design of combination therapies that are more efficient than the independent application of regular antibiotics or ancientbiotics. Moreover, the positive or negative effect of ancientbiotics on the human microbiota remains undetermined for many remedies and their active components, marking this as an area of research to be further explored in the future.

Given the current antibiotic resistance crisis and progress in the fields of ancientbiotic and molecular de-extinction, we foresee that within the next decade, some preparations based on ancientbiotics, as well as some resurrected molecules, will likely be in use in clinical practice to combat MDR infections, benefiting patients worldwide. We expect that some of those therapies will be robust enough to delay the development of antibacterial resistance and remain effective for a considerable time. This underscores the value of ancient human knowledge and the natural strategies that extinct organisms employed in their battles against ancient pathogenic bacteria.

Disclosure statement

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