Alternative Treatment Strategies for Secondary Bacterial and Fungal Infections Associated with COVID-19

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ABSTRACT

Antimicrobials are essential for combating infectious diseases. However, an increase in resistance to them is a major cause of concern. The empirical use of drugs in managing COVID-19 and the associated secondary infections have further exacerbated the problem of antimicrobial resistance. Hence, the situation mandates exploring and developing efficient alternatives for the treatment of bacterial and fungal infections in patients suffering from COVID-19 or other viral infections. In this review, we have described the alternatives to conventional antimicrobials that have shown promising results and are at various stages of development. An acceleration of efforts to investigate their potential as therapeutics can provide more treatment options for clinical management of drug-resistant secondary bacterial and fungal infections in the current pandemic and similar potential outbreaks in the future. The alternatives include bacteriophages and their lytic enzymes, anti-fungal enzymes, antimicrobial peptides, nanoparticles and small molecule inhibitors among others. What is required at this stage is to critically examine the challenges in developing the listed compounds and biomolecules as therapeutics and to establish guidelines for their safe and effective application within a suitable time frame. In this review, we have attempted to highlight the importance of rational use of antimicrobials in patients suffering from COVID-19 and boost the deployment of alternative therapeutics.

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Key Summary Points

Exaggerated usage of antimicrobials in the management of COVID-19 has aggravated the phenomenon of antimicrobial resistance (AMR)

There is an urgent need for alternative antimicrobials. Studies that have demonstrated the effectiveness of several alternatives against drug-resistant bacterial and fungal infections have been highlighted in this study

The alternatives include inorganic nanoparticles, bacteriophages, repurposed drugs, antimicrobial enzymes, peptides and small molecules

To explore diverse approaches to deal with the microbial infections is imperative given a continuous rise in the ineffectiveness of traditional antimicrobials

INTRODUCTION

The current outbreak of the novel coronavirus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) has taken over 4 million lives worldwide (as of 1 October 2021, WHO) (https://covid19.who.int/). Disease morbidity and mortality of patients around the globe are a matter of grave concern. In such viral outbreaks, while treatment of the primary disease is critical, attention to secondary bacterial and fungal infections is as imperative, especially if they are drug-resistant. As witnessed during the epidemics of viral infections in the past 2 decades, a large percentage of patients' deaths occurred because of drug-resistant secondary microbial infections [1]. Emerging reports have demonstrated that 90% of the COVID-19 patients have received antibiotic therapy though only a few actually required them. This impractical and empirical use of antibiotics further aggravates

the antibiotic-resistance crisis we are facing today [1–3]. Hence, it is of paramount importance to assess the morbidity due to secondary infections, to consider alternative clinical approaches to currently deployed therapeutics and to accelerate their research and development (Fig. 1).

The successful launch of several vaccines within a year has given us enough reasons to rethink the timelines. In this review, we discuss the alternative treatment strategies to target secondary infections caused by fungal or bacterial pathogens in COVID-19 patients. We also highlight the potential of antimicrobial strategies that we believe mandates further investigation (Table 1).

AIM AND METHODOLOGY

The study aims to present the secondary bacterial and fungal infections in patients suffering from COVID-19 that show high levels of antimicrobial resistance. We have attempted to summarize the various antimicrobial alternatives to treat the secondary infections, which are currently at various stages of research and development, and highlight their importance in preparedness for future pandemics. For a period of 5 months (October 2020–March 2021) the authors of this study performed a PubMed search using various combinations of the keywords: (1) COVID-19, (2) secondary infections, (3) bacterial and fungal infections, (4) antibiotic resistance, (5) antifungal resistance, (6) alternative therapeutic strategies and (7) novel molecules. Different research topics were assigned to the authors to generate a narrative review. The authors then retrieved full texts of the relevant papers and discussed and prepared separate drafts according to their topic. These drafts were merged into a final manuscript consisting of 9 figures, 4 tables and 161 references. The final manuscript was proofread and approved by all the authors of this study.

This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.
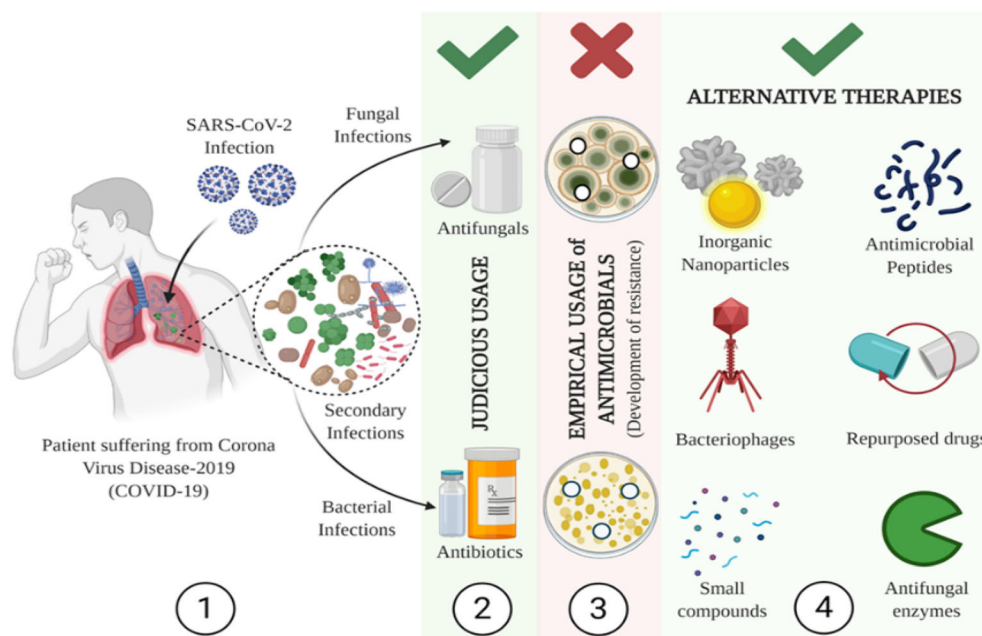
Fig. 1 Schematic representation of drug-resistant secondary infections in SARS-CoV-2-infected patients and the possible alternative therapies. (**1**) A patient suffering from COVID-19 and other bacterial and fungal secondary infections. (**2**) Current treatment regimen for secondary infections. (**3**) Empirical prescriptions and non-compliance with the prescribed antimicrobial course exacerbate the problem of drug resistance. (**4**) The untapped alternative therapeutic options available that can help combat drug resistance and treat infections

Table 1 Objectives and summary of the review

Why carry out this study?	What was learned from this study?	What are the future prospects of this study?
The mortality and morbidity rate due to secondary infections associated with COVID-19 is immense. The impractical use of antibiotics has laid down a perfect foundation for the development of drug-resistant pathogens	We have identified and overviewed several strategies that have the potential to be used against bacterial and fungal infections during COVID-19	Heavy reliance on traditional methods of targeting microbes and ignorance about innovations has led to the phenomenon of drug resistance. This study is a culmination of several alternate ways we can deal with this situation that mandates further analysis
Our narrative highlights the other effective therapies that can be introduced against these drug-resistant pathogens. This different perspective of the treatment of resistant pathogens is a bypass for the way we see medicine	The study describes the possible therapeutic applications of these alternate strategies by illustrating the various ways in which they were used successfully in in vitro and in vivo experiments against drug-resistant pathogens	Antibiotics will always be the underpinning of modern medicine; however, strategies described in this review can be an instrumental way to develop better therapeutic options. A multifaceted approach is always better than a direct one

SECONDARY BACTERIAL INFECTIONS AMONG COVID-19 PATIENTS

Secondary microbial infections in the respiratory tract are a common phenomenon in patients suffering from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and contribute significantly to high morbidity and mortality rates [1]. Opportunistic infections with bacterial and fungal pathogens often manifest as superinfections and are acquired mostly in hospitals. About 50% of the non-surviving patients suffering from COVID-19 have been reported to contract a bacterial infection, yet the required strategy to manage these infections in COVID-19 cases is not in place yet [4, 5].

An observational study on 52 critically ill patients with COVID-19 showed 7 subjects to have hospital-acquired infections and 4 out of those to have died because of infection. In another study done on the deceased patients, sepsis was found to have occurred in all the subjects (113/113) [6, 7]. A plethora of bacterial pathogens are reported to cause these infections including *Acinetobacter baumannii*, *Enterococcus faecium*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Haemophilus influenzae* and *Pseudomonas aeruginosa*. Metagenomic next-generation sequencing analysis of urine, catheters, lower respiratory and blood samples demonstrated 22 out of 38 (57.89%) patients had secondary bacterial infections with gram-negative bacteria being the most detected pathogens (50%) [8, 9]. In a study done in a city in Iran, all 19 patients admitted to ICU were found to be infected with *A. baumannii* (90%) and *Staphylococcus aureus* (10%) [10]. These reports highlight the high occurrence of secondary infections in patients suffering from the novel coronavirus disease and the urgent requirement for reliable preventive measures and solutions.

Antimicrobial Resistance in Secondary Infections

The complexity of diagnosis and treatment of secondary infections in patients with COVID-19 is a major issue that has propelled the empirical use of antibiotics [11]. Patients with invasive mechanical ventilation are prone to hospital and ventilator-acquired infections and therefore are given antibiotics [12]. A study on hospitalized patients found that 72% (1450/2010) of the patients received antibiotic treatment while only 8% (62/806) actually had any bacterial or fungal infections. This excessive use of antibiotics is further exacerbating the antimicrobial resistance menace [3, 13]. While some bacteria are naturally resistant to antibiotics, others can acquire resistance through chromosomal mutations (such as insertions and deletions) and horizontal gene transfer via transformation, conjugation or transduction by temperate phages [14, 15]. The resistance phenomenon however can increase rapidly on over-prescription of antibiotics [16]. Epidemiological studies have also illustrated a direct relationship between antibiotic use and the development and dissemination of drug-resistant pathogens [17, 18]. A worldwide increase in *Enterobacterales* producing carbapenemase (such as *K. pneumoniae* and *E. cloacae*) and clinical isolates of multiple and extremely drug-resistant strains (MDR and XDR) of *A. baumannii* and *P. aeruginosa* have been reported recently [19]. A similar trend was also observed in the high-risk clones (HiRCs) of (ST)131 *Escherichia coli* and ST258, ST307 and ST11 *K. pneumoniae*, which have developed effective resistance mechanisms against even the new antibiotics such as aminoglycoside and polymyxins [20]. Bacterial resistance to existing antibiotics has therefore threatened the underpinning of modern medicine, and the current pandemic caused by SARS-CoV2 may also have accelerated the cause and spread of antimicrobial resistance [3]. Hence, in this study, we have attempted to identify some of the effective

alternatives to antibiotics that appear promising to mitigate this global menace. We believe that by evaluating other antimicrobial solutions which could perhaps be leveraged for treating secondary infections in COVID-19 patients, indiscriminate use of antibiotics can also be curtailed (Table 2).

Table 2 Features of other alternative approaches proposed in this review

Features	Antibiotics	Inorganic NPs	Phages	Phage lysins	Repurposed drugs
Resistance	Bacterial pathogens can develop resistance to antibiotics [18]	The diversity in the action of NPs indicates fewer chances of bacterial resistance [138]	Resistance against lytic phages is rare [139]	Resistance against lysins appears bleak [140]	If resistance develops, the same approach could help us discover new drug candidates
Specificity	Antibiotics are of both broad and narrow ranges [18]	They are generally of a broad range [138]	Phages are highly specific [45]	Lysins are specific for a target [141]	Repurposed drugs are mostly of broad-spectrum [70]
Availability	Antibiotics are tougher to formulate and produce [142]	NPs can be prepared readily and studied against any pathogens [143]	Phages against any pathogenic bacteria can be isolated [45]	Endolysins have a moderate discovery rate [141]	Repurposed drugs are mostly FDA-approved drugs that are readily available [144]
Efficiency	Antibiotics are efficient against both types of bacteria [145]	NPs have efficient antimicrobial activity against both bacteria and fungi [146, 147]	Phages have been reported to be effective against both types of bacteria [148]	Lysins are also reported to be effective against both types of bacteria [149]	Can be highly effective against various bacterial pathogens [70, 150]
Environmental impact	Non-essential usage of antibiotics can affect downstream environments [151]	Excessive use of NPs can affect health and environmental conditions [152]	Being composed of protein and nucleic acid, phages have a low environmental impact [153]	No reported significant environmental impact	Excessive usage of even repurposed drugs can affect downstream environments [154]
Commerciality	The cost of developing a new antibiotic is quite high [155]	Synthesis of NPs is quite simple and cost-effective [156]	Isolation and pharmaceutical production of phages is relatively economic [153]	The cost of lysin production is high [141]	Repurposed drugs usually circumvent the cost of new drug formulation [144]

SECONDARY FUNGAL INFECTIONS AMONG COVID-19 PATIENTS

Fungal infections contribute heavily to human fatality, yet their role is not well recognized [21]. A retrospective study of SARS and influenza virus from China and worldwide suggests that COVID-19 patients, especially those who are severely ill and immunocompromised, are at a greater risk of suffering from invasive mycoses. Effective treatment and life-saving operations would require proper detection and responsible treatment of the secondary fungal infections [22].

Age and SOFA (sequential organ failure) scores are some of the most influential prognostic factors being monitored in COVID-19 patients. Other vital factors for the said disease are pre-existing diabetes and chronic ailments such as obesity, hypertension and chronic lung disease, etc. [5]. Although the presence of bacterial and/or fungal co-infection is an important and explicit factor affecting mortality, it has not received the appropriate clinical attention [23]. Diagnosis of COVID-19 in patients along with viral pneumonia is often escalated by secondary infections, which have contributed extensively to the inflating the mortality rate. According to a cohort study [9], 20 of the 90 SARS patients had infections involving the lower respiratory tract in 2003, which summed up to nearly 70.6% of SARS patients who were critically ill. Of these infections, the most common pathogenic agents were gram-negative bacteria and the fungi *Candida*. Other common secondary infections include mucormycosis, aspergillosis and cryptococcosis [22].

Fungicide Resistance in Primary and Secondary Fungal Infections

Heavy and unregulated use of fungicides has increased the incidence of invasive mycoses, which are resistant to several fungicides (Fig. 2). This has been noticed in both plant pathogenic fungi and strains that affect human health [24]. The effects of such non-judicious use are explicit in critically ill patients. In a cohort of 31 patients, 19.4% were found to be suffering from
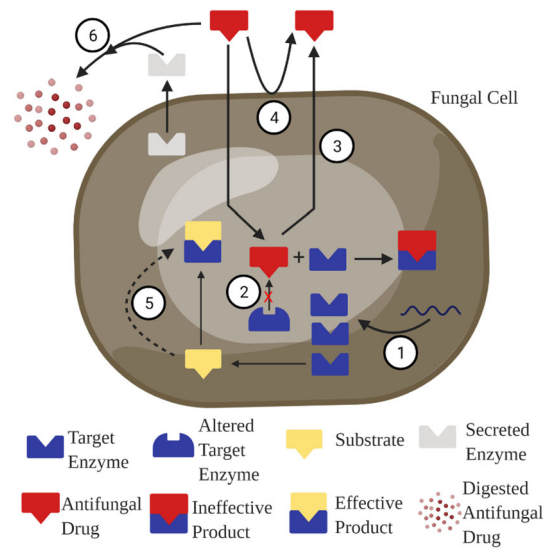

Fig. 2 Mechanisms of resistance in fungi against antifungal compounds. (**1**) Fungi tend to overproduce enzymes that are targeted by azoles and other drugs. This prevents the inhibition of vital biochemical reactions. (**2**) Altering the spatial structure of the targeted enzyme reduces the binding efficiency of azole exponentially. This is a classic case of enzyme-substrate mismatch. (**3**) Azoles and antifungal drugs are actively pumped out of the cell with the help of efflux pumps. (**4**) The drugs are not able to penetrate the fungal cell wall/membrane. (**5**) The cell bypasses the conventional pathway that the drug aims to target. (**6**) The fungal cells secrete extracellular enzymes that degrade the antifungal compounds

aspergillosis [25]. Recent studies conducted in hospitals in India dedicated to the treatment of COVID-19 reported candidemia to affect approximately 2.5% of the critically ill patients, of which 53% died. Additionally, 66% of patients who died were experiencing persistent fungemia despite having been treated with antifungal medication [26]. Oral triazole therapy has been established as the gold standard for the treatment of fungal infections and has been in use clinically for many years. However, a study conducted a decade ago by Denning et al. reported a high frequency of triazole resistance in non-culturable *Aspergillus fumigatus* in patients suffering from chronic fungal diseases (Fig. 2) [27]. Failure of common and traditional methods of therapy calls for examining the non-traditional solutions (Table 3).

Table 3 Comparison of various features of antifungal drugs with the other alternative antifungal therapies proposed in this review

Features	Antifungal drugs	Antifungal enzymes	Antifungal peptides	Repurposed drugs
Name	Azoles [27, 113, 114]	Chitotriosidases, lactoferrins, antileukoproteases and lysozymes [86, 92, 98, 157]	Defensins, cathelicidins, dermicidins, hGAPDH, synthetic peptides [117, 111, 122–124]	Haloperidol, anti-inflammatory drugs, atorvastatin [74, 76, 81]
Tested on fungi	Have been long in use and developed for every potentially pathogenic fungus	*Candida albicans, C. stellatoidea, C. tropicalis, C. pseudotropicalis, C. krusei, Aspergillus fumigatus*	*Candida albicans, C. stellatoidea, C. tropicalis, C. pseudotropicalis, C. krusei, Cryptococcus neoformans, Aspergillus, Fusarium oxysporum, Neurospora crassa*	*C. albicans, Cryptococcus, Saccharomyces, Aspergillus, C. neoformans, C. gattii, Lomentospora prolificans*
Range	Both broad and narrow ranged	Broad ranged [95]	Broad ranged [111]	Broad ranged
Sources	Mostly synthetic preparations	Natural plant and human-based sources [86, 92]	Natural as well as synthetic preparations	Repurposed traditional drugs
Reported cases of resistance	High [27, 158]	Not reported yet	Not reported yet	Not reported yet
Feasibility of therapeutics	Yes, in practice	Yes, tested [157, 87]	Yes, tested [111, 126]	Yes, tested

INORGANIC NANOPARTICLES AS ANTIMICROBIALS

Inorganic nanosized particles (10–15 nm dimension) have been gaining importance because of their efficiency as biocidal agents against both pathogenic bacteria and fungi [28–30].

Silver nanoparticles (AgNPs) are one of the most successful and widely used antimicrobial agents because of their multi-targeted actions on pathogens. Ag+ ions released by AgNPs interfere with electron transport and in the transduction of signals, leading to the damage of bacterial DNA, cell membranes and proteins (Fig. 3) [31, 32]. AgNPs have demonstrated antibacterial properties in vitro against ampicillin-resistant bacterial pathogens such as

E. coli, S. aureus (ATCC 25923), *K. pneumoniae* and *H. influenzae* [33, 34] and in vivo activity in a murein model against an MDR strain of *P. aeruginosa* [35]. The antifungal activity of AgNPs has also been well established, and studies by Bocate et al. and Bahrami-Teimoori et al. have demonstrated the efficacy of AgNPs against several pathogenic fungal species such as *Aspergillus ochraceus, A. flavus, A. parasiticus, A. nomius, A. melleus, Fusarium oxysporum, Macrophomina phaseolina* and *Alternaria alternata* [36, 37].

Zinc oxide (ZnO) NPs have also received much attention because of their biocidal activity [30, 38]. Their applications result in cell shrinkage, disorganization and cell death, as has been confirmed by confocal laser scanning microscopy (CLSM) and scanning electron
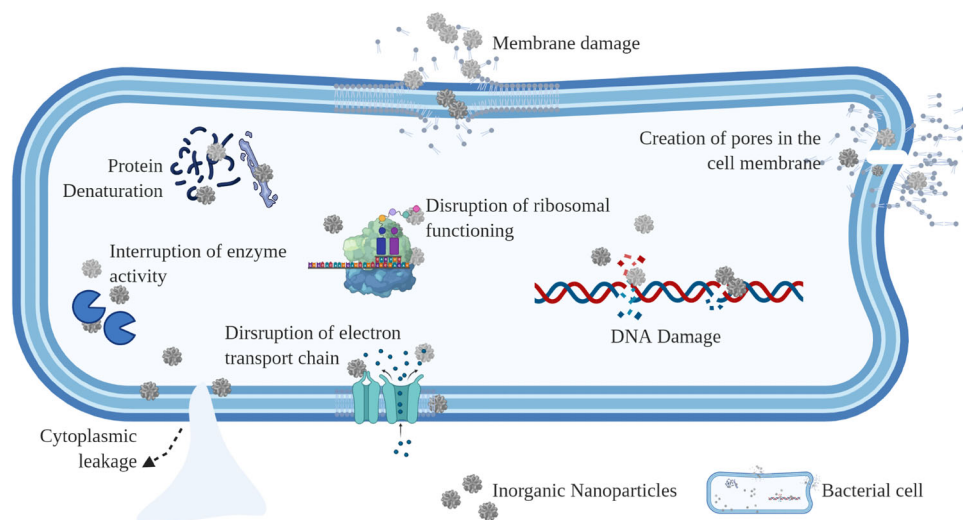
Fig. 3 Diagrammatic illustration of known antibacterial mechanisms of inorganic nanoparticles (NPs). Inorganic NPs cause membrane damage and form pores causing cytoplasmic leakage and also interruption of electron transport chain and activity of essential bacterial enzymes. Other antibacterial activities of NPs include damage to bacterial DNA and proteins, collectively leading to cell death

microscopy (SEM) analysis in some reports (Fig. 3) [39, 40]. These NPs have demonstrated growth inhibition and a decrease in infectivity in *K. pneumoniae* and increased permeability and disintegration of the cell membrane in *E. coli* [41]. The antimicrobial activity of ZnO NPs has been demonstrated experimentally in both in vitro and in vivo set-ups. These NPs are not just bactericidal; their antifungal efficacy has also been indicated by several studies. Green synthesis procedures of ZnO NPs also have added advantages and shown inhibitory effects against pathogenic *C. albicans* and *A. niger* [30, 42–44].

Given the promising potential of inorganic NPs, studying their safety and efficacy and developing them as therapeutics requires more attention. Important factors to consider should be measuring their toxicity, stability and immuno-modulatory response [32].

BACTERIOPHAGES AS ANTIBACTERIAL AGENTS

Bacteriophages (phages in short) are bacterial viruses that infect bacterial hosts through highly specific interactions [45, 46]. Phages typically follow two different cycles: lytic and lysogenic. In the lytic cycle, phage infection is followed by lysis of the host bacterium and the release of new viral particles (Fig. 4). The lysogenic cycle involves the integration of the viral genome into the genome of the host bacteria as a prophage, which remains integrated with the host until an event triggers the release of new virions [46, 47].

Phage therapy (using lytic bacteriophages to treat bacterial infections) dates back to the early 1900s when Frederick Twort (1915) and Felix d'Hérelle (1917) discovered phages independently. Felix d'Hérelle had successfully used phages for the first time to treat dysentery, a disease that did not have an effective treatment then. In the 1920s and 1930s, phage therapy was being commonly used in several parts in Europe and the erstwhile Soviet Union. However, the advent of antibiotics eclipsed the use of phage therapy in most parts of the world except for a few countries of Eastern Europe where it is still practiced, such as the Eliava Phage Therapy Center in Tbilisi, Georgia, and the Phage Therapy Unit (PTU) of Hirszfeld Institute of Immunology and Experimental Therapy, Wrocław, Poland, among others [48]. Over the years, recommended prerequisites for
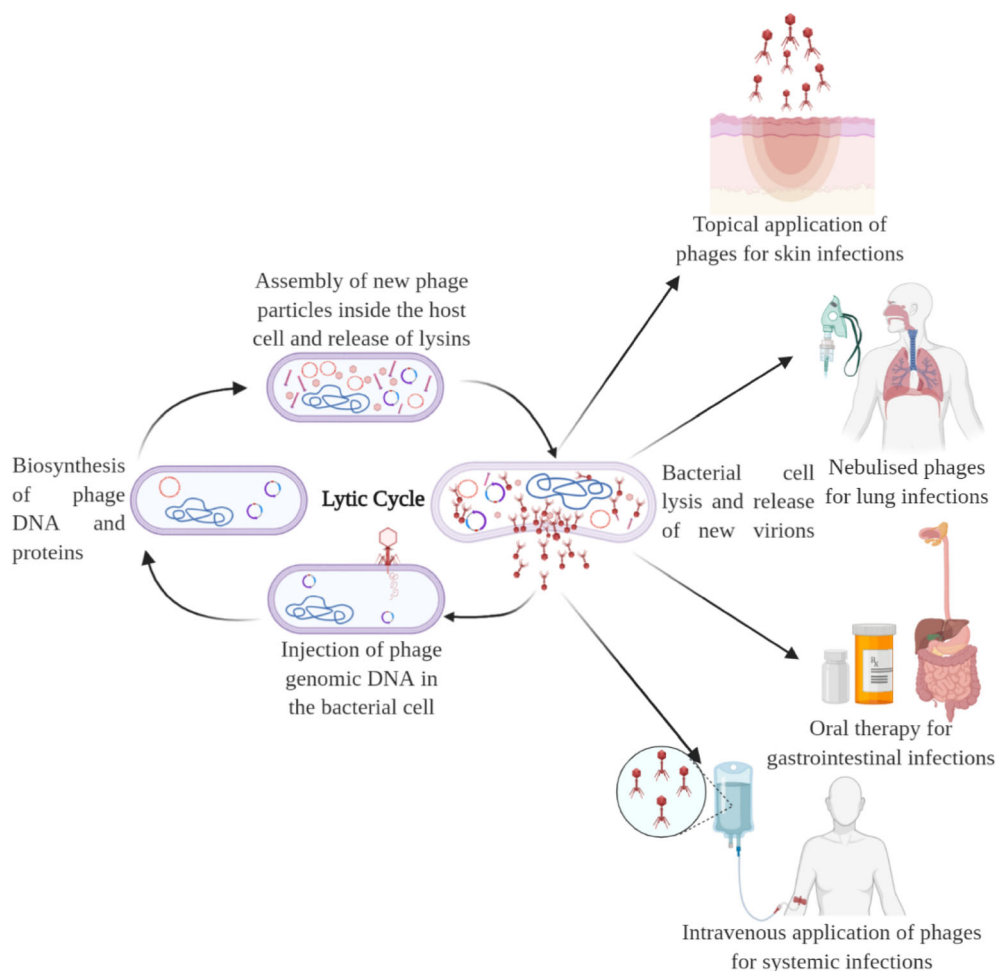
Fig. 4 Therapeutic usage of lytic bacteriophages to treat bacterial infections. The figure illustrates the lytic cycle of bacteriophages and the known methods for their administration for the treatment of bacterial infections

therapeutic application of phages have emerged which need to be adhered to, including the use of well-characterized infective phages with no or permissible levels of endotoxins [49–51]. The use of bacteriophages as investigative new drugs (IND) for treating patients who are unresponsive to available antibiotics has been approved by the FDA in several cases and is gaining wide acceptance [52, 53].

Recent successful cases of phage therapy in treating life-threatening infections in humans have reignited interest in phage therapy, and modern technology-fueled research is being carried out toward addressing gaps in our understanding of phage biology [54, 55]. A study by Schooley et al. describes how nine

different bacteriophages were used in personalized therapy for the treatment of a 68-year-old patient with pancreatitis accompanied by a MDR *A. baumannii* infection. Phages, in this case, were administered both intravenously and percutaneously within the abscess cavities, which led to clearance of the pathogen. Moreover, phage-resistant *A. baumannii* strains that developed during the therapy were found to have increased sensitivity to antibiotics, which dramatically improved the patient's condition [54]. The phage cocktails from the Eliava Institute, Tbilisi, comprise phages that infect several different species of pathogens. The pyophage preparation containing phages against *S. aureus*, *Streptococcus* sp., *P. aeruginosa*, *E. coli* and *Proteus*

has been used for patients suffering from urinary tract and digestive system infections along with bronchopneumonia, leading to successful clearance of the pathogens. The intestiphage mixture consisting of phages against pathogenic *E. coli*, *Shigella* sp., *Proteus* sp., *Staphylococcus* sp., *Pseudomonas* sp. and *Salmonella* sp., responsible for causing intestinal problems, was successfully used in a trial comprising 580 children [56]. Apart from these reports, the Eliava Phage Therapy Center has also successfully treated many patients suffering from MDR strains of *Achromobacter xylosoxidans* causing lung infection in cystic fibrosis [57]. These successes indicate phages could be potential game changer when dealing with drug-resistant secondary infections in COVID-19 patients as well. Unlike antibiotics, natural phages can be rapidly isolated in large amounts. Synthetic biology can also generate innumerable variants of a phage [58]. In addition, the delivery and administration of several phages as therapeutics are well established [59]. Ryan et al. have described successful routes for phage applications including inhalation of nebulized phages for lung infections, topical applications for local delivery and oral intake for gastrointestinal infections (Fig. 4) [60]. FDA approval of phage therapy against secondary infections for patients in critical care with COVID-19 in 2020 reinforces the role bacteriophages can play in managing the pandemic [61].

REPURPOSED DRUGS

The paucity of new antibiotics and the discovery and development of new drugs inherently being a lengthy process that incurs huge costs [62], repurposing of existing drugs is gaining interest and acceptance for treating multidrug-resistant (MDR) infections and other human diseases (Fig. 5). Overall, it involves lower costs and shorter development timelines [63, 64]. A review of the field shows several approved drugs have already been repurposed for new therapeutic uses [65–67]. Sildenafil, which was originally intended to treat hypertension, is being repurposed for the treatment of erectile dysfunction and pulmonary arterial hypertension.

Bupropion, thalidomide and minoxidil are also being repurposed [68]. Many of the drugs (remdesivir, hydroxychloroquine and ribavirin) used in the treatment of COVID-19 are fresh examples of repurposed drugs. Drug repurposing hence should be vigorously pursued as a promising approach for finding drugs against antibiotic-resistant secondary bacterial/fungal infections [69].

Repurposed Drugs for Treating Bacterial Infections

Ciclopirox is an off-patent topical antifungal drug that also has the potential to inhibit the growth of *A. baumannii*, *K. pneumoniae*, *P. aeruginosa* (including MDR isolates) and *P. mirabilis*. In *P. aeruginosa* ciclopirox inhibits the production of pyocyanin, a secondary metabolite, and also may decrease the production of pyoverdine, an iron-scavenging siderophore that contributes to the pathogenicity of *P. aeruginosa* [70, 71]. Statins also have been reported to reduce virulence factors of *P. aeruginosa* and in vivo attachment of *S. pneumoniae* to lung and vascular tissues and have shown broad-spectrum antibacterial effect though at relatively high concentrations [72, 73].

Repurposed Drugs for Treating Fungal Infections

For treating fungal infections (Table 4, Fig. 5), currently, the most prescribed drugs that show broad-spectrum antifungal activity are azoles, including ketoconazole, voriconazole, itraconazole, polyenes and echinocandins [74, 75]. Among the repurposed drugs, the antifungal activity of tamoxifen, a generic anticancer drug used in treating breast cancer patients, has been demonstrated by a library screening of FDA-approved drugs [76, 77]. Interestingly, estrogen-receptor independent mechanisms of the drug have been suggested to be related to its antifungal activity. Another example is the antirheumatic drug auranofin, found to be active against *C. albicans* biofilms [78]. Auranofin has recently also been reported against *Cryptococcus*, the causative agent of cryptococcosis, which is
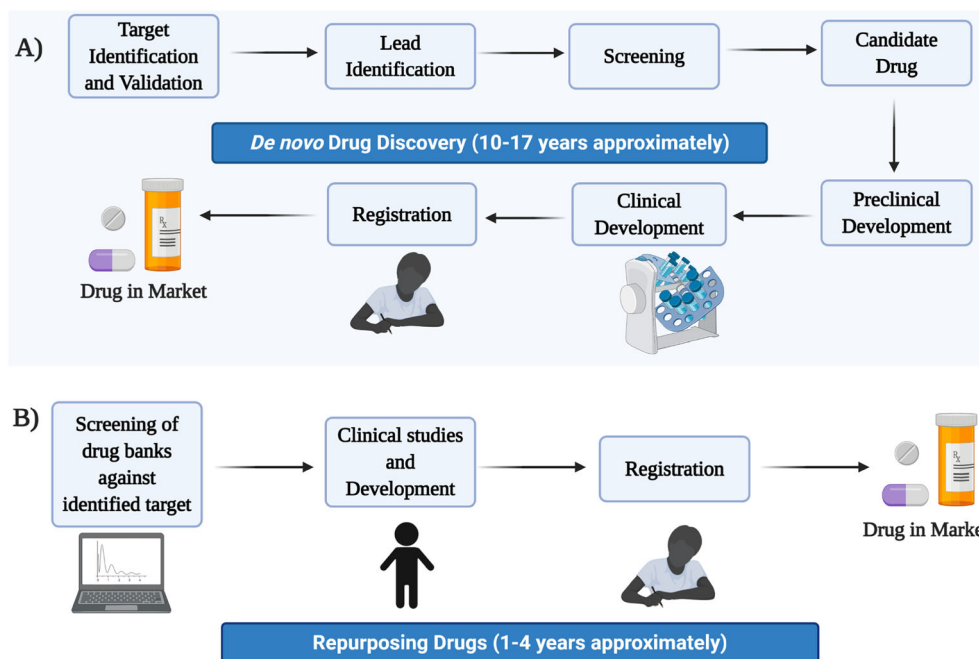
Fig. 5 Schematic representation of de novo drug discovery vs. repurposing of available drugs. De novo drug discovery and development can take up to 15 years for a drug to be available for clinical use. Drug repurposing by-passes time taken from drug discovery to pre-clinical stage

Table 4 Examples of the reported repurposed drugs against pathogenic bacteria and fungi

Drug	Initial usage	Repurposed against
Tamoxifen [63]	Anti-cancer drug	*Staphylococcus aureus*
Diflunisal [82]	Non-steroidal anti-inflammatory drug	*Staphylococcus aureus* (MRSA)
Nicosalmide [159]	Antihelminthic drug	*Psuedomonas aeruginosa*
Oxyclozanide [150]	Veterinary anthelmintic drug	*Staphylococcus aureus*
Chlorpromazine [160]	Antipsychotic drug	*Klebsiella pneumoniae*
Sertraline [160]	Antidepressant drug	*Enterococcus faecalis*
Nisoldipine, nifedipine, felodipine [80]	Calcium channel blockers	*Saccharomyces, aspergillus, cryptococcus*
Asprin, ibuprofen, tacrolimus [76]	Anti-inflammatory drug	*C. neoformans, C. gattii* and *E. rostratum*
Atorvastatin [81]	Cardiovascular drug	*C. gattii*
MMV665943 [161]	Preclinical malarial drug	*C. albicans, C. neoformans, C. gattii* and *Lomentospora prolificans*

one of the most crucial fungal infections affecting immunocompromised patients [79]. Additionally, nifedipine, nisoldipine and felodipine also inhibit *Cryptococcus*, where nisoldipine has been reported to be effective against *Candida*, *Saccharomyces* and *Aspergillus* [80]. Several anti-inflammatory drugs such as aspirin, ibuprofen, and tacrolimus have exhibited antifungal properties against *C. neoformans*, *C. gattii* and *E. rostratum*, respectively [76]. Atorvastatin, a cardiovascular drug, has been tested as an adjuvant to control fungal infections in a study which showed the potency against one strain of *C. gattii* [81]. MMV665943 is a preclinical antimalarial drug that has been reported exhibiting inhibitory response against *C. albicans*, *C. neoformans*, *C. gattii* and *Lomentospora prolificans* (Table 4) [82]. An increase in the biofilm-related infections worldwide and their recalcitrant response to classical antifungal drugs call for drug repurposing as a faster and cheaper approach to treat fungal infections (Fig. 5). A seeming lack of incentives for repurposing drugs compared to those for de novo drug development is however an impediment. Incentives like patent protection and tight regulations for entry by generic pharmaceutical manufacturers can help overcome some of the hurdles [68].

ENZYMES AS ANTIMICROBIAL AGENTS

Development of Antifungal Enzymes as Antimicrobial Agents

Lysozymes

Lysozymes are antimicrobial enzymes that hydrolyze 1, 4-beta-linkages of *N*-acetylmuramic acid and *N*-acetyl-ᴅ-glucosamine in the bacterial cell walls, leading to cell lysis (Fig. 6). The human body synthesizes and secretes lysozymes naturally as a defense against pathogenic bacteria and fungal pathogens such as *C. albicans*, *C. stellatoidea*, *C. tropicalis*, *C. pseudotropicalis* and others [83, 84]. Five antimicrobial peptides obtained on pepsin digestion of lysozyme have been found to be effective against *C. albicans* [85]. Lysozymes from different sources,

such as *Phaseolus mungo* and *Galleria mellonella*, have also been demonstrated to have antifungal potential and therefore could be potent sources for lysozymes for therapeutic purposes [84].

Chitotriosidase

When activated, macrophages and neutrophils in humans release the chitotriosidase enzyme, which is known to cleave chitin and kill invasive mycoses (Fig. 6) [86, 87]. High concentrations of chitotriosidase enzyme are expressed in the human eye, especially in the lacrimal glands, for defense against fungal organisms [88]. Chitotriosidase is currently used as a biomarker of macrophage activation caused by diseases such as sarcoidosis and various lipid storage diseases including Gaucher disease (GD) [89, 90]. Increased expression of chitotriosidase mRNA has been observed in animal models, suggesting them to be important mediators in immune responses [91].

Lactoferrins

Lactoferrins are proteases that bind to iron atoms and are omnipresent in almost all secretions of the body, including the salivary secretions. Lactoferrins produced by humans are found to be effective against *C. krusei* and *C. albicans* [92, 93]. Acceptable sources of bovine and recombinant human lactoferrins are accessible for commercial applications, although further research is required for its medicinal use [94]. Their microbial activity has been shown to be very broad in nature due to their ability to sequester ferric ions [95, 96]. Some clinical studies showed that oral administration of bovine lactoferrins can significantly reduce an already established candidiasis in mice. The therapeutic effect of orally administered lactoferrins also has exhibited a reduction in lesions in the oral cavity [97].

Antileukoproteases (ALP)

Antileukoproteases are serine-based mucous protease inhibitors and can be traced in bodily secretions of bronchial, cervical and nasal mucosa along with saliva and seminal fluids. The antifungal effect of recombinant ALP against *A. fumigatus* and *C. albicans* has been
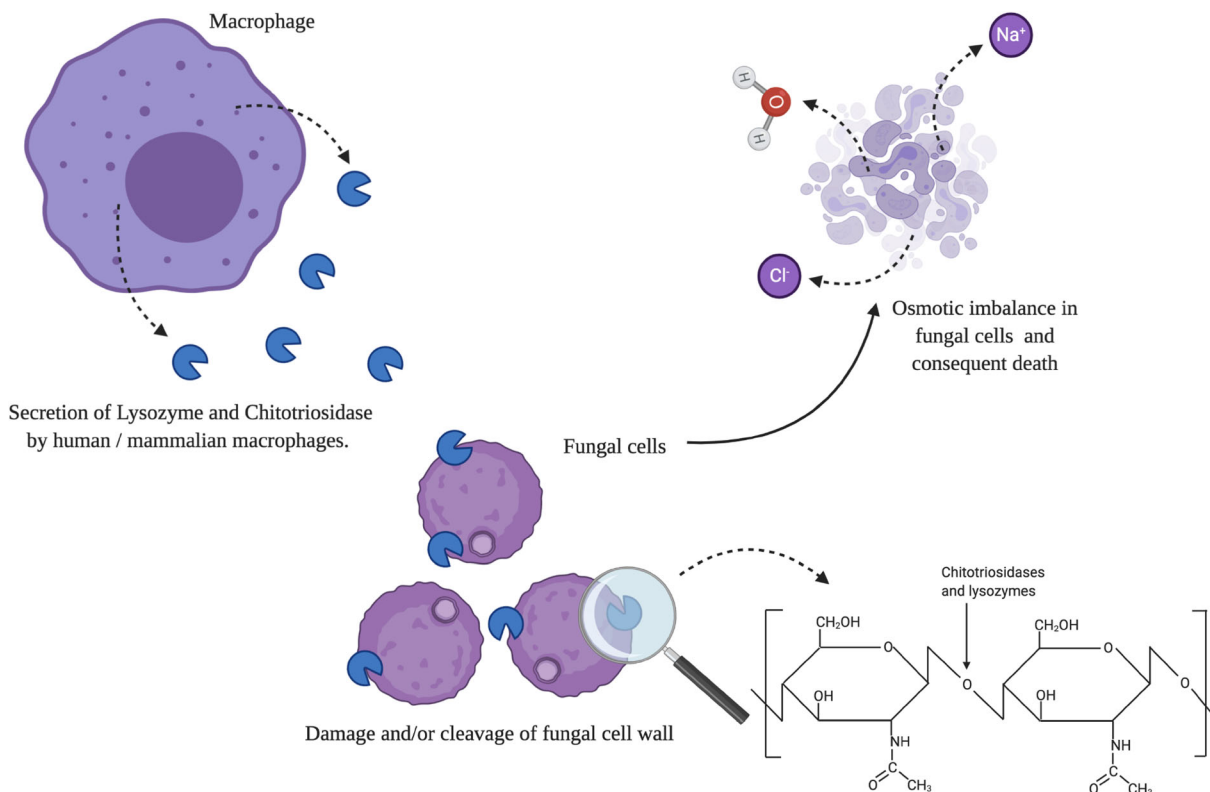
Fig. 6 Antifungal action of macrophage-secreted lysozyme and chitotriosidases. Various antifungal enzymes are secreted by the macrophages, for instance, the chitotriosidase cleaves the fungal cell wall and causes the rupture of cells. Other enzymes such as lysozymes, which may be secreted by both neutrophils and macrophages, are said to damage the fungal cell wall, thereby causing an osmotic imbalance leading to cell death

studied by Tomee et al. [98]. ALPs have been recommended as an alternative approach to traditional antifungal therapies and have also been suggested as a mode of treatment of fungal infections in immunocompromised patients and thus can play a key role in the management of secondary infections in COVID-19 patients [87, 98].

PHAGE LYSINS (ENZYBIOTICS)

The vast collection of whole-genome sequences of phages and characterization of their proteome have emerged as a blueprint from which several novel antibacterial agents can be developed. The bacteriophage-encoded lytic enzymes (lysins) are fast emerging [99, 100] as potential antibacterial agents due to their efficiency in cleaving the bacterial cell wall (Fig. 7). An increase in the number of new phage genomes/metagenomes in the database reveals the discovery of many novel lysin sequences. Compared to broad-spectrum, standard-of-care antibiotics, lysins are highly specific and do not cause harm to the healthy commensal microflora. They can be effective against both drug-sensitive and drug-resistant strains of pathogens and against dividing as well as non-dividing bacterial cells [100, 101]. Since peptidoglycan (PG) is the key integral component of the bacterial cell wall, the probability of bacteria modifying PG to thwart lysin attack is considerably low. Due to an efficient mechanism of action and their prospective use as the next-generation antimicrobials, phage lysins have been termed 'enzybiotics' [102].

On a promising note, lysins have exhibited a substantial response in human trials. A single dose of exebacase, an anti-staphylococcal lysin,
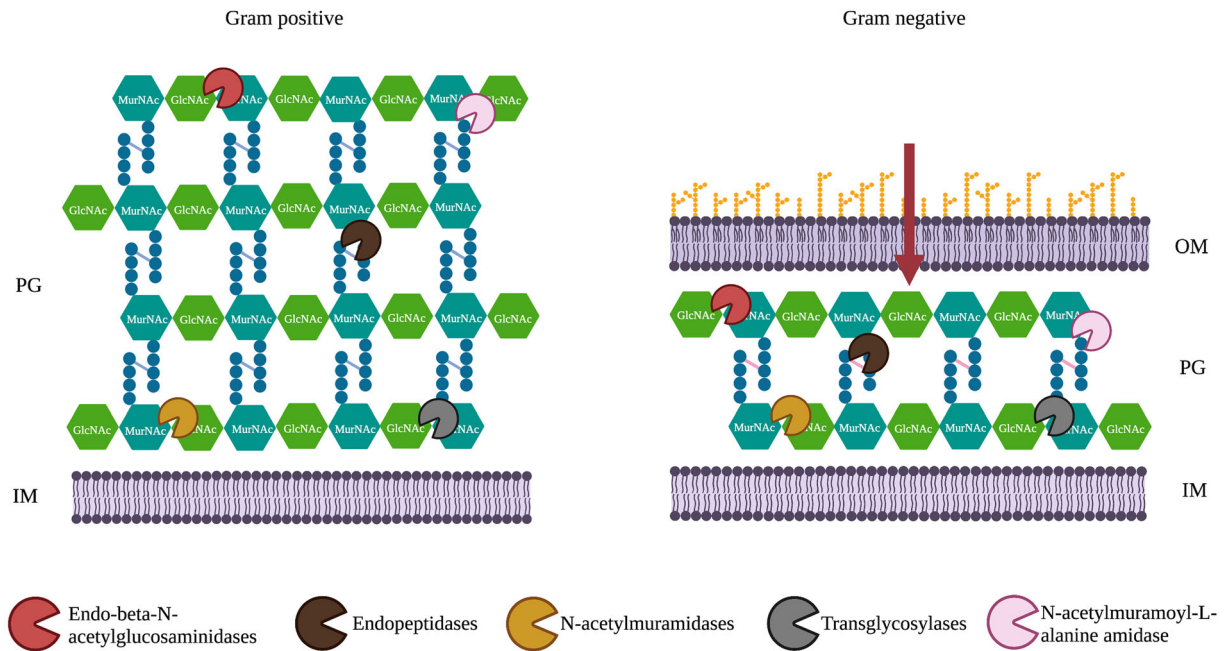
Gram positive

Gram negative

Endo-beta-N-acetylglucosaminidases   Endopeptidases   N-acetylmuramidases   Transglycosylases   N-acetylmuramoyl-L-alanine amidase

Fig. 7 Pictorial depiction of endolysin activity on the peptidoglycan layer (PG) of bacterial cells. PG layer here is represented by the repeating units of sugars *N*-acetylglucosamine (GlucNAc) and *N*-acetylmuramic acid (MurNAc). For gram-negative bacteria, endolysins (cleavage site represented as Pacman) have to transverse (red arrow) the outer membrane (OM). PG in gram-positive bacteria consists of tetrapeptide chains (blue spheres), which are cross-linked by interpeptide bridges (blue line), while in gram-negative bacteria tetrapeptide bridges are cross-linked by pentapeptide bridges (pink line). IM represents the inner membrane

showed a 70.4% responder rate when used in combination with antibiotics on patients suffering from bacterial endocarditis during its phase 2 clinical trial [103]. Lysins have also been developed for commercial applications. These include Staphefekt[TM] SA.100, which is available as a gel or cream, and Staphefekt[TM] XDR.300 (Gladskin), available as an aseptic solution. Both products specifically target MRSA infection of the skin (http://www.micreos.com/) [100]. Preclinical and early phase clinical trials have demonstrated the lytic efficiency of phage lysins. P128, a chimeric ectolysin, was demonstrated to have rapid antibacterial efficacy in vivo against MRSA and VRSA infections in a murine model [104]. Another study demonstrated the potency of a pneumococcal lysin against drug-resistant *S. pneumoniae*, the pathogen responsible for causing pneumonia, bronchitis and septicemia. Intravenous injection of lysin Cpl-1 (2 mg) in mouse models demonstrated reduction of the bacterial load

from 4 logs to undetectable levels ($\sim < 2$ logs) within 15 min of application, resulting in faster recovery from fatal pneumonia compared to the control group [105]. Another lysin (Pal) has given positive results against *S. pneumoniae* infection of the murine nasopharynx by eliminating the pathogen using a single dose with no post-treatment recolonization [101].

Secondary pneumococcal infections which are resistant to available antibiotics are a matter of great concern in COVID-19-infected patients. As antibiotics fail to meet the need for an urgent solution in treating drug-resistant infections, the bacteriolytic activity of lysins accompanied by an antibiotic synergistic effect should be explored further to realize their potential.

ANTIMICROBIAL PEPTIDES

Antimicrobial peptides (AMPs) have powerful antibacterial, antifungal and antiviral properties

[106]. Being produced by all organisms, natural and synthetic AMPs present a valuable source to treat infections. Their high effectiveness at inactivating pathogens with sometimes concurrent immunomodulatory effects makes AMPs promising candidate compounds for the treatment of infections. While many of the peptides kill bacteria by traversing the bacterial envelope to bind to intracellular targets, most of them insert into the membranes of the pathogen, creating perturbations and holes that lead to a collapse of the proton motive force and/or cause leakage of the cytoplasmic content [107–110]. In addition to AMPs that act against bacteria, many antifungal peptides have been discovered with both antimicrobial and immunomodulatory activities. Many of these peptides are also released by the human body as a natural defense against microbial infections and invasive mycoses. Peptides such as cathelicidin provide resistance against a broad range of infections [111]. A promising peptide, Jelleine-1, has been proven to exhibit very potent in vitro as well as in vivo antifungal activity [112]. A growing body of evidence remarkably hints at the antifungal potential of various antimicrobial peptides.

Defensins

Defensins are a group of short, positively charged peptides with cysteine residues that can be categorized based on the alignment of their disulfide bridges. Humans are known to express α- and β-defensins [113]. Although defensins are not restricted to humans and have also been found in a wide spectrum of organisms, ranging from simpler eukaryotes like *Pseudoplectania nigrella* to prokaryotes like *Anaeromyxobacter dehalogenans*, suggesting that the organisms evolved these mechanisms for protection against invasive mycoses in a hostile environment [114–116]. Researchers have also observed the anticandidal activity of human defensins along with the MFC (minimal fungicidal concentration) required to have an appropriate inhibitory effect in which, out of the three human defensins incorporated into the study, named human β-defensins 1–3, defensin-3

showed the strongest fungicidal activity against *C. albicans* with a low MFC of 2.5 μM [117]. Defensins sourced from plants and insects have also been successful in acting against pathogens that infect humans. Plant defensin 'HsAFP1' and insect-sourced defensin 'Heliomicin' both, for instance, have been very successful in eliminating colonies formed by *C. albicans* [118].

Cathelicidins

Cathelicidins are polypeptides that are stored in considerable concentrations in macrophages and polymorphonuclear leukocytes in humans and other animals. Their primary action is to provide an antimicrobial defense barrier for the skin and exposed surfaces of the body that are vulnerable to infection and invasion. They have a conserved 'cathelin' domain and a positively charged peptide at the C-terminal that confers an antimicrobial property to these molecules [111]. They are generally synthesized and secreted by mucosal epithelial and mast cells and are possible candidates for the treatment of fungal infections [119]. Culture-dependent antifungal activity has been shown by cathelicidins in both humans and mice; the human cathelicidin, 'LL-37', and the mouse cathelicidin, 'mCRAMP', not only impeded the growth of *C. albicans* but also eliminated it successfully in both the organisms [120]. In another study, five different cathelicidins showed significant activity in vitro against clinically isolated yeasts and *C. neoformans* in particular [121].

Dermicidins

Dermicidins are synthesized and produced by human sweat glands and are then transported to the skin surface. These peptides are processed before they gain antimicrobial activity. Dermicidins have been shown to possess high antifungal activity against *C. albicans* in laboratory conditions that were recreated to mimic the environment of human sweat [122].

hGAPDH (2-32)

A peptide that has been derived from a conserved protein, GAPDH, has been shown to confer some degree of protection to tissues against fungal infections. The hGAPDH peptide, a human GAPDH, has been shown to cause inhibition of growth of *C. albicans* in small concentrations along with simultaneous inhibition of candidal virulence factors [123].

Synthetic Peptides

Antifungal activity against *C. albicans* has been demonstrated for three chemically synthesized peptides, aurein 1.2, citropin 1.1 A and uperin 3.6, in a study by Kamysz et al. [124]. Especially in the case of secondary infections, where eliminating a resistant fungal infection could be challenging, peptides like VS2 and VS3 in combination with fluconazole were observed to have a significant impact on decelerating the growth of multidrug-resistant *Candida* spp., *Cryptococcus neoformans*, *Aspergillus. niger*, *Fusarium oxysporum* and *Neurospora crassa*. These peptides were also seen to induce necrosis by causing intracellular accumulation of reactive oxygen species (ROS) and exhibited rapid activity on the killing kinetics assay [125]. Other synthetic peptides such as KSL-W also demonstrated potent antifungal activity [126].

Next-Generation Antibiotics and Antifungal Agents

It is evident from the COVID-19 pandemic that the secondary infections caused by bacterial and fungal pathogens are a significant threat, especially to immunocompromised patients. Considering the increasing rise in antibiotic resistance, our focus on developing effective strategies based on several alternatives to manage infections is timely. It is also important here to highlight the drug discovery program underway toward developing the next generation of antimicrobials.

Small Molecule Inhibitors as Antibacterial Agents

Small chemical compounds that can target a variety of cellular loci, ranging from the cytoplasmic membrane, enzymes to respiratory functions and genetic material are good starting points in the drug discovery and development process [127]. There are novel classes of molecules that are providing new ways to assist the development of antimicrobial therapies [128]. In particular, we discuss the novel targets in pathogenic bacteria in the early stages of development (Fig. 8).

One such class of inhibitors that have an added advantage of selectivity over other antimicrobials are those which target DNA repair mechanisms as the molecular targets. Lim et al. discovered a compound, IMP-1700, that not only inhibited DNA repair in MRSA but was also found to sensitize bacteria to fluoroquinolone antibiotic ciprofloxacin, which is responsible for the induction of DNA damage [129]. Another compound, POL7080, has been demonstrated as an inhibitor of LptD, a protein involved in lipopolysaccharide insertion in the outer membrane of *P. aeruginosa*. LptD has low mutation rates and is well conserved across many bacterial species [129, 130].

IgA protease in *H. influenzae* is a virulence factor secreted by various gram-negative bacteria and is a promising target for new drugs for managing antibiotic-resistant *H. influenzae* strains [131]. Apart from targeting essential and unique enzymes in pathogens, structural variations in a host enzyme and pathogen enzyme are another aspect to limit microbial infection. Pseudouridimycin is one such recently reported inhibitor that binds selectively to bacterial RNA polymerase and is assumed to be efficacious against a variety of drug-resistant pathogens [132]. Nucleoside analogues often used to treat viral and fungal infections are also promising candidates for treating bacterial infections [133].
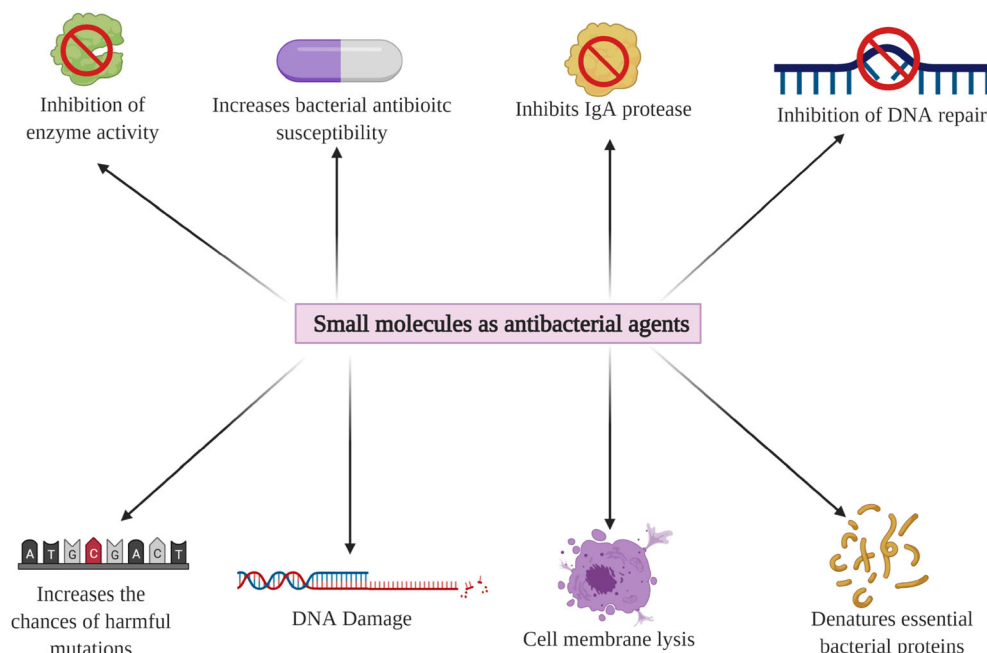
Fig. 8 Molecular antibacterial mechanisms of small molecules. Small molecules are known to inhibit bacterial DNA repair mechanisms and enhance bacterial susceptibility to the antibiotic ciprofloxacin, which further induces DNA damage. Also, several other small molecules (example: IMP-1700) are found to be efficacious against pathogens by inhibiting and denaturing the activity of certain essential enzymes required for the survival of the bacteria

Small Molecule Inhibitors as Antifungal Agents

The discovery of novel bioactive antifungal compounds and the identification of molecular targets are of extreme importance [134]. SARS-CoV-2 patients being immunocompromised have higher chances to suffer from invasive fungal infections causing aspergillosis (*Aspergillus* infection), candidiasis (*Candida* infection), cryptococcosis (*Cryptococcus* infection) and mucormycosis (*Mucorales* infection) [22].

Tertiary RNA structures are emerging as effective de novo targets that can lead to the discovery of pharmacologically active and non-toxic antifungal molecules. RNA structures such as self-splicing group-II intron are found in eukaryotes such as plants, yeast and fungi and are absent in human cells. These compounds are effective and comparable to amphotericin B in inhibiting the growth of *C. parapsilosis* [135].

A dominant virulence attribute of fungi such as *C. albicans* is yeast to hyphae transition, which is another promising drug target. In a study on the discovery of small molecule inhibitors against *Candida* infections by Wong et al., screening a library of 50,240 compounds led to the discovery of a novel compound called SM21, which exhibits strong activity against drug-resistant *Candida* isolates and biofilm. SM21 was also found to be non-toxic to human cells (Fig. 9) [136].

In addition to similar studies, several small molecules such as farnesol, rapamycin, geldanamycin, histone deacetylase inhibitors and cell cycle inhibitors in *C. albicans* that control hyphae production have been reported [137].

CONCLUSION

Secondary infections are the major cause of death in immunocompromised SARS-CoV-2-infected patients. There is an urgent need to review the current drug regime and fast-track research and pre-clinical/clinical studies
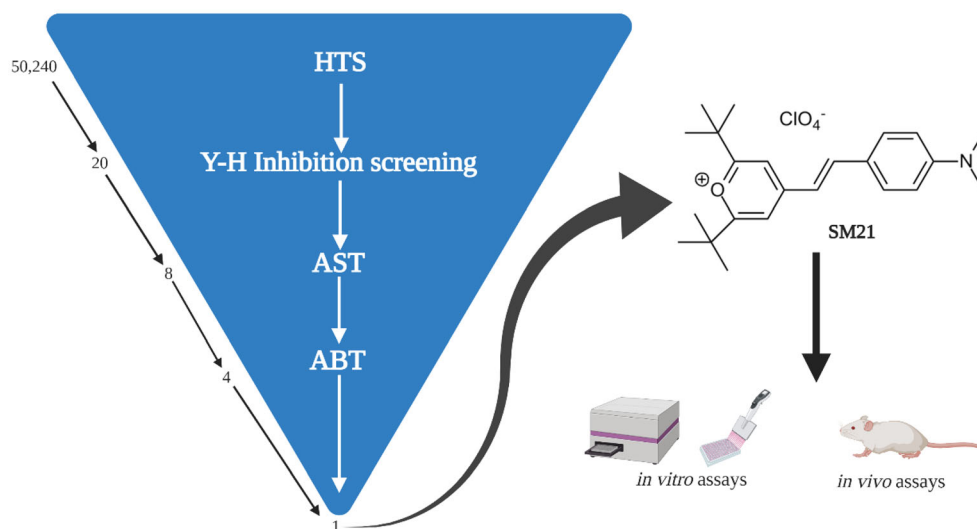
Fig. 9 Screening of novel antifungal small molecules. Wong et al.'s study is a potent example of how novel antifungal molecules are screened from a library of molecules. High-throughput screening (HTS) of a library of 50,240 small molecules was done for Y-H inhibitors, which yielded 20 active compounds that were further validated by assessing their activity in a dose-dependent manner. Eight molecules were identified as potent Y-H inhibitors, which were further analyzed in an antifungal susceptibility test (AST). The four most potent molecules were selected for an anti-biofilm test (ABT), which led to SM21, the most potent of all, that was chosen for further in vitro and in vivo assays

required for the development of credible alternatives to treat primary or secondary drug-resistant infections. This pandemic impels us to explore therapeutic options beyond the conventional and tap into less studied methods of treatment. Several promising developments in the field of antimicrobials have been made lately, as traced in this review, and we believe a multipronged approach can be a better way to deal with secondary infections instead of depending on antibiotics alone. However, validation of the merits, safety and efficacy of these strategies through clinical trials needs to be expedited. Entailing mechanisms for compassionate use of the suggested treatments need to be brought into mainstream discussion as well. Preparedness for future global crises such as the current coronavirus pandemic is imperative.

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Compliance with Ethics Guidelines. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

Data Availability. Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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