Antibacterial Compounds from Mushrooms: A Lead to Fight ESKAPEE Pathogenic Bacteria?

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ABSTRACT

Infectious diseases are among the greatest threats to global health in the 21st century, and one critical concern is due to antibiotic resistance developed by an increasing number of bacterial strains. New resistance mechanisms are emerging with many infections becoming more and more difficult if not impossible to treat. This growing phenomenon not only is associated with increased mortality but also with longer hospital stays and higher medical costs. For these reasons, there is an urgent need to find new antibiotics targeting pathogenic microorganisms such as ESKAPEE bacteria. Most of currently approved antibiotics are derived from microorganisms, but higher fungi could constitute an alternative and remarkable reservoir of anti-infectious compounds. For instance, pleuromutilins constitute the first class of antibiotics derived from mushrooms. However, macromycetes still represent a largely unexplored source. Publications reporting the antibacterial potential of mushroom extracts are emerging, but few purified compounds have been evaluated for their bioactivity on pathogenic bacterial strains. Therefore, the aim of this review is to compile up-to-date data about natural products isolated from fruiting body fungi, which significantly inhibit the growth of ESKAPEE pathogenic bacteria. When available, data regarding modes of action and cytotoxicity, mandatory when considering a possible drug development, have been discussed in order to highlight the most promising compounds.

Introduction

Nowadays, 97 % of approved antibiotics have a microbial origin, from either soil fungi (e.g., penicillin) or bacteria (e.g., streptomycin) [1]. These antibacterial agents have revolutionized the treatment of infections such as tuberculosis, pneumonia, leprosy, and gonorrhea. Unfortunately, the overuse and misuse of antibiotics has gradually led to acquired drug resistance in bacterial strains, an ever-increasing phenomenon. Almost a century after Fleming's discovery of penicillin, there is a sore need to identify new antibacterial drugs capable of fighting infections caused by multidrug-resistant (MDR) bacteria.

Alternative sources could represent an interesting reservoir of such molecules. Over the past decade, search for antibacterial compounds has been extended to marine microorganisms or endophytic fungi, which also need to activate a whole antimicrobial chemical arsenal to impose themselves in their highly competitive environment [2, 3]. In comparison to cultured bacteria and fungi, there has been considerably less attention devoted to investigating macroscopic fungi. Indeed, as they are also exposed to various biotic stresses, both at the underground (mycelium) and aerial (fruiting body) level, they synthesize a variety of antibacterial molecules. In Western countries, mushrooms are considered above all for their culinary and nutritional value, like truffles or boletus mushrooms that deliver a sought-after flavor, or white mushrooms, low in calories but providing a good source of dietary fibers, proteins, vitamins, and minerals. In Asia, where traditional Chinese medicine prevails, many mushrooms are not only popular

ingredients in cuisine, but they are also reputed for their health benefits, like shiitake or *Ganoderma lucidum*, with numerous studies reporting their immunomodulatory properties and anticancer potential [4, 5]. Yet, the interest in macromycetes as an alternative source of antibacterial agents seems to emerge somewhat [6, 7], and a new class of mushroom-derived antibiotics, pleuromutilins, has been released recently [8].

Although numerous recent publications report the antibacterial activity of mushroom extracts, studies dealing with purified and identified compounds are still scarce. Therefore, this review aims at establishing a state of the art on mushroom molecules that affect bacteria development. Some publications report antibacterial activity against nonpathogenic bacteria, such as the Gram-positive model *Bacillus subtilis*. We have considered that it would be much more valuable to pay attention to molecules inhibiting the growth of pathogenic bacteria only. For this reason, we have decided to focus this review on fungal compounds exhibiting an antibacterial activity toward bacteria that represent a major threat in this context of antibiotic resistance, namely ESKAPEE bacteria: *Enterococcus faecium* (▶ **Table 1**), *Staphylococcus aureus* (▶ **Table 2**), *Klebsiella pneumoniae* (▶ **Table 3**), *Acinetobacter baumannii* (▶ **Table 4**), *Pseudomonas aeruginosa* (▶ **Table 5**), *Enterobacter* species (▶ **Table 6**), and *Escherichia coli* (▶ **Table 7**) [9].

The applied search strategy consisted in the systematic literature reporting mushroom antibacterial compounds active against ESKAPEE bacteria, without any time limitation. Electronic databases including SciFinder, Web of Science, Science Direct, PubMed, and Google Scholar were screened using the following keywords: “antibacterial, antimicrobial, mushroom, fungi, compounds, metabolites, Enterococcus, Staphylococcus, Klebsiella, Acinetobacter, Pseudomonas, Enterobacter, Escherichia coli”. Careful attention was paid to select publications referring only to purified compounds isolated from macromycetes and evaluated against one or several of these 7 pathogenic strains.

ESKAPEE Pathogenic Bacteria

As mentioned above, the excessive or inappropriate use of antibiotics has progressively led to the emergence of multidrug resistance. After the golden age of antibiotics in the middle of the 20th century, bacteria began to evolve by developing a whole arsenal of resistance genes, leading, for example, to drug inactivation and biofilm formation [10]. As a result, MDR infections were responsible of more than 33 000 deaths in Europe in 2015 [11], and in the United States, more than 2.8 million patients suffer from antibiotic-resistant infections each year [12]. Global data are alarming: drug-resistant infections are responsible for one death every 45 s in the world [13]. Besides this high increase in mortality and morbidity rates, antibiotic resistance also generates a high additional economic burden, reaching up to 20 billion dollars for the U. S. healthcare system [12, 14, 15].

In 2017, the WHO published a list of antibiotic-resistant bacteria, divided into 3 categories according to their degree of pathogenicity and resistance; it consists of strains responsible for several difficult-to-treat infections in humans, including nosocomial infections [16]. The most critical bacteria associated with resistance and virulence are *E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa*, and *Enterobacter* species, bacteria whose initials have given the acronym ESKAPE. This designation also highlights the ability of these pathogens to escape from many antibiotics, leading to severe diseases, such as bloodstream infections and pneumonia, associated with a high degree of mortality [17]. Although *E. coli* is not included in the ESKAPE group, this Gram-negative Enterobacteriaceae can produce enzymes involved in antibiotic resistance, such as carbapenemase and extended-spectrum beta-lactamases (ESBL). These resistance mechanisms make it a critical priority pathogen regarding the WHO list. On these arguments, we have decided to include in this review mushrooms metabolites that inhibit *E. coli* growth, leading to a review focusing on the extended ESKAPEE pathogenic bacteria.

Even if these microorganisms are often associated with MDR, we will see that many studies presented in this review have not necessarily been performed on resistant strains. For instance, all compounds reported to be active against *K. pneumoniae, P. aeruginosa*, and *Enterobacter* species have been evaluated on various strains not showing any special resistance to usual medication. On the contrary, all compounds inhibiting *E. faecium* growth have been tested on vancomycin-resistant strains. For *S. aureus* and *E. coli*, some molecules were active against ESBL-producing or methicillin-resistant strains; few metabolites were even active against a multiresistant *A. baumannii* strain. Some antibacterial assessments have also been carried out in the presence of phenylalanine-arginine β-naphthylamide (PAβN), an efflux inhibitor that permeabilizes the outer membrane of Gram-negative bacteria such as *K. pneumoniae, A. baumannii, P. aeruginosa*, and *Enterobacter* species, allowing bioactive compounds to reach their intracellular target. MIC and IC_{50} presented in ▶ **Tables 1**–**7** are expressed in µg/mL, as recommended by standardized guidelines; however, for a better comparison of the efficacy of these secondary metabolites, these data are also available in µM (**Table 1S**, Supporting Information).

Antibacterial Bioassays

Various bioassays can be used to assess the antibacterial potential of extracts or single compounds [18]. The most widespread include microdilution techniques and agar diffusion methods, but bioautography on thin-layer chromatography, flow or image cytometry, or bioluminescence or biofilm assays can be also applied. Each of these techniques presents some advantages and drawbacks, but one of the main difficulties is to compare efficacy of compounds tested with different methods. Indeed, each method will lead to one or several specific measurement units. For example, antibacterial effect of a compound evaluated by disk diffusion method will be expressed in millimetres as the diameter of the growing inhibition zone; on the other hand, the antibacterial effect of the same compound tested with broth dilution method will be expressed as a concentration (µg/mL) that can refer to several different values:

i) the minimum concentration that inhibits the growth of the microorganism (MIC);
ii) the concentration required for inhibiting 50% of microbial growth (IC_{50});

▶ **Table 1** Compounds isolated from mushrooms with antibacterial activity against *Enterococcus faecium*.

Chemical class Compound	Inhibition	Source	Ref
Diterpenoids			
cyathinin A	IC_{50} = 50 µg/mL [a]	*Cyathus subglobisporus* R. L. Zhao, Desjardin & K. D. Hyde (mycelium)	[43]
cyathinin D	IC_{50} = 50 µg/mL [a]		
striatal A	IC_{50} = 25 µg/mL [a]		
striatal B	IC_{50} = 6.25 µg/mL [a]		
striatal D	IC_{50} = 50 µg/mL [a]		
striatin C	IC_{50} = 25 µg/mL [a]		
Peptides			
copsin	MIC = 4 µg/mL	*Coprinopsis cinerea* (Schaeff.) Redhead, Vilgalys & Moncalvo (mycelium)	[60]
	MIC = 2 µg/mL [b]		
	MBC = 8 µg/mL		
	MBC = 2 µg/mL [b]		
plectasin	MIC ≥ 16 µg/mL	*Pseudoplectania nigrella* (Pers.) Fuckel (mycelium)	[57]
	MIC ≥ 32 µg/mL [b]		

[a] tested on a multidrug-resistant strain; [b] tested on a vancomycin-resistant strain

iii) or the lowest concentration that kills 99.9 % of the microorganisms, defined as the minimal bactericidal concentration (MBC), or more seldom the IC_{100} value.

The MIC value is generally preferred to IC_{50} to express antibacterial potential: although it is easy to observe the concentration that prevents bacterial development, it is harder to calculate thoroughly the concentration inhibiting the growth of half of the bacteria. Microdilution methods generally consist of series of 2-fold dilutions, and the inhibitory effect is usually observed over a very narrow concentration range. Consequently, a graph plotting bacterial growth *versus* concentration of the active compound may be too steep to calculate the IC_{50} value accurately.

Concerning the agar method, this one can be carried out by depositing the compound to be evaluated either in a well dug in the agar sterilely, or on a paper disc brought into contact with the agar. In any cases, only few publications mention the diameter of the disc or well, yet this is fundamental for determining whether an inhibition diameter is substantial or not. If a zone of inhibition measures 14 mm, it does not have the same meaning with a disk of 6, 9, or 12 mm of diameter. In addition, it is important to be precise as to whether the whole diameter is measured, including the central disk, or if the measurement is done from the edge of the paper disk. For all these reasons, it can be challenging to discuss the antibacterial potential of compounds when experimental procedure is not clearly mentioned, as well as results for a positive control.

Antibacterial activity of mushroom compounds presented in this review (▶ **Tables 1**–**7**) are expressed either as growth inhibition zone diameter (ZI), half maximal inhibitory concentration (IC_{50}), minimum inhibitory concentration (MIC), or minimum bactericidal concentration (MBC). Regarding diffusion assays, values were obtained for a quantity of 50 µg of compound, unless otherwise stated. Most of the inhibitory values presented herein have been determined following validated methods, such as Clinical & Laboratory Standards Institute (CLSI) guidelines or methods described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). As the aim of this review is to highlight the potential of fungal metabolites to inhibit the growth of ESKAPEE bacteria, we have decided to set a threshold in order to select the more interesting compounds in terms of activity: only metabolites exhibiting an MIC below or equal to 250 µg/mL toward sensitive strains have been retained (for IC_{50}, no value exceeds 50 µg/mL). When data were also available for resistant strains, they have been included even if values were then slightly higher. We have selected this maximal value of 250 µg/mL based on the MIC distribution data collected by EUCAST, reporting modal MIC around 100 µg/mL for several bacteria, such as *K. pneumoniae, E. coli*, and *Enterobacter* spp. [19]. Concerning diffusion methods, the minimum threshold diameter has been set to 12 mm for 50 µg of compound. However, these values should not be taken at face value. For example, according to the EUCAST guidelines, *Enterobacter* species are considered to be sensitive to ampicillin if the inhibition diameter is higher than 14 mm but sensitive to imipenem only if the diameter is larger than 50 mm [19].

We have also reckoned that activity observed for high amounts of compounds only could be associated with cytotoxicity toward eukaryotic cells; yet efficacy and safety are mandatory for considering an antibacterial compound as a potential antibiotic-candidate. While most of the publications do not mention anything regarding potential cytotoxicity, some authors have investigated both antibacterial and cytotoxic effects. These data are discussed below, as well as the mechanisms of action when appropriate.

▶ **Table 2** Compounds isolated from mushrooms with antibacterial activity against *Staphylococcus aureus*.

Chemical class Compound	Inhibition	Source	Ref
Anthraquinones			
(1S,3R)-austrocortilutein	IC_{50} = 12 µg/mL	*Cortinarius basirubescens* Cleland & J. R. Harris (fruiting body)	[20]
(1S,3S)-austrocortilutein	IC_{50} = 8 µg/mL		
(1S,3S)-austrocortirubin	IC_{50} = 3 µg/mL		
Torosachrysone	IC_{50} = 10 µg/mL		
Emodin	IC_{50} = 0.7 µg/mL		
parietin (physcion)	IC_{50} = 23 µg/mL MIC = 0.5 µg/mL		
Pyranoquinones			
multiformin A	ZI = 18 mm	*Hypoxylon multiforme* (Fr.) Fr. (stromata)	[25]
multiformin B	ZI = 20 mm		
multiformin C	ZI = 18 mm		
multiformin D	ZI = 15 mm		
sassafrin A	ZI = 19 mm	*Creosphaeria sassafras* (Schwein.) Y. M. Ju, F. San Martín & J. D. Rogers (stromata)	[24]
sassafrin B	ZI = 18 mm		
sassafrin C	ZI = 22 mm		
sassafrin D	ZI = 17 mm		
Terphenyl quinone			
spiromentin C	MIC = 250 µg/mL [a]	*Tapinella atrotomentosa* (Batsch) Šutara (fruiting body)	[27]
Lactone derivatives			
Osmundalactone	MIC = 250 µg/mL [a]	*Tapinella atrotomentosa* (Batsch) Šutara (fruiting body)	[27]
5-hydroxy-hex-2-en-4-olide	MIC = 250 µg/mL [a]		
coloratin A	ZI= 15 mm	*Xylaria intracolorata* (J. D. Rogers, Callan & Samuels) J. D. Rogers & Y. M. Ju (stromata)	[76]
skeletocutin A	MIC = 37.5 µg/mL MIC = 150 µg/mL [b]	*Skeletocutis* sp. (fruiting body)	[32]
skeletocutin B	MIC = 150 µg/mL MIC = 300 µg/mL [b]		
skeletocutin C	MIC = 150 µg/mL MIC = 300 µg/mL [b]		
skeletocutin D	MIC = 150 µg/mL MIC = 300 µg/mL [b]		
skeletocutin E	MIC = 37.5 µg/mL MIC = 150 µg/mL [b]		
skeletocutin J	MIC = 150 µg/mL MIC = 150 µg/mL [b]		
skeletocutin K	MIC = 37.5 µg/mL MIC = 75 µg/mL [b]		
skeletocutin L	MIC = 18.75 µg/mL MIC = 75 µg/mL [b]		
tyromycin A	MIC = 150 µg/mL MIC = 150 µg/mL [b]		
Furan derivative			
5-hydroxy-methylfurfural	ZI = 28 mm MIC = 250 µg/mL	*Morchella esculenta* (L.) Pers./*Verpa bohemica* (Krombh.) J. Schröt. (fruiting body)	[37]

cont.

▶ **Table 2** *Continued*

Chemical class Compound	Inhibition	Source	Ref
Phenolic acids			
cinnamic acid	MIC = 1.5 µg/mL	*Ganoderma lucidum* (Curtis) P. Karst. (fruiting body)	[35]
p-hydroxybenzoic acid	MIC= 3 µg/mL		
Sesquiterpenoids			
7-acetyl-4-methyl-azulene-1-carbaldehyde	ZI = 15 mm	*Lactarius deliciosus* (L.) Gray (fruiting body)	[77]
enokipodin A	ZI = 25.2 mm	*Flammulina velutipes* (Curtis) Singer (mycelium)	[40]
enokipodin B	ZI = 14 mm		
enokipodin C	ZI = 21 mm		
phellodonic acid	MIC = 10 µg/mL	*Phellodon melaleucus* (Sw. ex Fr.) P. Karst. (mycelium)	[39]
stereumamide A	MIC = 12.5 µg/mL	*Stereum hirsutum* (Willd.) Pers. (mycelium)	[38]
stereumamide D	MIC = 12.5 µg/mL		
sterostrein Q	MIC = 25 µg/mL		
Diterpenoids			
psathyrelloic acid	MIC = 16 µg/mL	*Psathyrella candolleana* (Fr.) Maire (mycelium)	[46]
sarcodonin L	ZI = 18 mm with 35 µg	*Sarcodon scabrosus* (Fr.) P. Karst. (fruiting body)	[45]
sarcodonin M	ZI = 22 mm with 35 µg		
psathyrin A	MIC = 14.3 µg/mL	*Psathyrella candolleana* (Fr.) Maire (mycelium)	[47]
psathyrin B	MIC = 22.7 µg/mL		
Triterpenoids			
ganoderic acid	MIC = 250 µg/mL	*Ganoderma lucidum* (Curtis) P. Karst. (mycelium)	[36]
Meroterpenoids			
ganomycin A	MIC = 25 µg/mL	*Ganoderma pfeifferi* Bres. (fruiting body)	[42]
ganomycin B	MIC = 25 µg/mL		
Steroid			
ergosterol	ZI = 25 mm MIC = 250 µg/mL	*Morchella esculenta* (L.) Pers./*Verpa bohemica* (Krombh.) J. Schröt. (fruiting body)	[37]
Alkaloids (piperazine derivatives)			
emestrin	IC_{50} = 4.55 µg/mL IC_{50} = 2.21 µg/mL[b] MIC = 20 µg/mL MIC = 10 µg/mL[b]	*Desarmillaria tabescens* (Scop.) R. A. Koch & Aime (mycelium)	[55]
epicorazine A	ZI = 35 mm with 100 µg	*Podaxis pistillaris* (L.) Fr. (mycelium)	[54]
epicorazine B	ZI = 30 mm with 100 µg		
epicorazine C	ZI = 25 mm with 100 µg		
Sulfur derivative			
bis((methylsulfonyl) methyl) disulfide	MIC = 12.5 µg/mL	*Lentinus edodes* (Berk.) Singer (fruiting body)	[73]
Fatty acid			
pentadecanoic acid	ZI = 17 mm MIC = 250 µg/mL	*Morchella esculenta* (L.) Pers./*Verpa bohemica* (Krombh.) J. Schröt. (fruiting body)	[37]
Polyacetylene			
drosophilin D	MIC = 4 µg/mL	*Drosophila subatrata* (Batsch) Quél. (mycelium)	[50]
Polysaccharide			
polysaccharide PL2	MIC = 25 µg/mL	*Lentinus edodes* (Berk.) Singer (spent mushroom substrate)	[66]

cont.

▶ **Table 2** *Continued*

Chemical class Compound	Inhibition	Source	Ref
Peptides			
boletusin	13 ≤ ZI ≤ 15 mm	*Boletus* sp. (fruiting body)	[61]
chrysospermin B	14 ≤ ZI ≤ 16 mm		
chrysospermin D	13 ≤ ZI ≤ 15 mm		
plectasin	4 ≤ MIC ≤ 32 µg/mL 16 ≤ MIC ≤ 32 µg/mL[b]	*Pseudoplectania nigrella* (Pers.) Fuckel (mycelium)	[57]
Proteins			
Cordyceps sinensis antibacterial protein	50 < MIC < 75 µg/mL	*Cordyceps sinensis* (Berk.) Sacc. (mycelium)	[62]
F2 protein	IC_{50} < 100 µg/mL[b]	*Agaricus bisporus* (J. E. Lange) Imbach (fruiting body)	[63]

[a] tested on an ESBL-producing strain; [b] tested on a methicillin-resistant strain

▶ **Table 3** Compounds isolated from mushrooms with antibacterial activity against *Klebsiella pseumoniae*.

Chemical class Compound	Inhibition	Source	Ref
Pyranoquinones			
multiformin A	ZI = 18 mm	*Hypoxylon multiforme* (Fr.) Fr. (stromata)	[25]
multiformin B	ZI = 20 mm		
multiformin C	ZI = 18 mm		
multiformin D	ZI = 16 mm		
sassafrin A	ZI = 20 mm	*Creosphaeria sassafras* (Schwein.) Y. M. Ju, F. San Martín & J. D. Rogers (stromata)	[24]
sassafrin B	ZI = 20 mm		
sassafrin C	ZI = 22 mm		
sassafrin D	ZI = 17 mm		
Indole-quinone polymer			
Melanin	ZI = 25 mm	*Schizophyllum commune* Fr. (mycelium)	[78]
Aromatic lactone			
coloratin A	ZI = 22 mm	*Xylaria intracolorata* (J. D. Rogers, Callan & Samuels) J. D. Rogers & Y. M. Ju (stromata)	[76]
Diterpenoids			
pleuromutilin	MIC = 1 µg/mL	*Pleurotus passeckerianus* Pilát (mycelium)	[48]
striatal A	IC_{50} = 50 µg/mL [a]	*Cyathus subglobisporus* R. L. Zhao, Desjardin & K. D. Hyde (mycelium)	[43]
striatal B	IC_{50} = 25 µg/mL [a]		
striatin C	IC_{50} = 50 µg/mL [a]		
Chlorinated derivative			
drosophilin A	MIC = 64 µg/mL	*Drosophila subatrata* (Batsch) Quél. (mycelium)	[50]
Polysaccharides			
exopolysaccharides	MIC = 9.2 µg/mL	*Lentinus edodes* (Berk.) Singer (mycelium)	[65]

[a] measured in the presence of phenylalanine-arginine β-naphthylamide (PAβN)

▶ **Table 4** Compounds isolated from mushrooms with antibacterial activity against *Acinetobacter baumannii*.

Chemical class Compound	Inhibition	Source	Ref
Lactone derivatives			
5-hydroxy-hex-2-en-4-olide	MIC = 6 µg/mL [a]	*Tapinella atrotomentosa* (Batsch) Šutara (fruiting body)	[27]
Osmundalactone	MIC = 10 µg/mL [a]		
Terphenyl quinone			
spiromentin C	MIC = 20 µg/mL [a]	*Tapinella atrotomentosa* (Batsch) Šutara (fruiting body)	[27]
Diterpenoids			
cyathinin D	IC_{50} = 6.25 µg/mL [b]	*Cyathus subglobisporus* R. L. Zhao, Desjardin & K. D. Hyde (mycelium)	[43]
striatal A	IC_{50} = 12.5 µg/mL [b]		
striatal B	IC_{50} = 6.25 µg/mL [b]		
striatin C	IC_{50} = 3.13 µg/mL [b]		
striatoid C	IC_{50} = 12.5 µg/mL [b]		
Alkaloid			
2-aminoquinoline	MIC = 128 µg/mL	*Leucopaxillus albissimus* (Peck) Singer (fruiting body)	[79]

[a] tested on a multiresistant strain; [b] measured in the presence of phenylalanine-arginine *β*-naphthylamide (PA*β*N)

Antibacterial Mushrooms Metabolites

In recent decades, the antimicrobial potential of mushrooms has been highlighted, and several antibacterial compounds have been reported, mostly from higher basidiomycetes and some ascomycetes. Active compounds are mainly secondary metabolites (terpenoids, quinone or lactone derivatives, alkaloids, etc.), but high molecular weight molecules like polysaccharides or proteins seem to contribute as well to anti-infectious properties of mushrooms. Some chlorinated and sulfurated derivatives, often encountered in macromycetes, are also parts of the bioactive compounds. The collected data are presented in ▶ **Tables 1**–**7** for each targeted ESKAPEE bacteria, and some structures of bioactive compounds are presented in ▶ **Fig. 1**.

Quinone Derivatives

A large part of antibacterial compounds identified in macromycetes are quinones and their derivatives, known to exhibit antimicrobial activity. Major antibacterial quinoids from macrofungi are anthraquinone and pyranoquinone derivatives, but some *p*-terphenyl quinones and indole-quinone polymers (melanins) have also been reported to have antibacterial activities.

Beattie et al. [20] described the purification of 8 antibacterial anthraquinoid pigments from *Cortinarius basirubescens* (Cortinariaceae); emodin shows the best antibacterial activity against both *S. aureus* and *P. aeruginosa*, with IC_{50} values of 0.7 and 1.5 µg/mL, respectively. On the other hand, torosachrysone, austrocortirubin, and austrocortiluteins possess more specific antibacterial activity against *S. aureus*, contrary to 6-methylxanthopurpurin-3-*O*-methyl ether, which displays more remarkable activity against *P. aeruginosa* than against *S. aureus*. Parietin (**1**) found in *Cortinarius* species is also a potent growth inhibitor of *S. aureus* and *P. aeruginosa* (MIC of 0.5 µg/mL), notably acting through photosensitized generation of $^1O_2$ and superoxide radical anion $O_2^{\bullet -}$ [20, 21]. In another study, the antibiofilm activity of parietin has been demonstrated against *S. aureus* and *E. faecalis* [22], which is particularly interesting as biofilms are implicated in 80 % of human microbial infections. Generally speaking, anthraquinone derivatives–also widespread in plants–are well-known for their antimicrobial properties, and several mechanisms have been already identified [23], but they are also known for their toxic and laxative effects, which might cause undesirable side effects if drug development would be considered. Therefore, a full set of cytotoxic experiments should be consistently performed to confirm both antibiotic potential and safety of anthraquinone derivatives.

The fungal pyranoquinones multiformins and sassafrins (azaphilones) isolated from the Ascomycetes *Hypoxylon multiforme* and *Creosphaeria sassafras*, respectively, also possess antibacterial activities [24, 25]. In a disk diffusion assay, they display moderate antibacterial activity against *S. aureus, E. coli, P. aeruginosa*, and *K. pneumoniae* when tested at 50 µg. Sassafrin C (**2**) followed by multiformin B show the best inhibition activity against all tested bacteria, even though the inhibition diameters of all azaphilones are below the values of antibiotic standards. Interestingly, multiformin D displays bacteriostatic effect against *S. aureus, P. aeruginosa*, and *E. coli* and has a weak bactericidal effect against *K. pneumoniae*. However, cytotoxic activities of some sassafrin and multiformin azaphilones have been reported at concentrations up to 30 µM [26].

Spiromentin C (**3**), a *p*-terphenyl quinone purified from the velvet roll-rim *Tapinella atrotomentosa* (Tapinellaceae, Basidiomycetes), exerts an interesting inhibition against ESBL-producing *E. coli*, as well as against multiresistant *A. baumannii*, with MIC values of 10 and 20 µg/mL, respectively [27]. Even though *A. baumannii* is defined as sensitive to conventional antibiotics for MIC below 8 µg/mL [18], this result is nevertheless encouraging, as

▶ **Table 5** Compounds isolated from mushrooms with antibacterial activity against *Pseudomonas aeruginosa*.

Chemical class Compound	Inhibition	Source	Ref
Anthraquinones			
6-methylxantho-purpurin-3-*O*-methyl ether	IC_{50} = 31 µg/mL	*Cortinarius basirubescens* Cleland & J. R. Harris (fruiting body)	[20]
Emodin	IC_{50} = 1.5 µg/mL		
parietin (physcion)	IC_{50} = 2 µg/mL		
parietin (physcion)	MIC = 0.5 µg/mL		[21]
Pyranoquinones			
sassafrin A	ZI = 18 mm	*Creosphaeria sassafras* (Schwein.) Y. M. Ju, F. San Martín & J. D. Rogers (stromata)	[24]
sassafrin B	ZI = 19 mm		
sassafrin C	ZI = 22 mm		
sassafrin D	ZI = 19 mm		
multiformin A	ZI = 14 mm	*Hypoxylon multiforme* (Fr.) Fr. (stromata)	[25]
multiformin B	ZI = 19 mm		
multiformin C	ZI = 18 mm		
multiformin D	ZI = 17 mm		
Lactone derivatives			
coloratin A	ZI = 16 mm	*Xylaria intracolorata* (J. D. Rogers, Callan & Samuels) J. D. Rogers & Y. M. Ju (stromata)	[76]
Coprinuslactone	MIC = 150 µg/mL	*Coprinus comatus* (O. F. Müll.) Pers. (mycelium)	[33]
Cyclohexenyl derivative			
lentinoid B	MIC = 160 µg/mL	*Lentinus strigellus* Berk. (mycelium)	[80]
Phenolic acids			
cinnamic acid	MIC = 0.7 µg/mL	*Ganoderma lucidum* (Curtis) P. Karst. (fruiting body)	[35]
p-hydroxybenzoic acid	MIC = 3 µg/mL		
Steroid			
Ergosterol	ZI = 18 mm MIC = 200 µg/mL MBC = 250 µg/mL	*Morchella esculenta* (L.) Pers./*Verpa bohemica* (Krombh.) J. Schröt. (fruiting body)	[37]
Alkaloid			
2-aminoquinoline	MIC = 128 µg/mL	*Leucopaxillus albissimus* (Peck) Singer (fruiting body)	[79]
Chlorinated derivatives			
drosophilin A	MIC = 250 µg/mL	*Drosophila subatrata* (Batsch) Quél. (mycelium)	[50]
gymnopalyne A	MIC = 67 µg/mL	*Gymnopus sp.* (mycelium)	[71]
Polyacetylene			
drosophilin D	MIC = 250 µg/mL	*Drosophila subatrata* (Batsch) Quél. (mycelium)	[50]

▶ **Table 6** Compounds isolated from mushrooms with antibacterial activity against *Enterobacter* spp.

Chemical class Compound	Inhibition	Bacterial species	Source	Ref
Terphenyl quinone				
atromentin	50 ≤ MIC ≤ 100 µg/mL	*E. aerogenes*[a]	*Hydnaceae* sp. (fruiting body)	[28]
Phenolic acids				
cinnamic acid	MIC = 1.5 µg/mL	*E. cloacae*	*Ganoderma lucidum* (Curtis) P. Karst. (fruiting body)	[35]
p-hydroxy-benzoic acid	MIC = 6 µg/mL			

[a] now classified as *Klebsiella aerogenes*

▶ **Table 7** Compounds isolated from mushrooms with antibacterial activity against *Escherichia coli*.

Chemical class Compound	Inhibition	Source	Ref
Pyranoquinones			
multiformin B	ZI = 19 mm	*Hypoxylon multiforme* (Fr.) Fr. (stromata)	[25]
multiformin C	ZI = 18 mm		
multiformin D	ZI = 15 mm		
sassafrin A	ZI = 20 mm	*Creosphaeria sassafras* (Schwein.) Y. M. Ju, F. San Martín & J. D. Rogers (stromata)	[24]
sassafrin B	ZI = 14 mm		
sassafrin C	ZI = 22 mm		
sassafrin D	ZI = 19 mm		
Terphenyl quinones			
spiromentin B	MIC = 100 µg/mL [a]	*Tapinella atrotomentosa* (Batsch) Šutara (fruiting body)	[27]
spiromentin C	MIC = 10 µg/mL [a]		
atromentin	50 ≤ MIC ≤ 100 µg/mL	*Hydnaceae* sp. (fruiting body)	[28]
Indole-quinone polymer			
melanin	ZI = 10.3 mm with 10 µg	*Armillaria mellea* (Vahl) P. Kumm. (rhizomorph)	[30]
	MIC = 160 µg/mL	*Auricularia auricula-judae* (Bull.) Quél. (fruiting body)	[31]
Lactone derivatives			
5-hydroxy-hex-2-en-4-olide	MIC = 10 µg/mL [a]	*Tapinella atrotomentosa* (Batsch) Šutara (fruiting body)	[27]
osmundalactone	MIC = 10 µg/mL [a]		
coloratin A	ZI = 16 mm	*Xylaria intracolorata* (J. D. Rogers, Callan & Samuels) J. D. Rogers & Y. M. Ju (stromata)	[76]
Ketone derivatives			
2-octanone	MIC = 125 µg/g	*Pleurotus ostreatus* (Jacq.) P. Kumm. (fruiting body)	[34]
3-octanone	MIC = 125 µg/g		
Phenolic acids			
cinnamic acid	MIC = 7 µg/mL	*Ganoderma lucidum* (Curtis) P. Karst. (fruiting body)	[35]
p-hydroxybenzoic acid	MIC = 30 µg/mL		
Sesquiterpenoids			
creolophin E	MIC = 5 µg/mL	*Creolophus cirrhatus* (Pers.) P. Karst. (mycelium)	[41]
phellodonic acid	MIC = 100 µg/mL	*Phellodon melaleucus* (Sw. ex Fr.) P. Karst. (mycelium)	[39]
stereumamide A	MIC = 12.5 µg/mL	*Stereum hirsutum* (Willd.) Pers. (mycelium)	[38]
stereumamide D	MIC = 12.5 µg/mL		
sterostrein Q	MIC = 12.5 µg/mL		
Diterpenoids			
pleuromutilin	MIC = 250 µg/mL	*Pleurotus passeckerianus* Pilát (mycelium)	[50]
cyathinin A	IC_{50} = 25 µg/mL [b]	*Cyathus subglobisporus* R. L. Zhao, Desjardin & K. D. Hyde (mycelium)	[43]
cyathinin D	IC_{50} = 12.5 µg/mL [b]		
striatal A	IC_{50} = 6.25 µg/mL [b]		
striatal B	IC_{50} = 3.13 µg/mL [b]		
striatal D	IC_{50} = 50 µg/mL [b]		
striatoid C	IC_{50} = 50 µg/mL [b]		
Steroids			
ergosterol	ZI = 21 mm MIC = 300 µg/mL	*Morchella esculenta* (L.) Pers./*Verpa bohemica* (Krombh.) J. Schröt. (fruiting body)	[37]

cont.

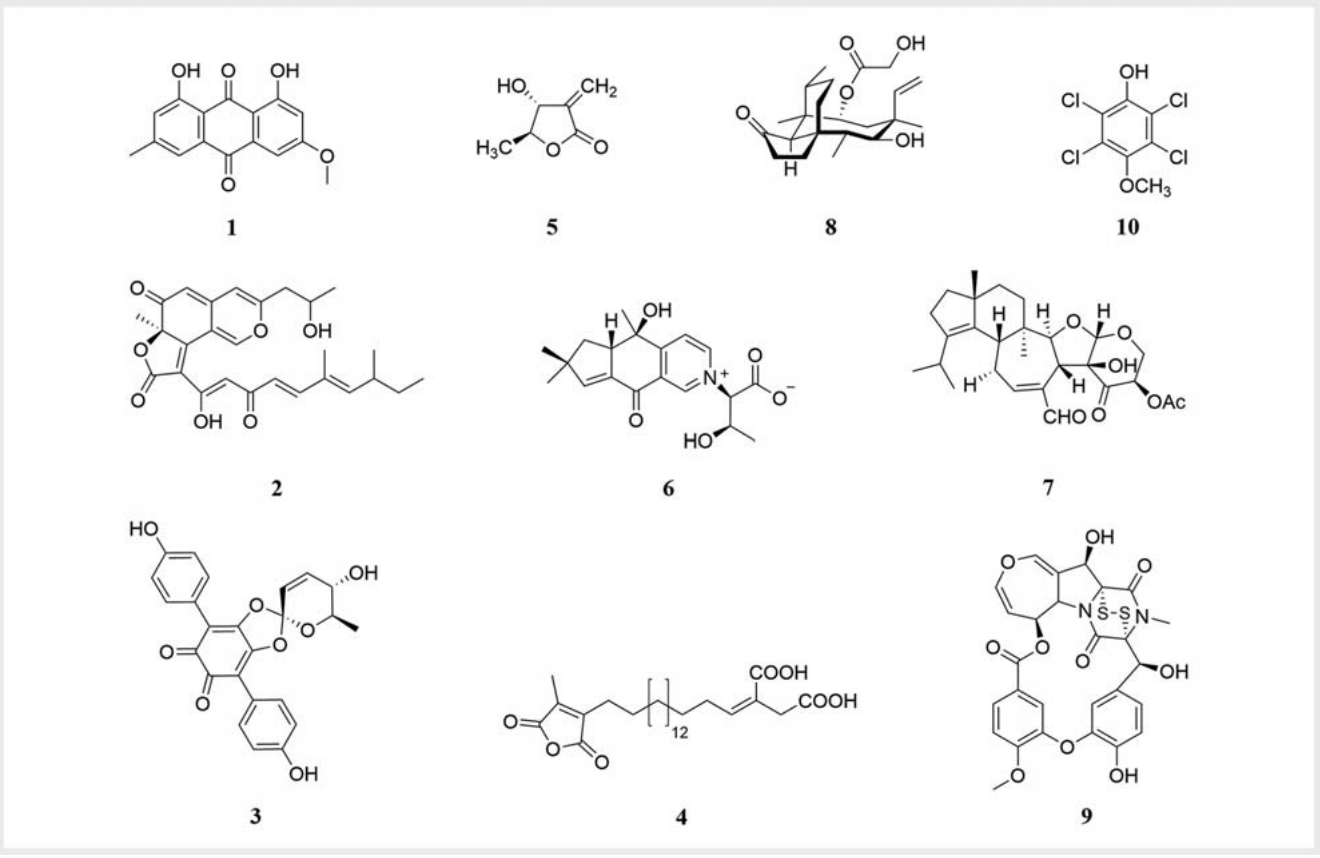
▶ **Table 7** *Continued*

Chemical class Compound	Inhibition	Source	Ref
Alkaloids (piperazine derivatives)			
emestrin	IC_{50} = 2.21 µg/mL	*Armillaria tabescens* (Scop.) R. A. Koch & Aime (mycelium)	[55]
epicorazine A	ZI = 25 mm with 100 µg	*Podaxis pistillaris* (L.) Fr. (mycelium)	[54]
epicorazine B	ZI = 24 mm with 100 µg		
epicorazine C	ZI = 18 mm with 100 µg		
Sulfur derivative			
bis((methylsulfonyl) methyl)disulfide	MIC = 100 µg/mL	*Lentinus edodes* (Berk.) Singer (fruiting body)	[73]
Chlorinated derivatives			
drosophilin A	MIC = 250 µg/mL	*Drosophila subatrata* (Batsch) Quél. (mycelium)	[50]
tetrachloropyro-catechol	MIC = 20 µg/mL	*Mycena* sp. (mycelium)	[72]
tetrachloropyro-catechol methylether	MIC = 50 µg/mL		
Alcohols			
octan-1-ol	MIC = 125 µg/g	*Pleurotus ostreatus* (Jacq.) P. Kumm. (fruiting body)	[34]
octan-3-ol	MIC = 125 µg/g		
Fatty acid			
pentadecanoic acid	ZI = 21 mm MIC = 250 µg/mL	*Morchella esculenta* (L.) Pers./*Verpa bohemica* (Krombh.) J. Schröt. (fruiting body)	[37]
Polyacetylene			
drosophilin D	MIC = 250 µg/mL	*Drosophila subatrata* (Batsch) Quél. (mycelium)	[50]
Polysaccharides			
polysaccharides	MIC = 12.5 µg/mL	*Lentinus edodes* (Berk.) Singer (spent mushroom substrate)	[66]
exopolysaccharides	ZI = 15 mm	*Lentinus edodes* (Berk.) Singer (mycelium)	[65]
Protein			
Cordyceps sinensis antibacterial protein	MIC = 75–100 µg/mL	*Cordyceps sinensis* (Berk.) Sacc. (mycelium)	[62]

[a] tested on an ESBL-producing strain; [b] measured in the presence of phenylalanine-arginine-naβphthylamide (PAβN)

the data regarding bioactivity of mushroom extracts and metabolites against *A. baumannii* are rare. In addition, spiromentin C also inhibits methicillin-resistant *Staphylococcus aureus* (MRSA) growth to a lesser extent (MIC = 250 µg/mL), and its analogue spiromentin B also affects the growth of ESBL *E. coli*. These compounds have been also investigated in combination with cefuroxime against MRSA, but no synergistic effect has been observed. Yet, further toxicological tests are warranted to guarantee the safety of these compounds for human cells, to confirm therefore their potential interest as future antibiotics. Another terphenyl quinone from several *Hydnaceae* species, atromentin, has shown antibacterial activity against *E. coli* and *Enterobacter aerogenes* (now included in the *Klebsiella* genus), a nosocomial bacteria leading to opportunistic infections, and known to acquire resistance mechanisms [28]. Even if MIC values are quite high (50–100 µg/mL) compared to penicillins against clinical strains, this result remains quite promising. Atromentin has been reported to inhibit the enoyl-acyl carrier protein (ACP) reductase of *S. pneumoniae*, an enzyme essential for the biosynthesis of fatty acids, but without any effect on the enoyl-ACP reductase of *S. aureus* or *E. coli*. Therefore, its mode of action explaining the inhibition of *E. coli* and *E. aerogenes* still has to be elucidated [29].

Melanins are particular quinone derivatives, commonly described as high molecular weight insoluble pigments, derived from the oxidation of phenols leading to heterogeneous indole-quinone polymers. Although fungal melanin structures are still not well characterized, they have been studied for biological activities. Raw melanin isolated from *Armillaria melea* (Agaricale, Basidiomycetes) shows antibacterial activities against *P. aeruginosa*, and, to a less extent, toward *S. aureus* and *E. coli* [30]. However, once purified, its inhibitory activity against *P. aeruginosa* decreases, and disappears completely with regard to *S. aureus* and *E. coli*. This could be due to the linkage of melanin with antimicrobial polysaccharides in the raw fraction. However, melanin from the jelly ear mushroom (*Auricularia auricula-judae*) has been reported to inhibit the growth of *E. coli* and *P. aeruginosa*, with MIC values of 160 µg/mL and 640 µg/mL, respectively. In addition, it was also found to inhibit biofilm formation at values below MIC [31]. This antibiofilm effect is regulated by quorum sensing and does not interfere with bacteria growth. Authors suggest that



▶ **Fig. 1** Chemical structures of some compounds isolated from mushrooms displaying antibacterial activities against some ESKAPEE pathogenic bacteria.

the quorum quenching activity by melanin could result from the close conformations between synthesis intermediates of melanin and acylhomoserine lactones, proteins implicated in quorum sensing. They presume a possible competitive inhibition by melanin intermediates by binding to the acylhomoserine lactone receptors, thus quenching the intercellular communication. The melanin antibacterial effect could also result from damages of the cell bacteria membrane. Further investigations are needed to better understand the mode of action of fungal melanins.

Lactone, Ketone, and Phenolic Derivatives

Thirteen lactones from a polypore species of *Skeletocutis* (Basidiomycetes) have been tested for their activity against methicillin-sensitive and -resistant *S. aureus*: skeletocutins K (**4**) and L show the best antibacterial effect against both strains [32]. Interestingly, skeletocutin I inhibits the biofilm formation of *S. aureus* but has no significant effect on bacterial growth (the antibiofilm activity of skeletocutins K and L has not been evaluated in this study). Furthermore, these lactone derivatives did not show any cytotoxicity against mouse fibroblast and HeLa cell lines when tested at concentrations of 37 µg/mL, warranting further investigations. Two lactones isolated from the velvet roll-rim also possess interesting antibacterial activities: they strongly inhibit multiresistant *A. baumannii* and ESBL-producing *E. coli*, with MIC ≤ 10 µg/mL, and have also an effect on MRSA [27]. Nevertheless, cytotoxicity and stability assessments should be carried out prior to considering these molecules as potential drug-candidates. Activity-directed fractionation of *Coprinus comatus* (Psathyrellaceae) led to the isolation of coprinuslactone (**5**), which shows moderate bacteriostatic and antibacterial effects against *P. aeruginosa* and *S. aureus*. This lactone also exerts a bactericidal activity at twice its MIC value and dissolves *in vitro*-established biofilms of *P. aeruginosa* and *S. aureus* at concentrations below MIC [33]. Coprinuslactone has shown interesting quorum quenching activity against *E. coli* at subtoxic concentration, which could explain its ability to disrupt bacterial biofilm; less than 4 µg/mL was sufficient to completely abolish the quorum sensing response of a specific autoinducer. Coprinulactone also interferes with peptidoglycan synthesis by binding to the MurA enzyme and inhibiting it. Moreover, observation of the structure of the cell membrane after treatment with this compound suggests that it can destabilize the latter. Finally, this lactone has showed weak cytostatic but no cytotoxic effect against a mouse fibroblast cell line [33]. Lactones are often reactive molecules, with the risk of ring-opening and loss of efficacy; however, all these lactones (skeletocutins, coprinulactone, 5-hydroxy-hex-2-en-4-olide, and osmundalactone) are γ- or δ-lactones, which

could be stable enough to be considered for further investigations regarding their antibiotic potential.

Some volatile ketones and their corresponding alcohols have been isolated from the edible oyster mushroom *Pleurotus ostreatus*. A mixture of these compounds in concentrations imitating the composition of fruiting bodies of *P. ostreatus* has inhibited the growth of all tested bacterial strains, including *K. pneumoniae* and *P. aeruginosa* [34]. However, none of these compounds displays remarkable antibacterial effects, and drug development is difficult with volatile compounds, limiting thereby the potential of these molecules.

With regard to phenolic compounds, no molecule specific to the fungi kingdom has been reported to exert antibacterial effects toward ESKAPEE bacteria. The ubiquitous cinnamic and *p*-hydroxybenzoic acids (isolated from *Ganoderma lucidum*) were evaluated for antibacterial activity on *S. aureus, P. aeruginosa, E. coli*, and *Enterobacter cloacae*, and they showed significant effects with MIC values comprised between 0.7 and 30 µg/mL [35]. Based on literature suggesting that phenolic compounds are rapidly metabolized and mostly glucuronidated after human consumption, authors produced acetylated glucuronide derivatives from the parent compounds: these derivatives showed a still high antibacterial activity but nevertheless lower than that of the original phenolic acids.

Terpenoids

Antibacterial terpenoids identified in mushrooms are mainly sesqui- and diterpene derivatives.

Regarding larger compounds, only ganoderic acid has been identified as a weak antibacterial triterpene against *S. aureus* but leading to a strong cytotoxicity toward HeLa and U87 glioma cancer cells [36]. The ubiquitous ergosterol (isolated from morel mushrooms) is also the only steroid reported to be active toward pathogenic bacteria, with a minimal bactericidal concentration of 250 µg/mL against *P. aeruginosa*, twice less active against *E. coli* [37].

Regarding sesquiterpenoids, 9 fungal compounds have been reported to inhibit the growth of *S. aureus* or *E. coli*. Stereumamides A (**6**) and D, resulting from the conjugation of sesquiterpenes with an α-amino acid, were found to have a similar activity on *S. aureus* and *E. coli* growth (MIC = 12.5 µg/mL), while sterostrein Q was slightly less active on *S. aureus* than against *E. coli* [38]. Phellodonic acid, an hirsutane-type sesquiterpene, has also shown a potent antibacterial activity against *S. aureus* (MIC = 10 µg/mL), less against *E. coli* with a 10-times higher MIC value [39]. However, phellodonic acid is toxic toward several mammalian cells lines, including HeLa human cells (IC_{50} of 10 µg/mL), thus preventing its development without appropriate structural modifications. Among other active sesquiterpenoids are found enokipodins A–D, cuparene derivatives isolated from the mushroom *Flammulina velutipes* (Physalacriaceae, Basidiomycetes) [40]. Enokipodins A and C show a higher antibacterial activity against *S. aureus* (ZI of 25.2 and 21 mm, respectively) compared to enokipodins B and D, which are respective benzoquinone-type derivatives resulting from the oxidation of enokipodins A and C. However, none of these compounds inhibited the growth of Gram-negative bacteria such as *E. coli*. On the contrary, creolophin E, a norhirsutane sesquiterpene from the rare *Creolophus cirrhatus*, inhibits the development of *E. coli* with a minimal inhibitory concentration of 5 µg/mL [41]. Unfortunately, as for phellodonic acid, this compound is also strongly cytotoxic toward various human cell lines ($IC_{50} \leq 7$ µg/mL). Ganomycins A and B from *Ganoderma pfeifferi* are meroterpenoids exhibiting a farnesyl hydroquinone structure; they were evaluated both by diffusion and microdilution assay on 4 different strains of *S. aureus*, as well as on *P. aeruginosa* and *E. coli*, with ampicillin used as a positive control. Both ganomycins inhibit *S. aureus* growth with an MIC of 25 µg/mL, but no evaluation of toxicity toward eukaryotic cells has been carried out [42].

Several mushroom diterpenoids have also been identified as significant antibacterial compounds. An interesting publication reports the evaluation of cyathane diterpenes isolated from *Cyathus subglobisporus* toward several ESKAPEE bacteria, including a multi-resistant *E. faecium* strain, an ESBL-producing *K. pneumoniae*, and the other Gram-negative *E. coli, P. aeruginosa*, and *A. baumannii* [43]. Evaluations have been performed according to CLSI guidelines, and in the presence of PAβN, a pump efflux inhibitor, in the case of Gram-negative bacteria. All compounds showed various antibacterial activities. Striatal A (**7**) was found to inhibit significantly *E. faecium, K. pneumoniae, A. baumannii,* and *E. coli* growth, with IC_{50} comprised between 6 and 50 µg/mL. Striatal B also strongly inhibited the growth of *E. faecium* and *A. baumannii* (with PAβN), while striatin C and striatal C were very active against *A. baumannii* and *E. coli*, respectively, in the presence of PAβN. However, all the active compounds also displayed cytotoxicity against various cancerous and noncancerous cells with IC_{50} values in the µM range, which nuances the interest for these molecules as potential antibiotics. Two other cyathane derivatives isolated from the edible mushroom *Strobilurus ohshimae* have also shown weak bioactivity against *P. aeruginosa* but again are associated with cytotoxicity toward cancer cells [44]. Also with respect to cyathane derivatives, sarcodonins M and L displayed antibacterial activity against *S. aureus* in a disk diffusion assay, where disks of 6 mm of diameter were soaked with increasing concentrations of test compounds; no evaluation on mammalian cells has been reported for these molecules [45].

Several diterpenoids isolated from cultures of the basidiomycete *Psathyrella candolleana* possess antibacterial properties against Gram-positive bacteria. Psathyrelloic acid inhibits *S. aureus* but has no effect on *P. aeruginosa* and *E. coli* [46]. Similarly, psathyrins A and B, 2 tetracyclic diterpenoids, also inhibit weakly the growth of *S. aureus* without any effect toward *P. aeruginosa* [47]. While the first publication reports an MIC value, the second one mentions MIC_{50}. While an MIC value is determined for a given isolate, the MIC_{50} corresponds to the lowest drug concentration that inhibit 50% of a large panel of isolates of a given species, namely *S. aureus* in this study. It seems that only one ATCC strain has been evaluated in this work; therefore, the data should be considered as MIC and not MIC_{50}. None of these compounds from *P. candolleana* have been submitted to cytotoxic assay to evaluate their potential safety for human cells.

Another important antibacterial diterpene is pleuromutilin (**8**). This compound, firstly isolated in 1951 from *Pleurotus mutilus*

(now renamed *Clitopilus scyphoides*), has also been identified in other Agaricales species, mostly from the genus *Clitopilus* as well as from the closely-related *Omphalina mutila* [48, 49]. Pleuromutilin shows high antibacterial activity against *S. aureus* and *K. pneumoniae* (MIC of 0.25 and 1 µg/mL, respectively), while its activity against *E. coli* and *P. aeruginosa* is very weak [48, 50]. Also, pleuromutilin is not toxic to mice at a dose of 50 mg/kg of body weight, evaluated by intravenous and intraperitoneal route. Semi-synthetic derivatives of pleuromutilin have been further developed and form now the antibiotic class of pleuromutilins [51]. Initially used in veterinary medicine, some pleuromutilins have been approved for human medicine; for example, retapamulin is used as a topical agent for the treatment of impetigo or infected wounds caused by *S. aureus*, and more recently, lefamulin has been approved to treat some community-acquired bacterial pneumonia [8]. Other indications, including pediatric use, are currently undergoing phase I trials. Pleuromutilins inhibit bacterial proteins synthesis by binding to the 50S ribosomal subunit and thus inhibit the formation of peptide bonds. Due to this unique interaction with the bacterial ribosome, there is no cross-resistance with current antibiotics targeting the biosynthesis of proteins, like tetracyclines or macrolides [52]. Pleuromutilins show a wide spectrum of action against *S. aureus* and *E. faecium*, including resistant strains, and recent "extended spectrum" pleuromutilins display an interesting activity against Enterobacteriaceae, including carbapenem-resistant strains. Recent investigations aim to further extend the antibacterial spectrum to include more ESKAPE pathogens. During its clinical evaluation against community-acquired bacterial pneumonia, lefamulin was globally well tolerated by patients, with the most frequent side effect being diarrhea, often observed with all antibiotics, even though studies have shown that lefamulin has a rather low impact on the intestinal microbiome [51, 53]. Similarly, some vaginal fungal infections can also appear. No genotoxicity has been reported, but lefamulin has induced some teratogenic effects on rodent fetus *in vivo*; therefore, it should not be used by pregnant and breastfeeding patients, and women of childbearing potential should use effective contraception during treatment. In addition, it has to be noted that lefamulin is primarily metabolized by CYP3A4; hence it can interact with many other drugs. Up to now, pleuromutilins represent the only class of marketed antibiotics derived from macromycetes.

Alkaloids

Certain macrofungi alkaloids have interesting antibacterial activities against ESKAPEE bacteria. Epicorazines A–C from *Podaxis pistillaris* (Podaxaceae) are piperazine alkaloids containing a disulfide bridge. They inhibit the growth of *S. aureus* in a disk diffusion assay, as well as the one of *E. coli*, to a lesser extent [54]. It seems that the presence of a hydrogen on the beta position of C6′ as well as the double bond between C2′ and C3′ increase their antibacterial activity. However, all of these compounds also show cytotoxic activities against human cells at antibacterial concentrations, which is a break for further development. Another antibacterial piperazine alkaloid isolated from the plant pathogen *Armillaria tabescens*, emestrin (**9**), exerts a remarkable inhibition activity against *E. coli* (IC_{50} = 2.21 µg/mL) as well as against both sensitive and methicillin-resistant *S. aureus* [55]. Emestrin has also been reported to have antifungal properties, but no toxicological test toward human cell lines has been carried out to our knowledge. Cytochalasins are fungal metabolites consisting of a substituted isoindole scaffold fused with a macrocyclic ring. Several of these compounds, isolated from macromycetes but also from micromycetes, have been evaluated on *S. aureus* growth and biofilm production. Though they have not any significant effect on bacterial growth, some of them were found to strongly interfere with biofilm formation (up to 90% of inhibition when tested at 256 µg/mL) [56]. Cytochalasan derivatives are therefore supposed to act on the quorum sensing of *S. aureus*, but the mechanism of action remains to be elucidated. Nevertheless, these compounds are known to be toxic agents, binding to actin filaments and blocking their polymerization, thus limiting their use in humans.

Peptides and Proteins

Beside small secondary metabolites, some polypeptides and proteins with antibacterial activity have also been identified from macromycetes, referred to as fungal defensins. The most interesting one is plectasin, a peptide composed of 40 amino acids and isolated from the ebony cup fungi, *Pseudoplectania nigrella* (Pasthyrellaceae, Basidiomycetes). Plectasin shows interesting activity against both *S. aureus* and *E. faecium*; according to the strains tested (including clinical isolates, MRSA, and vancomycin-resistant *E. faecium*), MIC values range from 4 µg/mL to 128 µg/mL. Activity results from the ability of plectasin to bind directly to lipid II, the essential precursor of the cell wall [57, 58]. Furthermore, no cytotoxicity has been reported against human epidermal keratinocytes and murine fibroblasts. In 2008, a derivative of plectasin, NZ2114, developed by Novozymes and Sanofi-Aventis, is more efficient and less toxic than the original peptide, and active against a broad range of Gram-positive bacterial strains resistant to existing antibiotics [59]. There are numerous recent studies dealing with bioassays, optimization, and large-scale production of NZ2114 and other plectasin derivatives, highlighting the promising potential of these fungal defensins. Another fungal defensin with the same mode of action, copsin, was identified from *Coprinopsis cinerea* (Sarcosomataceae, Ascomycetes). This 57 amino acids peptide shows high bactericidal activity against *E. faecium* (MBC = 4 µg/mL), even more on a vancomycin-resistant strain (MBC = 2 µg/mL), but has no effect on *S. aureus* at the maximum tested concentration of 64 µg/mL [60]. The cytotoxicity of copsin has not been investigated yet. Some peptaibols from boletus also inhibit the growth of sensitive and methicillin-resistant *S. aureus* in a disk diffusion assay [61].

Besides those polypeptides, some proteins with antibacterial effects have also been identified from macromycetes. The *Cordyceps sinensis* antibacterial protein (CSAP) isolated from *Cordyceps sinensis* (now renamed *Ophiocordyceps sinensis*, Ascomycetes) exhibits a moderate inhibitory activity on the growth of *E. coli* and *S. aureus* [62], as well as F2 protein from the common mushroom *Agaricus bisporus* (Agaricaceae), inhibiting the growth of both methicillin-sensitive and resistant *S. aureus* [63]. A ribonuclease isolated from the edible mushroom *Lentinus sajor-caju* (formerly known as *Pleurotus sajor-caju*) was also found to inhibit the

growth of *S. aureus* and *P. aeruginosa*, but its further usage as antibiotic is excluded due its antimitogenic effects on normal and cancer cell lines [64].

Polysaccharides

Several polysaccharides from macromycetes have shown activity against ESKAPEE bacteria, including polysaccharides isolated from *Lentinus edodes* and from their spent substrate. These polysaccharides, not structurally described, have displayed some activity against *K. pneumoniae* and *E. coli* [65, 66]. Several other publications report antibacterial mushroom polysaccharides but generally with MIC values in the mg/mL range [67–69], which is too weak to consider any further development. In addition, polysaccharides are complex compounds whose molecular structure is generally not always fully elucidated. Nevertheless, new formulation strategies can reinforce the antibacterial potential of fungal polysaccharides. For instance, silver nanoparticles with glucans from *Pleurotus florida* have been shown to be effective against a multiresistant strain of *K. pneumoniae*, with an MIC value of 40 µg/mL [70].

Other Compounds

Basidiomycetes are known for their capacity to produce organohalogen compounds, mostly chlorinated metabolites. These compounds exhibit various properties, including antibacterial effects. For instance, the polychlorinated phenol drosophilin A (**10**), isolated from *Drosophila subatrata* (now *Parasola conopilus*), displays significant antibacterial activity against *S. aureus* and *K. pneumoniae* (MIC comprised between 4 and 64 µg/mL), while its inhibitory effect is more moderate on the growth of *P. aeruginosa* and *E. coli* (MIC = 250 µg/mL) [50]. Gymnopalyne A, a chlorinated isocoumarin from the cultures of *Gymnopus* sp. (Basidiomycetes), inhibits *P. aeruginosa*, with an MIC value equivalent to gentamycin; this compound is quite selective as it does not affect *S. aureus* and *E. coli* growth [71]. Two other chlorinated compounds from *Mycena* species (Tricholomataceae), tetrachloropyrocatechol and its methylated derivative, exert antibacterial effects against *E. coli* (MIC values of 20 and 50 µg/mL, respectively); substitution of a hydroxyl group by a methyl function seems to decrease the bioactivity [72].

Sulfur compounds are also particular metabolites occurring in mushrooms. One of them isolated from the edible mushroom *Lentinus edodes* shows high and moderate antibacterial effect against *S. aureus* and *E. coli*, respectively, but no significant activity against *P. aeruginosa* [73].

Pentadecanoic acid, a saturated fatty acid purified from wild morel species, displays a weak antibacterial activity against *S. aureus* and *E. coli*; it probably acts as a bactericidal agent as MBC are close to MIC values for these 2 bacterial strains [37].

Finally, a polyacetylene from *Parasola conopilus*, drosophilin D, shows high inhibitory effects on *S. aureus*, moderate against *P. aeruginosa* and *E. coli*, and insignificant on *K. pneumoniae* [50].

Concluding Remarks and Perspectives

Bacterial infections still represent a major health threat for all countries, even industrialized ones with access to modern therapies. In the same way that bacteria have adapted to our antibiotics, it is now our turn to adapt through the development of new drugs, to block the growing phenomenon of multidrug resistance.

Nature has proven to be a huge reservoir of drug candidates, and mushrooms could be an underexplored resource for novel antibacterial products with original scaffolds, as illustrated with pleuromutilins. However, one of the main obstacles encountered in the development of natural molecules is the supply of active ingredients, and mushrooms could be problematic in terms of biomass and overall yield. Some species can grow rapidly; otherwise, optimized cultivation under sterile conditions could be considered, as well as high-producing strains selection. Another option would be to identify the genes involved in the biosynthesis of the metabolite of interest and introduce them in the plasmid of a yeast cell, for example. A genetic engineering process is generally suitable to meet the industrial scale-up. This strategy has been successfully applied to produce pleuromutilin; the gene cluster was inserted within an *Aspergillus* strain, enabling a significant increase of pleuromutilin production (2106%) [74]. For small compounds having a rather simple chemical structure–like coprinuslactone or drosophiline A–production by total chemical synthesis could be more attractive from an economic point of view. Finally, active compounds identified in mushrooms can be specific to the fungi kingdom but also more widespread like the anthraquinone parietin, occurring in some lichens or plants. In this case, isolation of the active compound could be carried out from plants if global yield, process, and cost are more profitable. It has also to be noted that macromycetes can host endophytic microorganisms with potential properties. For example, an endophytic *Enterobacter* species isolated from the common mushroom *Agaricus bisporus* was found to produce several antibacterial compounds [75]. The production of such molecules through the cultivation of endophytic organisms could be another interesting alternative.

Regarding bioactivity, one of our main expectations is that the chemical diversity of mushrooms could be associated with original modes of action, as illustrated with coprinulactone or plectasin. Very few of the publications mentioned in this review have gone so far as to study the mechanisms involved; however, this must be the subject of in-depth studies, whether for the direct effect on bacterial growth or for biofilm inhibition, which represents a promising area of investigation. Also, the characterization of these modes of action is crucial to anticipate the emergence of new resistance mechanisms toward active fungal metabolites. Finally, as already mentioned, cytotoxicity must be evaluated on eukaryotic cells before considering further development as antibiotics, as well as *in vivo* validation of efficacy and safety.

In conclusion, the information summarized in this review emphasizes the potentiality of some fungal compounds as alternative agents against pathogenic bacteria. Despite global awareness of the antibiotic crisis and intense research from the scientific community, new effective antibiotics are struggling to emerge. We are aware that the road leading to a novel drug-candidate is long and tedious. Nevertheless, we are convinced that mushrooms repre-

sent a yet unexplored source of promising antibacterial compounds, which deserves to be investigated while being preserved.

Supporting Information

The antibacterial activities presented in this review (IC_{50} or MIC) are also available in µM in Supporting Information.

Contributors' Statement

Conception and design: A. Urbain, M. Bourjot, C. Huguet; data collection and analysis: V. Hamers, A. Urbain, C. Huguet; draft manuscript: V. Hamers, A. Urbain, C. Huguet; critical review: A. Urbain, C. Huguet, M. Bourjot, V. Hamers.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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