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Review Article

Biodegradation of antibiotics: The new resistance determinants – part I

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ABSTRACT

History shows that the discovery of, and the resistance to, antibiotics go hand in hand. While knowledge of resistance mechanisms, their impact and distribution is vast, over the years, the topic of antibiotic degradation has often been overlooked and regarded as being discrete from the research on resistance. As a result, understanding of the degradation of antibiotics and the impact of antibiotic degraders on the environment and human health are, for most classes, neither thoroughly documented nor understood. Current information on the biodegradation of antibiotics is described in two review articles. This first part focuses on sulfonamides, trimethoprim, aminoglycosides, amphenicols and tetracyclines. Detailed metabolic and molecular aspects as well as the role of the degraders in natural microbial communities are discussed. An integrated analysis of the accumulated data indicates that appreciation of the interplay between resistance and degradation is quite fragmented, and closing this gap will require novel experimental approaches.

Introduction

The discovery of antibiotics in the early 20th century marked the beginning of modern medicine. Over the past decades, their continuous use has created lasting effects not only on human and animal health, but also on the environment. Current medical and animal farming practices rely on the availability of antibiotics. This dependency has led to their intensive and sometimes imprudent use. Because antimicrobials often leave the body unaltered, antibiotic residues as well as antibiotic-resistant bacteria (ARB) and genes (ARG) enter soils and water bodies through the application of manure onto agricultural fields and through the wastewater treatment process [1–3]. As a result, both wastewater treatment plants (WWTP) and animal farming are considered important sources of ARB and ARG. These contaminated habitats possess high densities of commensal and environmental bacteria and provide the perfect settings for the selection, development, and spread of antibiotic resistance [4–6] (Fig. 1). As a consequence, the timeline of antibiotic discovery and the occurrence of resistance go hand in hand [7], and antibiotics, as well as ARBs and ARGs, are now regarded as emerging pollutants [8–12].

Information on antibiotic resistance is extensive [13]. However, only a few studies have focused on the degradation of antibiotics, and even fewer have investigated microorganisms that can use them as carbon and energy sources, i.e. antibiotrophs [14,15]. Thus, the role of these degraders in the environment remains poorly understood. Beyond their value as tools for bioremediation and biological treatment, investigating these organisms may also help researchers understand the evolution of resistance (Fig. 1). Recent studies suggest that degraders can protect susceptible members of the microbiota by reducing the concentration of the antibiotic and thus abolishing the need for the susceptible bacteria to acquire resistance genes of their own [16,17]. This mechanism, known as indirect resistance, poses severe risks in clinical settings and is often linked to antibiotic therapy failures [16]. Moreover, it may even influence how resistance and susceptibility evolve in natural communities.

This study outlines the current knowledge of biological degradation, distinguishing between organisms capable of modifying (biotransformation), cleaving (biodegradation), or mineralizing (subsistence) these micropollutants. Furthermore, it draws together information on the central degradation pathways and genes characterized

Abbreviations: 3A5MI, 3-amino-5-methylisoxazole; AMO, ammonia monooxygenase; AOB, ammonia-oxidizing bacteria; ARB, antibiotic-resistant bacteria; ARDB, antibiotic resistance database; ARG, antibiotic-resistant genes; ATU, allylthiourea; CARD, Comprehensive Antibiotic Resistance Database; CBT, Closed Bottle Test; CFU, colony-forming unit; DAPC, 2,4-diaminopyrimidine-5-carboxylic acid; DHFR,, dihydrofolate reductase; DHPS, dihydropteroate synthetase; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; MRSA, methicillin-resistant *Staphylococcus aureus*; NAS, nitrifying activated sludge; NOB, nitrite-oxidizing bacteria; SMX, sulfamethoxazole; SRT, solids retention time; WWTP, wastewater treatment plants; ZWT, Zahn-Wellens Test

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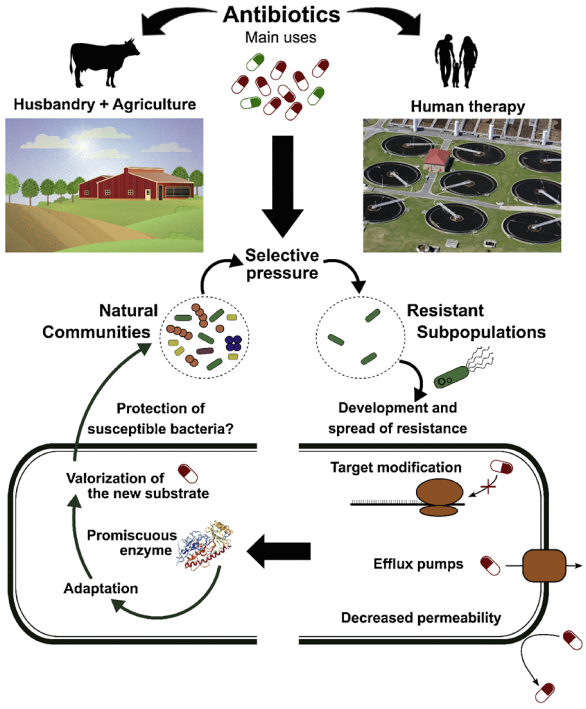


Fig. 1. WWTP, animal farms and agricultural fields as "hotspots" for the development of antibiotic resistance and degradation mechanisms. The WWTP image is courtesy of the Integration and Application Network (ian.umces.edu/symbols); vectors graphics are from FreePik (www.freepik.com); the molecular structure is from *tetX* enzyme – PDB ID 2XDO [191] – obtained from RCSB PDB (www.rcsb.org).

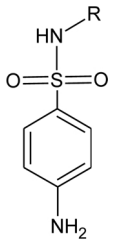
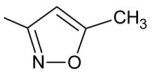
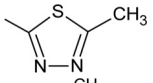
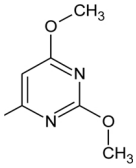
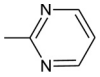
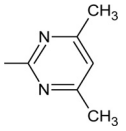
to date. It differs from other reviews on this topic [18–20] in comparing abiotic and biotic degradation pathways, details specific biological pathways and discusses the feasibility of developing biotechnological approaches to remediating contaminated sites. In addition, critical knowledge is summarized regarding the role of these degraders in natural microbial communities as well as emphasizing gaps in the methodology.

The review is divided into two parts and cites literature from all major and some minor classes of antibiotics. The first part examines the biodegradation and biotransformation of sulfonamides, trimethoprim, aminoglycosides, amphenicols and tetracyclines. In the second part, beta-lactams, macrolides, quinolones, ionophore antibiotics and other minor antibiotic classes (e.g., oxazolidinones, nitroimidazoles, and others) are discussed. Both parts provide information on the chemical structure, mode of action and mechanisms of resistance to a given antibiotic class. Subsequently, the commonest abiotic routes of transformation in the environment are summarized, and reports on biotransformation and biodegradation are discussed, beginning with complex microbial communities and concluding with axenic microbial cultures and responsible enzymes.

Sulfonamides

Sulfonamides are synthetic antibiotics that act as competitive inhibitors of dihydropteroate synthetase (DHPS) and block bacterial synthesis of folic acid [21]. Since the discovery of Prontosil (sulfamidochrysoidine), the first of the sulfonamide antibiotics introduced, more than 5000 different sulfonamides have been developed [22]. Antibiotics of this class differ in the heterocyclic group (Table 1), and are presently most commonly used for veterinary purposes [23] and as growth promoters in animal husbandry [24]. In human medicine, these antibiotics, specifically sulfamethoxazole, are still highly relevant when used in combination with trimethoprim, a combination known as co-trimoxazole [25]. This combination is primarily administered orally and is the drug of choice for treatment and prophylaxis of *Pneumocystis jiroveci* pneumonia in HIV-infected patients [21]. High doses of co-trimoxazole have been shown to be effective against methicillin-resistant *Staphylococcus aureus* (MRSA) infections [26]. Mechanisms of resistance mainly constitute insensitive versions of DHPS (encoded by*sul1*, *sul2*, *sul3*, and *sul4* [27–29]) and, on occasion, can be mediated by genes encoding efflux pumps, such as *smeDEF* [30]. Detailed reviews on this topic can be found elsewhere [28,31–33].

Table 1
General chemical structure of the main sulfonamide antibiotics, composed by an aniline moiety, a sulfonamide group and a heterocyclic moiety (R), adapted from Ingerslev and Halling-Sørensen [41].

General structure	Sulfonamide	Heterocyclic moiety (R)
	Sulfanilamide	—H
	Sulfamethoxazole	
	Sulfamethizole	
	Sulfadimethoxine	
	Sulfadiazine	
	Sulfamethazine	
	Sulfathiazole	

Sulfonamides adsorb weakly to sediments or sludge [34] and can quickly reach and contaminate groundwater. The main physicochemical properties for the primary antibiotics of this class are listed in suppl. Table S1.

Abiotic degradation

Sulfonamides are susceptible to photolysis both by exposure to natural light and UV irradiation [35,36] (suppl. Fig. S1). Nevertheless, these processes alone were shown to generate persistent and toxic intermediates, showing that natural conditions are insufficient for ensuring complete environmental removal [37].

Biotransformation and biodegradation

Comprehensive reviews on the biodegradation of sulfonamides were published in 2012 [38] as well as more recently [39,40] in an attempt to synthesize the vast amount of dispersed knowledge on the their degradation. Here, prior knowledge is highlighted and the most relevant studies on the degradation and transformation of sulfonamide antibiotics discussed. Over the years, some high-throughput studies have highlighted the potential of microorganisms to subsist on antibiotics as their sole carbon and energy source [14,15]; a detailed overview of the metabolic pathways proposed thus far is provided to assess the

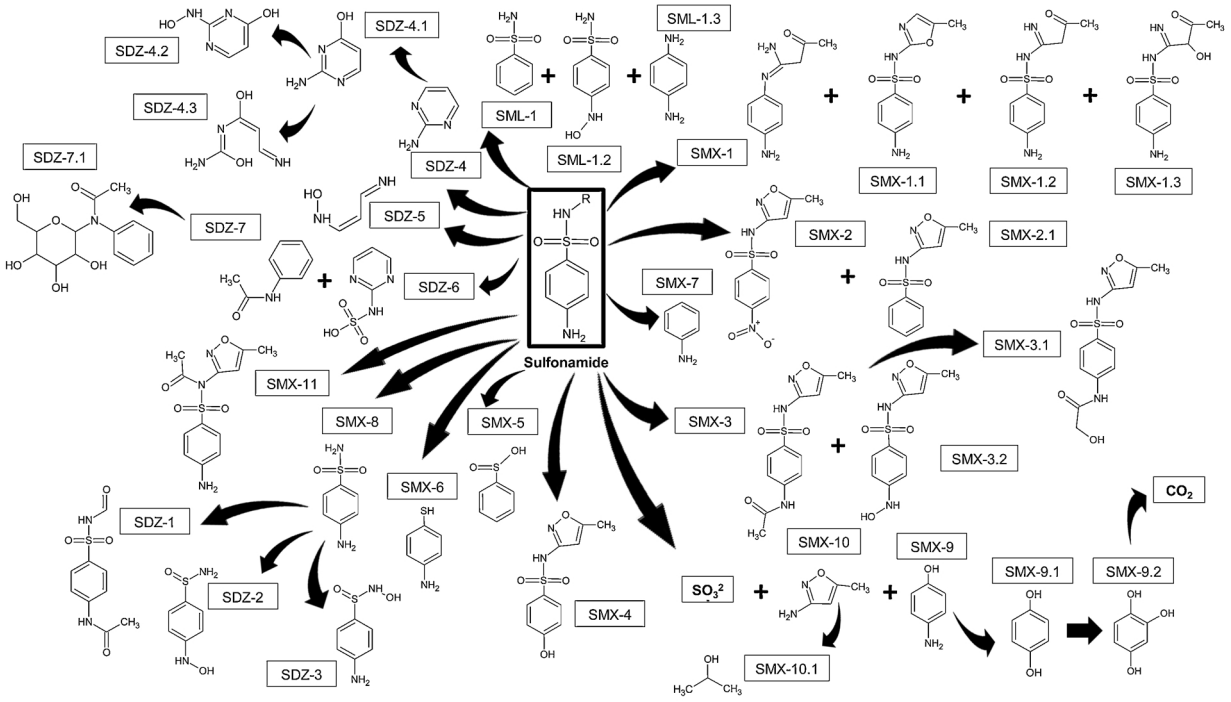


Fig. 2. Summary of the main metabolites detected during degradation of sulfanilamide (SML), sulfamethoxazole (SMX) and sulfadiazine (SDZ) by individual bacteria and complex microbial communities under aerobic and anaerobic conditions.

likelihood of such claims.

Biological degradation of sulfonamides was first reported in activated sludge [41,42]. More recently, it was further shown that these communities could degrade sulfonamides either in presence or absence of additional carbon and nitrogen sources [43]. Nevertheless, the transformation pathway was dependent on nitrogen availability. In the presence of additional nitrogen sources, sulfamethoxazole (SMX) was fully converted into 3-amino-5-methylisoxazole (3A5MI, SMX-10, Fig. 2), which lacks antibiotic activity [44]. In nitrogen starvation, sulfamethoxazole was only partially degraded into 3A5MI, and an additional product was detected, which appeared to result from the hydrolysis of the primary amine of sulfamethoxazole (SMX-4, Fig. 2), but its identity was not further confirmed. Degradation of other sulfonamide antibiotics, namely sulfamethazine and sulfanilamide (Table 2) has also been described. However, none of the previous studies assessed mineralization of the parent drug.

In anaerobic conditions, the degradation of sulfonamides seems to depend on the structure of the heterocyclic ring independently of the nature of the electron acceptor. For instance, in anaerobic digesters inoculated with sludge and manure, sulfadiazine was extensively transformed by hydroxylation of the pyrimidine ring, whereas sulfamethazine, with two methyl groups attached to the pyrimidine ring, was not transformed at all [45,46]. Under anaerobic Fe(III)-reducing conditions in soils [47], both sulfamethoxazole and sulfisoxazole, with an N—O bond within the heterocyclic ring, were quickly degraded, while sulfamethizole and sulfathiazole, which do not contain this bond, were not degraded. Thus it was proposed that this transformation can be initiated by reductive cleavage of the N—O bond in the isoxazole group to form an unstable, radical anion and yield several stable, dead-end products (SMX-1 to -1.3, Fig. 2). The same mechanism was observed in abiotic conditions with Fe(II) and goethite, suggesting it is a mere byproduct of the Fe(III) reduction carried out by soil microbiota and not the result of catalysis by specific enzymes. Another report also showed the extensive transformation of the isoxazole moiety of sulfamethoxazole in activated sludge under anaerobic conditions with skimmed milk and bicarbonate [48]. Furthermore, it was observed that reduction is accompanied by limited mineralization (between 1.2% and 2.2%) of the molecule, implying that the aniline moiety may remain intact under anaerobic conditions.

This instability of the isoxazole moiety of sulfamethoxazole was also observed in anoxic conditions [49]. This moiety may be effectively degraded in microbial fuel cells with potassium ferricyanide as an electrolyte in the cathode chamber [49]. Here, the accumulation of 3A5MI upon sulfamethoxazole degradation was exclusively transient, thus yielding isopropanol as a final product (SMX-10.1, Fig. 2). Furthermore, in water/sediment tests with NO_3^- as an electron acceptor, several reports [34,50,51] found that two major sulfamethoxazole metabolites were formed: 4-nitro-sulfamethoxazole and desamino-sulfamethoxazole (SMX-2 and -2.1, Fig. 2). Both products were formed concomitantly with sulfamethoxazole degradation, and nitrate reduction, specifically, 4-nitro-sulfamethoxazole, was found to be more toxic than the parent compound [44]. Nevertheless, once the nitrite was entirely consumed, the 4-nitro-sulfamethoxazole reverted back to its original parental form, suggesting that sulfamethoxazole concentration in the environment may fluctuate depending on nitrate availability.

Similar products were also reported in anoxic conditions with both ammonium (NH_4^+) as a nitrogen source and activated sludge enriched for ammonia-oxidizing bacteria (AOB) [52]. Under these conditions, allylthiourea (ATU) completely suppressed the transformation of sulfamethoxazole, strongly indicating that copper-containing enzymes, such as ammonia monooxygenase (AMO), may be involved in this process. Recently, many aerobic heterotrophic bacteria from phyla *Actinobacteria* or *Proteobacteria* (Table 2) have been shown to transform or even mineralize sulfonamide antibiotics, and the knowledge of the specific metabolic pathways has become quite extensive.

The biotransformation of sulfonamides (Fig. 2) by bacterial strains was first reported for *Rhodococcus* and *Pseudomonas* strains. For instance, *R. rhodochrous* [53] was found to hydrolyze sulfamethoxazole,

Table 2
Microbial communities and single bacterial strains able to degrade sulfonamide antibiotics. *N.d.* Not determined. [a] This strain can degrade other sulfonamides; *Genome sequences are available for these strains.

Class	Order	Organism	Origin	Antibiotic	Conditions	Identified metabolites	Reference
Complex microbial community			Activated sludge	Sulfamethoxazole	Aerobic	3A5MI (SMX-10) and an additional uncharacterized metabolite	[43]
			Activated sludge	Sulfamethazine	Aerobic	Sulfanilic acid; sulfamethazine dimers	[192]
			Activated carbon filter	Sulfanilamide	Aerobic	benzenesulfonamide; hydroxylamine benzene sulfonamide; *p*-phenylenediamine (SML-1 to 3)	[193]
			Soil	Sulfamethoxazole	Anaerobic (Fe^{3+} reducing)	Cleavage of the N-O bond in the oxazole moiety (SMX-1 to 1.3)	[47]
			Water/ sediment	Sulfamethoxazole	Anoxic (nitrate reducing)	4-nitro-sulfamethoxazole (SMX-2); desamino-sulfamethoxazole (SMX-2.1)	[34,50]
			Anaerobic digester sludge	Sulfamethoxazole	Anoxic (MFC)	benzenesulfinic acid (SMX-5); 3A5MI (SMX-10) and isopropanol (SMX-10.1)	[49]
			Farm, urban and pristine soils	Sulfamethizole Sulfisoxazole	Aerobic	*N.d.*	[14]
Actinobacteria	*Actinomycetales*	*Rhodococcus* sp. BR2	Membrane reactor	Sulfamethoxazole	Aerobic	*N.d.*	[60]
		Microbacterium sp. BR1	Membrane reactor	Sulfamethoxazole	Aerobic	4-aminophenol (SMX-9); SMX-9.1; SMX-9.2; 3A5MI (SMX-10), sulfite and CO_2	[60–62]
		Microbacterium sp. C448*	Agricultural soil	Sulfamethazine	Aerobic	2-amino-4,6-dimethylpyrimidine	[194,195]
		Microbacterium lacus SDZm4	Manure	Sulfadiazine	Aerobic	2-aminopyrimidine (SDZ-4)	[196]
		Rhodococcus rhodochrous ATCC 13808	Culture collection	Sulfamethizole Sulfamethoxazole	Aerobic	Hydroxyl-*N*-(5-methyl-1,2-oxazole-3-yl)benzene-1-sulfonamide (SMX-4)	[53]
		Rhodococcus equi ATCC 13557	Culture collection	Sulfamethoxazole	Aerobic	SMX-3.1	[54]
		Arthrobacter sp. D2 and D4*	Activated sludge	Sulfadiazine	Aerobic	Sulfanilamide (SMX-8) and all SDZ metabolites in Fig. 2	[65]
		Kribbella sp. SDZ-3S-SCL47	Sediment	Sulfadiazine	Aerobic	2-aminopyridine (SDZ-4); 2-amino-4-hydroxypyrimidine (SDZ-4.1)	[197]
		Gordonia sp. SMX-W2-SCD14	Activated sludge	Sulfamethoxazole	Aerobic	4-aminophenol (SMX-9); hydroquinone (SMX-9.1); 3A5MI (SMX-10)	[64]
Alphaproteobacteria	*Rhodobacteriales*	*Paracoccus* sp. SDZ-PM2-BSH30	Pig manure	Sulfadiazine	Aerobic	2-aminopyridine (SDZ-4); 2-amino-4-hydroxypyrimidine (SDZ-4.1)	[197]
	Rhizobiales	*Methylobacterium* sp. SDZ-W2-SJ40	Activated sludge	Sulfadiazine	Aerobic	2-aminopyridine (SDZ-4); 2-amino-4-hydroxypyrimidine (SDZ-4.1)	[197]
		Ochrobactrum sp. SMX-PM1-SA1	Pig manure	Sulfamethoxazole	Aerobic	4-aminophenol (SMX-9); hydroquinone (SMX-9.1); 3A5MI (SMX-10)	[64]
		Labrys sp. SMX-W1-SC11	Wastewater				
Betaproteobacteria	*Burkholderiales*	*Alcaligenes faecalis* CGMCC 1.0767	Culture collection	Sulfamethoxazole	Aerobic	N^4-Acetylsulfamethoxazole (SMX-3); Sulfamethoxazole hydroxylamine (SMX-3.2)	[56]
		Ralstonia sp. HB1 and HB2 *Achromobacter* sp. BR3	Membrane reactor	Sulfamethoxazole	Aerobic	*N.d.*	[60]
		Achromobacter sp. S-3	Activated sludge	Sulfamethazine	Aerobic	*N.d.*	[198]
		Achromobacter denitrificans PR1*	Activated sludge	Sulfamethoxazole[a]	Aerobic	3A5MI (SMX-10)	[59,199]
Gammaproteobacteria	*Pseudomonadales*	*Pseudomonas aeruginosa* PA01*	Culture collection	Sulfamethoxazole	Aerobic	N^4-Acetylsulfamethoxazole (SMX-3)	[54,200]
		Pseudomonas psychrophila HA-4	Activated sludge	Sulfamethoxazole	Aerobic	Sulfanilamide (SMX-6); 3A5MI (SMX-10); Aniline (SMX-8); 4-aminothiophenol (SMX-7)	[201]
		Acinetobacter sp. HS51	Seawater	Sulfapyridine Sulfathiazole Sulfadimidine Sulfadoxine Sulfadiazine	Aerobic	*N.d.*	[202]
		Acinetobacter sp. W1	Activated sludge	Sulfamethoxazole Sulfadiazine Sulfamethazine	Aerobic	SMX: phenolsulfonic acid, glycolamide and 3-amino-3-(hydroxyamino)propanoic acid	[67]
	Enterobacteriales	*Escherichia* sp. HS21	Seawater	Sulfapyridine Sulfathiazole	Aerobic	*N.d.*	[202]
		Salmonella sp.	Culture collection of clinical, non-clinical and food samples	Sulfisoxazole	Aerobic	*N.d.*	[15]

forming the same dead end as the one described previously [43](SMX-4). Conversely, *Pseudomonas aeruginosa* and *R. equi* could form different metabolites when sulfamethoxazole was fed in combination with glucose [54]. It was proposed that both strains could transform this antibiotic into N^4-acetylsulfamethoxazole (SMX-3), while *R. equi* would metabolize it and thus further lead to the formation of an alcohol derivative that accumulated at low amounts (SMX-3.1). Indeed, the equimolar transformation of sulfamethoxazole into N^4-acetylsulfamethoxazole was recently described for several *Proteobacteria* strains isolated from mineral water [55]. In addition, it was observed [56] that the co-metabolic transformation of sulfamethoxazole by *Alcaligenes faecalis* yielded hydroxylamine sulfamethoxazole (SMX-3.2) and N^4-acetylsulfamethoxazole (SMX-3), both of which have also been reported as human metabolites of sulfonamide antibiotics [24,57] (Fig. 2).

Particular consideration should be given to such biotransformation products when evaluating elimination rates. In some cases, only minor changes of the molecule occur with no elimination of the antibiotic activity [44], while in others the metabolites can retransform back into the parental form. The latter has been demonstrated [58] for N^4-acetylsulfamethoxazole in microcosm experiments with river water and sediments. In this way, despite exhibiting lower toxicity compared to its parental form [44], this transformation does not guarantee complete detoxification of the drug, as it is quickly reverted.

Conversely, other bacterial strains have been found to use sulfonamides as a source of carbon and energy [59,60]. Most often, the sulfonamide bond is cleaved (Fig. 2), and in some cases, the heterocyclic moieties are released as dead-end products (Table 2). For instance, it was demonstrated that the degradation of sulfamethoxazole by *Microbacterium* sp. BR1 was initiated by *ipso*-hydroxylation of the aniline moiety, resulting in the cleavage of the sulfonamide bond and accumulation of 3A5MI [61]. Sulfite and 4-aminophenol were only transiently accumulated and the latter was further channeled into the citric acid cycle via 1,2,4-trihydroxybenzene (SMX-9.1 and -9.2, Fig. 2) [62,63]. More recently, the formation of 4-aminophenol and 3A5MI was also reported for three other sulfamethoxazole-degrading strains (Table 2) [64], suggesting the same underlying mechanism as strain BR1. Furthermore, in this study, hydroquinone was also detected, suggesting that 4-aminophenol may be channeled to the citric acid cycle through this intermediate. However, contrary to strain BR1, all three strains could further degrade 3A5MI via an unknown pathway, suggesting that this moiety may not accumulate in the environment even if released as a dead-end product of sulfamethoxazole degradation by some bacterial strains. Degradation of the heterocyclic group was also observed for sulfadiazine, a pyrimidine-substituted sulfonamide. For instance, *Arthrobacter* strains D2 and D4 [65], a *Terrabacter* sp. strain [66] and *Acinetobacter* sp. strain W1 [67], were found to hydroxylate 2-aminopyrimidine (SDZ-4 to -4.2, Fig. 2) and further utilize it as a carbon source.

The *ipso*-hydroxylation of sulfonamides appears to be shared among several members of the *Micrococcaceae* family and has been recently linked to the presence of a conserved sulfonamide degradation cluster [68]. The *sad* gene cluster encoding two flavin-dependent monooxygenase (SadA and SadB) and a flavin mononucleotide (FMN) reductase (SadC) provides reduced co-factors to the first two enzymes. The first monooxygenase (SadA) was shown to be responsible for the initial attack of sulfonamide molecules by releasing 4-aminophenol (SMX-9) [68], while SadB transforms this metabolite into 1,2,4-trihydroxybenzene (SMX-9.1, Fig. 2). Interestingly, transformation of sulfonamides depends on the nature and bulkiness of the heterocyclic group, which influences the affinity of each parent molecule for the active site. This property may explain the different degradation rates observed for different sulfonamides while assuming the same underlying mechanism for all [59,61].

The members of the *Microbacterium* and *Arthrobacter* genera that harbor the *sad* cluster were also shown to harbor the extensively described *sul*1 gene [68], a widespread resistance gene encoding a sulfonamide-insensitive DHPS. The co-existence of both a degradation mechanism and a resistance gene in the same strains raises important questions concerning the co-evolution of these traits. Thus, future studies should also investigate whether antibiotic degraders require additional resistance genes to grow in the presence of sulfonamide antibiotics or whether they alone suffice for the antibiotic resistance phenotype.

Despite recent advances in the molecular characterization of these sulfonamide degraders, few studies have focused on assessing their applicability for biotechnological treatments. Since the link between antibiotic degradation and resistance remains unexplored, the direct application of these degraders may promote the undesirable spread of resistance. Furthermore, these strains may not perform ideally under environmental conditions, as observed in membrane bioreactors spiked with *Microbacterium* sp. BR1 [69]. This specialized strain did not improve sulfamethoxazole removal compared to removal in the control experiments, as it was unable to thrive at low temperatures and to degrade the antibiotic at environmentally relevant concentrations. However, the ability to degrade environmental concentrations may also depend on the regulation of this metabolic pathway in each strain, since *Achromobacter denitrificans* PR1 was able to degrade sulfamethoxazole in batch experiments at concentrations as low as 600 ng/l [70].

Sulfonamides are also susceptible to degradation by ligninolytic enzymes. For instance, the versatile peroxidase of *Bjerkandera adusta* [71] degrades sulfamethoxazole yielding 3A5MI as a stable product, carboxylic acids (acetic and oxalic acid), and anions (nitrate, nitrite, and sulfate), suggesting an effective degradation of an aniline ring. Moreover, laccase [72,73] was shown to degrade sulfonamides with distinct heterocyclic groups. As observed in previous reports under both anaerobic and aerobic conditions, all the sulfonamides had different elimination rates. In addition, for sulfapyridine, 2-aminopyrimidine and aniline were identified as transformation products. Although complete degradation by these enzymes alone may be costly and inefficient, their use as a pre-treatment may be a suitable application because they render intermediates more amenable to biological degradation [74].

Limited research has been done on the topic of sulfonamide degradation and its link to indirect resistance. For instance, the correlation was investigated between sulfamethoxazole degradation and the spread of known resistance genes (*sul*1 and *sul*2) in mesocosms with river water and biofilm from pristine and polluted environments [75]. In this study, an interesting effect was that, at high sulfamethoxazole concentrations (5 μg/l) in waters from pristine environments, the rapid degradation of the antibiotic led to a reduced spread of the known resistance genes. This suggests that degradation might actually decrease the horizontal gene transference of resistance genes and the proliferation of antibiotic-resistant bacteria. This study did not take into account a robust assessment of the microbial diversity, and the amount of mineralization was also overlooked. Nevertheless, it raises new and interesting implications for the role of these antibiotic degraders in achieving the equilibrium between antibiotic resistance and susceptibility in natural communities.

Trimethoprim

Trimethoprim, a diaminopyrimidine, is often used in combination with sulfamethoxazole. Despite being structurally different, it shares most of the antibacterial spectrum and mechanism of action with sulfonamides by inhibiting dihydrofolate reductase (DHFR), a downstream enzyme also involved in the synthesis of folic acid [31]. Resistance also predominately occurs through mutated and horizontally transferred versions of DHFR [31]. Presently, more than 20 genes encoding mutated versions of DHFR have been described and can be found in both the Antibiotic Resistance Database (ARDB) and the Comprehensive Antibiotic Resistance Database (CARD) [76,77]. Despite being used in combination with sulfonamides and sharing a similar mechanism of

action, trimethoprim is vastly different in physicochemical properties (see suppl. Table S1). Trimethoprim adsorbs weakly to sediments or sludge, and therefore its mobility among environmental compartments is slightly more restricted than that of sulfonamides [78].

Abiotic degradation

This antibiotic is susceptible to photolysis, but to a lesser extent than sulfonamides. This process was reported to yield photostable products, mainly trimethoxybenzoylpyrimidine (TMP-a, suppl. Fig. S1), a ketone derivative [79,80].

Biotransformation and biodegradation

In contrast to sulfonamides, which are quickly degraded in aerobic conditions by activated sludge, trimethoprim is typically recalcitrant to degradation or transformation under the same conditions [42,78,81,82]. However, significant transformation of trimethoprim by aerobic-activated sludge was reported [83] for sludge collected from a system with an extended Solids Retention Time (SRT) as well as by several authors for nitrifying activated sludge (NAS) [42,84–86]. NAS is an activated sludge process specializing in efficient nitrogen removal. Due to an increased SRT compared with conventional activated sludge systems [87], this system is enriched in autotrophic nitrifiers, namely AOB and nitrite-oxidizing bacteria (NOB). These obligate aerobic bacteria use inorganic carbon (e.g., CO_2) as their primary carbon source and generate energy by the oxidation of ammonia or nitrite respectively [88]. Trimethoprim degradation in aerobic NAS systems has been attributed mainly to AOB activity because ATU significantly reduced the degradation of this drug in batch tests (Table 3) [84,86]. Two products of trimethoprim oxidation by NAS were identified [85] and were further confirmed in experiments performed with 20 mg/l and 0.5 mg/l trimethoprim, respectively [89]. These degradation products were consistent with typical reactions catalyzed by ammonia monooxygenases (TMP-1 and -1.1, Fig. 3). However, at lower concentrations (5 µg/l), additional products were detected [89], suggesting a different pathway for trimethoprim elimination. At this concentration, trimethoprim transformation started with demethylation of the parent compound (TMP-3, Fig. 3) and resulted in the accumulation of 2,4-diaminopyrimidine-5-carboxylic acid (DAPC, TMP-3.3), which is only slowly metabolized in this system.

The assumption that AOB are solely responsible for trimethoprim biotransformation was challenged in similar experiments [90,91]. Both studies implied that heterotrophic bacteria were crucial for trimethoprim transformation in NAS. Specifically, it was found that ATU did not inhibit trimethoprim degradation in batch tests when added halfway through the incubation time [90]. Moreover, axenic cultures of the ammonia-oxidizing *Nitrosomonas europaea* were unable to transform this drug, while the heterotrophic aerobic bacteria enriched from NAS were able to cleave the trimethoprim molecule (TMP-2 and -2.1, Fig. 3), thus accumulating recalcitrant metabolites that were likely not further degraded. The metabolites described in [90] for heterotrophic bacteria enriched from a NAS system and in [85] for NAS are fundamentally different. Considering that one observed cleavage of the trimethoprim structure [90], it is likely that this pathway involves specific enzymes. In contrast, the other [85] only observed oxidation without further transformation, supporting the idea that unspecific enzymes such as ammonia monooxygenases may be involved in this process. In this way, both mechanisms appear to be equally important for the removal of TMP from the environment, although more studies are required in order to identify the microorganisms and enzymes responsible for these transformations.

An additional metabolite was detected for TMP degradation in soils consisting in the hydroxylation of the molecule at C6 position (TMP-4, Fig. 3). Interestingly, trimethoprim removal was also reported to be effective in anaerobic digesters [45], but neither pathway nor mechanism has been described to date.

Aminoglycosides

Streptomycin, the firstly aminoglycoside described, was isolated from the soil-dwelling bacteria *Streptomyces griseus* [92]. It became invaluable due to its ability to inhibit *Mycobacterium tuberculosis*, which is presently re-emerging due to high levels of resistance [93,94]. Several other natural aminoglycosides were isolated from other Actinomycetes (*Streptomyces* sp. and *Micromonosphora* sp.), and many semi-synthetic analogues were subsequently developed and introduced for both clinical and veterinary use [95]. Presently, the most commonly prescribed antibiotics of this class are gentamicin, tobramycin, and amikacin, while streptomycin is still utilized due to its effectiveness in the treatment of tuberculosis [96]. Each of these consists of a complex mixture of closely related derivatives that can render the detailed characterization of degradation and transformation mechanisms difficult.

These antibiotics (Fig. 4) typically consist of an aminocyclitol nucleus (mainly 2-deoxystreptamine, as in compounds a, b, c, e; or streptidine, as in d, Fig. 4) linked to amino sugars by glycosidic bonds [97]. They possess concentration-dependent bactericidal ability and act to inhibit protein synthesis in bacteria [97]. Aminoglycosides are also often used in combination with beta-lactams [95,97]. They are still in use with human and veterinary medicine [23,25,98], albeit at a much lower scale compared to other antibiotic classes, such as tetracyclines, sulfonamides, or beta-lactams. Thus, contamination with them is rarely detected in wastewaters [99], but resistance levels are highly prevalent among clinical and environmental isolates [100–105], the predominant resistance mechanisms being enzymatic modification (acetylation, adenylation, phosphorylation), efflux pumps and target modification (16S rRNA methylation). All gene sequences and detailed mechanisms of action can be found elsewhere [76,77,97,106] and the main psychochemical properties of this class can be found in suppl. Table S1.

Abiotic degradation

Stability of aminoglycosides is difficult to assess due to their multicomponent nature and established presence of impurities. It was reported [107] that, at room temperature, gentamicin was quickly degraded (48 h) in a solution of dextrose, commonly combined with antibiotics for intravenous administration. Several degradation products were detected, one of which was identified as sisomicin (AMG-b,

Table 3
Microbial communities and single bacterial strains able to degrade sulfonamide antibiotics and trimethoprim.

Class	Order	Organism	Origin	Antibiotic	Conditions	Identified metabolites	Reference
Complex microbial community			NAS	Trimethoprim	Aerobic ammonia oxidizing	TMP-1 and TMP-1.1	[85]
			NAS (heterotrophs)	Trimethoprim	Aerobic	TMP-2 and TMP-2.1	[90]
			NAS	Trimethoprim	Aerobic	TMP-1; TMP-1.1; 4-desmethyl-TMP (TMP-3); TMP-3.1; TMP-3.2; DAPC (TMP-3.3)	[89]
			Farm, urban and pristine soils	Trimethoprim	Aerobic	*N.d.*	[14]

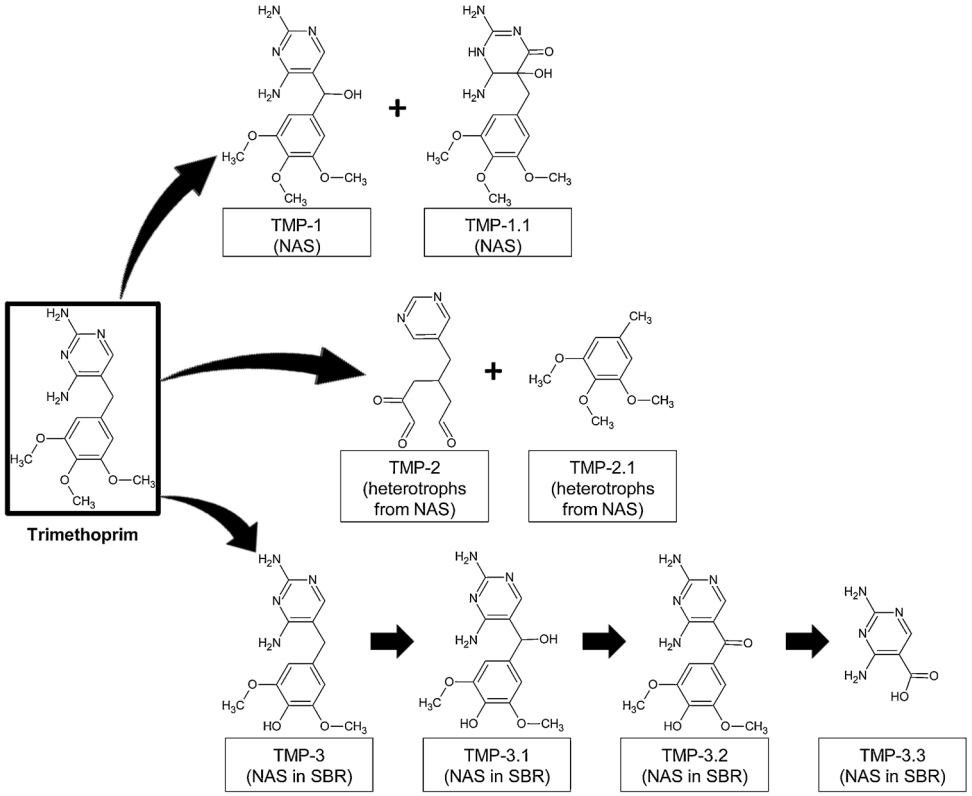


Fig. 3. Summary of the main degradation pathways of trimethoprim by NAS.

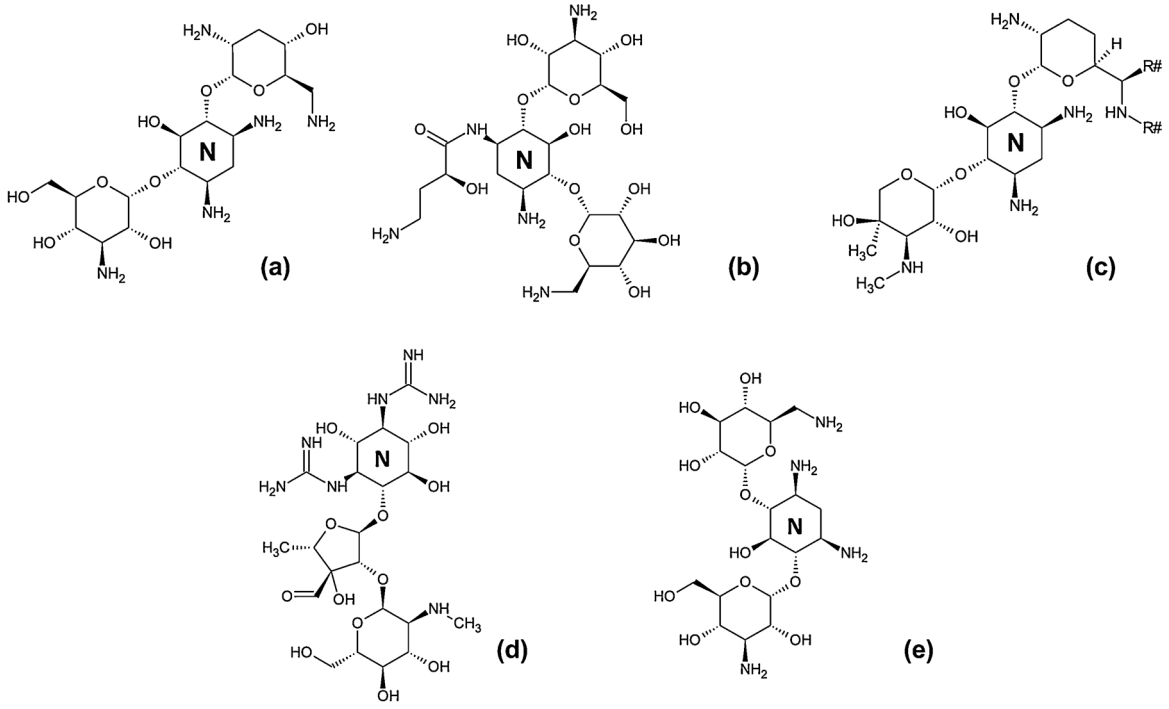


Fig. 4. Chemical structures of the main aminoglycoside antibiotics, with the aminocyclitol nucleus (N): (a) gentamicin, (b) tobramycin, (c) amikacin (d), streptomycin (d) and kanamycin (e).

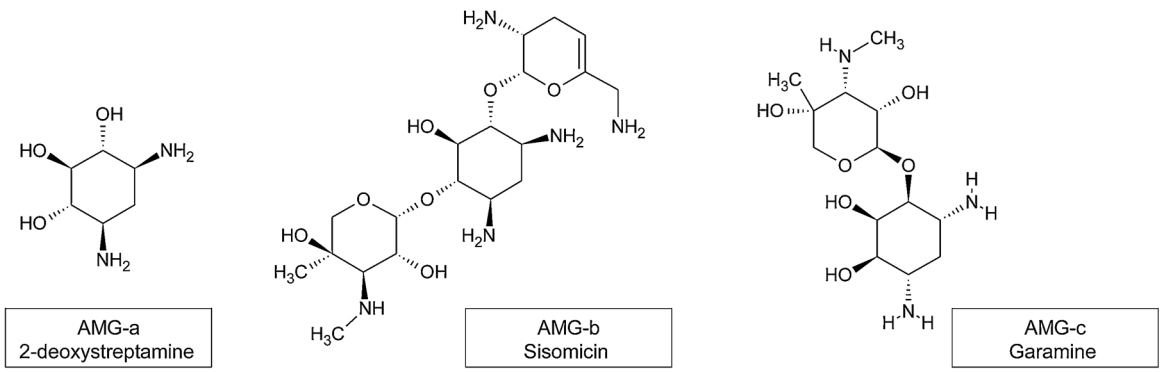


Fig. 5. Chemical structure of some of the common impurities and degradation products of gentamicin and kanamycin.

Fig. 5), a molecule with known antibacterial activity [108]. This and other intermediates (AMG-b and -c, Fig. 5) are also established by-products of aminoglycoside synthesis in *Micromonospora purpurea* [109], indicating that these compounds are part of the producing strain's metabolism [110,111].

Degradation of the parent compounds in natural matrices was shown to be significantly enhanced at higher temperatures [112] and low pH. For instance, under extremely acidic conditions (6 N HCl), both gentamicin and kanamycin were revealed to both lose antibacterial activity completely and release 2-deoxystreptamine (Fig. 5) [109,113,114]. In contrast, direct photolysis of aminoglycosides is negligible because they do not absorb light from the solar spectrum [115]. Significant photodegradation occurs mostly in the presence of natural organic matter, which can bind and make them more susceptible to radical attacks [115].

Biotransformation and biodegradation

In most studies, aminoglycosides are generally considered non-biodegradable in both aerobic (Closed Bottle Test [CBT], Zahn-Wellens Test [ZWT], and CO_2-evolution test) and anaerobic conditions for experiments executed with activated sludge [116–118]. Moreover, a significant amount was adsorbed to the sludge, but not degraded, suggesting that they may become unavailable for biodegradation after adsorption. In soil, it was also found that kanamycin was moderately persistent when incubated for 63 days [119]. The specific properties of each soil significantly influenced degradation rate, with higher rates occurring in soils with high organic matter content and water retention ability. However, neither the specific mechanism for the elimination of these drugs nor the antimicrobial activity of the degradation products were assessed. Conversely, enzymatic modification of these drugs by bacterial strains is well known, as it represents the central mechanism of resistance to antibiotics of this class [97,106]. Enzymes that mediate these reactions are intracellular and classified as nucleotidyltransferases, phosphotransferases or acetyltransferases; they catalyze the derivatization of different amino or hydroxyl groups in the aminoglycoside structure (Fig. 6). Although these modifications result in significant decrease of antibacterial activity, no cleavage of the molecule itself occurs [120].

Few studies have investigated the ability of isolated microorganisms and complex microbial communities to degrade these antibiotics. It was found [14] that soil microbiota from different sources (farmland, urban soil, and undisturbed pristine soils) were able to use aminoglycosides (amikacin, gentamicin, kanamycin, and sisomicin) as a sole carbon source. Furthermore, others observed the subsistence phenotype (increase in colony-forming unit [CFU] counts over time with the antibiotic as sole carbon source) in more than 50 *Salmonella* sp. isolates from clinical, non-clinical, and food samples [15]. However, the metabolic pathway and toxicity of the degradation products were not assessed and no direct proof of mineralization was provided. These observations were even challenged in similar experiments [121,122]. The subsistence phenotype was investigated in soils under different anthropogenic influence [122]. Although an increase in CFU counts over time was also observed in media with streptomycin as the single carbon source, actual degradation of this antibiotic was not found. Similarly, others investigated the aminoglycoside subsistence phenotype in aerobically grown gut bacteria [121]. Nine *E. coli* and *Cellulosimicrobium* sp. isolates obtained in this study displayed the subsistence phenotype, however no degradation of the antibiotic was observed. These conflicting results highlight the need for combining high-throughput screening techniques with a detailed assessment of the mechanistic aspects of antibiotic removal.

To date, only one bacterial strain has been identified as using streptomycin as both a carbon and energy source [123]. This *Stenotrophomonas maltophilia* strain enriched from soil was able to degrade the antibiotic via streptamine into pyridine, which was further metabolized via an unknown pathway (SMC-1 and SMC-1.1, Fig. 6). Degradation occurred concomitantly with the release of volatile nitrogenous compounds, methylamine and ammonia (Fig. 6). No other authors have investigated this particular transformation mechanism and the enzymes involved in this process as well as their relevance in environmental settings remain unidentified.

Thus, although enzymatic modification is widespread and aminoglycoside-transformation strains are highly prevalent, the impact of enzymatic modification on the development and evolution of resistance in susceptible bacteria has not been thoroughly assessed. For instance, bacteria engineered to express aminoglycoside-modifying enzymes do not protect susceptible subpopulations, as recently exemplified [16,17]. These results, contrary to those observed for chloramphenicol-degrading strains (see next section), may result from the low permeability of these drugs that require active uptake before undergoing intracellular transformation. However, as no bioremediation studies have employed aminoglycoside-modifying enzymes, the real effect of aminoglycoside transformation on the development of *de novo* resistance remains unclear.

Amphenicols

Chloramphenicol is a natural antibiotic isolated from the soil-dwelling bacterium *Streptomyces venezuelae*. Due to its severe side effects and suspected carcinogenesis, it is currently banned from use in the treatment of food-producing animals in the EU, USA, and China, among other regions. However, it is still used to treat a small number of infections in clinical settings [25,124]. Several synthetic analogs of

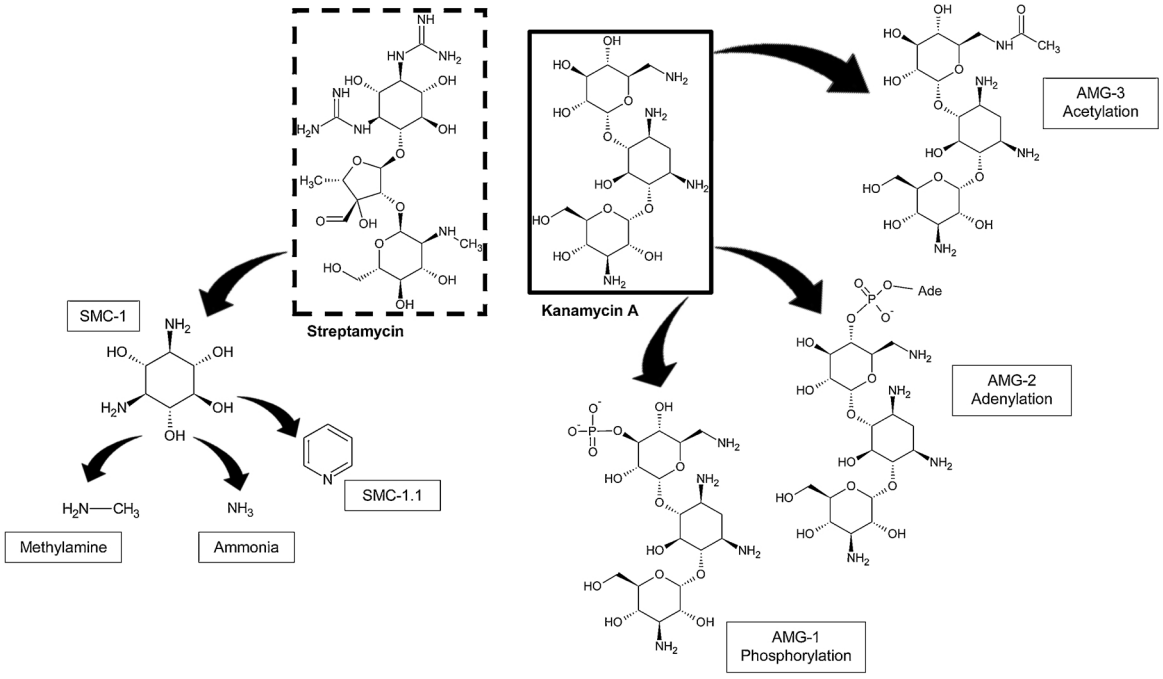


Fig. 6. Degradation of streptomycin by *Stenotrophomonas maltophilia*, formerly *Pseudomonas maltophilia* [123] and examples of common modifications of kanamycin A (AMG-1 to 3), adapted from Magalhães and Blanchard [106].

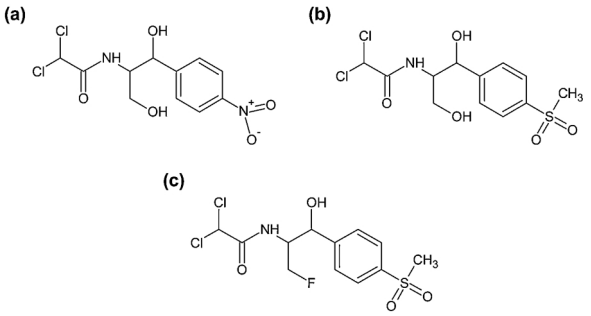


Fig. 7. Chemical structure of amphenicol antibiotics: (a) chloramphenicol, (b) thiamphenicol and (c) florfenicol.

chloramphenicol, namely thiamphenicol and florfenicol, have been developed and are presently in use [124,125] (Fig. 7). These antibiotics act as bacteriostatic agents by preventing amino acid chain elongation during protein synthesis [126] and possess both a dichloroacetamide group and an aromatic moiety (Fig. 7). However, chloramphenicol contains a nitrobenzene group, whereas in thiamphenicol and florfenicol, the *p*-nitro group in the benzene ring is replaced by a sulfomethyl group. This substitution was reported to reduce toxicity and eliminate the development of aplastic anemia in humans and animals [126] (suppl. Table S1).

Despite the low levels of usage, chloramphenicol is naturally produced and abundant in soils. Consequently, it can be translocated into plants that serve as food supply for livestock animals [127,128], leading to high levels (μg/kg) of contamination in animal products even in countries where its use has been banned [129–131]. Resistance to chloramphenicol frequently occurs through its enzymatic modification by acetylation or phosphorylation [124]. Thiamphenicol is also a substrate of these transferases, while florfenicol, due to its fluor residue, remains untransformed (Fig. 7). Consequently, many strains that are resistant to chloramphenicol may be susceptible to florfenicol [124,132], with cross-resistance occurring only by efflux pumps [124].

Abiotic degradation

Chloramphenicol and florfenicol can be modified by hydroxylation and dechlorination (suppl. Fig. S2) under acidic or basic pH and high temperatures (60 °C) [133]. Nevertheless, their aromatic moieties are not affected. Chloramphenicol can also be degraded by photolysis to yield hydroxylated byproducts; however, while this transformation eliminated antimicrobial activity, increased toxicity against *Artemia salina* was observed [134]. Photolysis can also transform thiamphenicol and florfenicol through similar reactions, but the toxicity of the by-products has yet to be assessed [135].

Biotransformation and biodegradation

The literature assessing the biodegradation of chloramphenicol by complex communities and isolated microorganisms is scattered, and there is a lack of recent, detailed studies. Several authors reported [136,137] that chloramphenicol was degraded in soil, with higher degradation rates occurring under aerobic rather than anaerobic conditions, but the metabolic pathway and activity of the degradation products were not assessed. In addition, it was observed that a reduced persistence of the fluorinated analog “florfenicol” occurred during anaerobic digestion [46]. The degradation consisted of dechlorination, alkyl fluorine hydrolysis and demethylation reactions, but the chemical structures of these metabolites were not identifed. The metabolites showed an increased persistence (> 40 days) compared to the parent molecule and moderate toxicity against anaerobic sludge microbiota.

Many bacteria, including antibiotic-producing strains, can resist chloramphenicol through enzymatic inactivation by acetylation (CAP-5) or phosphorylation (CAP-6, Fig. 8) [124,138,139]. However, this reaction may be quickly reversed by esterases from various sources,

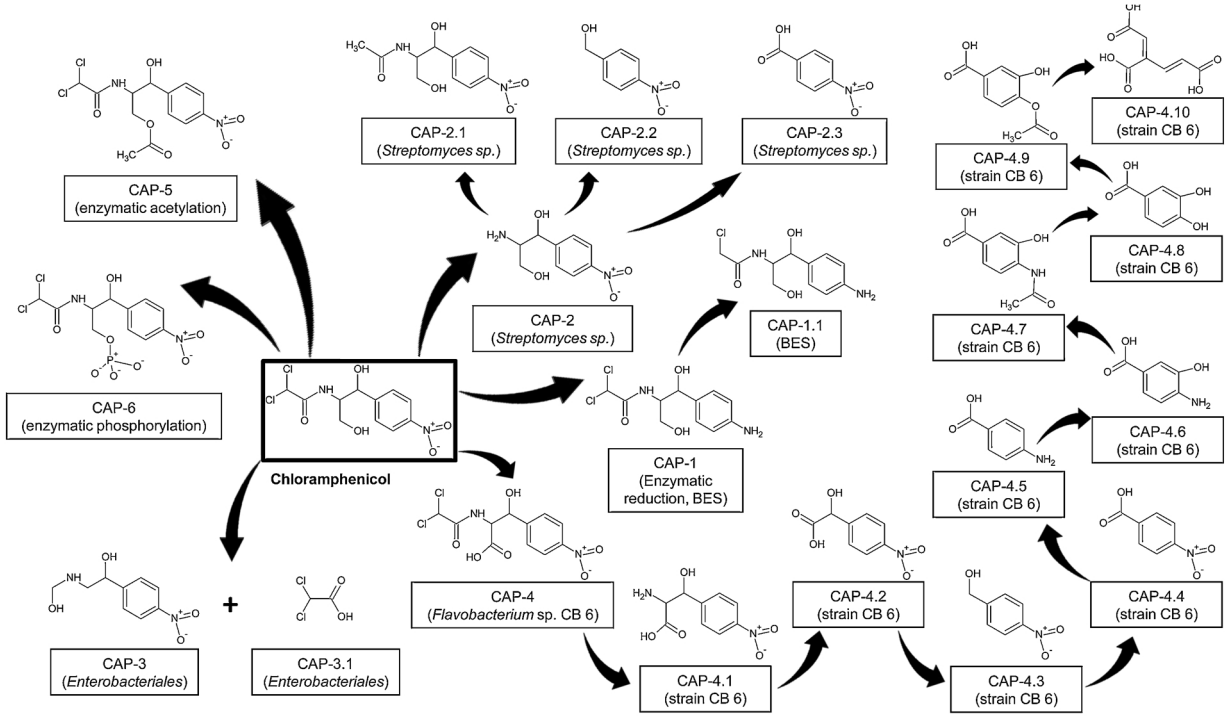


Fig. 8. Summary of degradation and transformation reactions of chloramphenicol carried out by bacterial strains.

including soil, serum, and pathogenic bacteria [140–142]. Esterases obtained from a soil metagenome [141,143], besides their ability to reverse acetylation of chloramphenicol, may also hydrolyze both chloramphenicol and florfenicol parental forms. The hydrolyzed product of chloramphenicol was further identified as *p*-nitrophenylserinol (CAP-2, Fig. 8), resulting from the hydrolysis of the *N*-dichloroacetyl group. Some chloramphenicol-producing actinomycetes have also exhibited a similar hydrolytic activity. For instance, a *Streptomyces* sp. strain was identifed as capable of transforming chloramphenicol by hydrolytic cleavage of the *N*-dichloroacetyl group (*p*-nitrophenylserinol) that was either subsequently transformed by acetylation to *N*-acetyl-*p*-nitrophenylserinol (stable dead-end product) or further hydrolyzed in small, parallel reactions into *p*-nitrobenzyl alcohol (CAP-2.2) and *p*-nitrobenzoic acid (CAP-2.3, Fig. 8) [144] Several bacterial strains were also shown to reduce the *p*-nitro group of chloramphenicol to an amino group. This reaction, possibly mediated by nitroreductases, was reported to eliminate antimicrobial activity [145,146].

As yet, only a few strains have been reported as using amphenicol antibiotics as their sole carbon source (Table 4). A *Streptomyces* sp. strain capable of doing so was described previously [147], but a degradation pathway has not been proposed and *Flavobacterium* sp. CB 60 was found to acetylate this drug by constitutively expressed enzymes and further degrade this product into unknown metabolites [148,149]. Nevertheless, actual mineralization of the molecule was not assessed. Another strain of the *Flavobacterium* genus (CB 6) was reported to use chloramphenicol as a sole carbon source [150]. Degradation was performed by inducible enzymes and was initiated by oxidation of the primary alcoholic group in the C-3-position. Eventually, β-carboxy-*cis,cis*-muconic acid was formed as the final product of the primary degradation pathway (CAP-4 to -4.10, Fig. 8). This carboxylic acid, a common metabolite from xenobiotic degradation, can readily be metabolized in central pathways, thus strongly supporting the assumption that this strain exhibits the subsistence phenotype [151,152]. Six other strains (Table 4) in the order *Enterobacteriales* [15,153] have been reported as capable of subsisting on amphenicol antibiotics as sole carbon source, but no sufficient proof of the subsistence phenotype has been provided in any of these studies, as they lack detailed characterization and CO_2 evolution tests.

To the best of our knowledge, studies regarding biotechnological applications are infrequent. However, several authors [154,155] have exemplified that it is possible to harness microbial dechlorination of amphenicol antibiotics through combination with an electrochemical system, which was able to reduce (CAP-1, Fig. 8) and further dechlorinate (CAP-1.1, Fig. 8) the *p*-nitro group of chloramphenicol.

With the exception of the extensive transformation of chloramphenicol by *Flavobacterium* sp. CB 6, common elimination routes of this antibiotic leave the *p*-nitrobenzene moiety intact. Therefore, most degradation products are still expected to retain some toxicity. However, since chloramphenicol is naturally produced in soil, other unknown and overlooked routes may be involved in its elimination.

Conversely, some authors investigated the link between antibiotic degraders and the onset of resistance. One study [16] showed that, when *E. coli* expressed a chloramphenicol acetyltransferase (CatA1) in co-culture with a susceptible strain, it prevented this strain from developing or acquiring resistance. Further investigation [17] was made of the influence of this phenotype co-occurring with opportunistic human pathogens, *Streptococcus pneumoniae,* and *Staphylococcus aureus* both *in vitro* and in mouse infection models. In both cases, susceptible populations were protected by indirect resistance during chloramphenicol treatment. Surprisingly, in the co-infection studies in mice, the resistant subpopulation was outcompeted by the susceptible bacteria, suggesting unexpected fitness costs to the resistant cells. This explanation is plausible because resistant cells maintain the ability to grow quickly in the presence of chloramphenicol and thereby become preferred targets of host defense factors [156]. This unexpected and paradoxical effect of antibiotic resistance indicates a general lack of knowledge and research on the fitness costs and implications of antibiotic degraders in natural populations.

Table 4
Bacterial strains able to degrade or transform amphenicol antibiotics. *N.d.* Not determined.

Class	Order	Species	Origin	Antibiotic	Conditions	Identified metabolites	Reference
Complex microbial communities			Farm, urban and pristine soils	Florfenicol	Aerobic	*N.d.*	[14]
			Activated sludge	Chloramphenicol	Anaerobic (BES)	AMCl2 (CAP-1), AMCl (CAP-1.1)	[154,155]
Actinobacteria	*Actinomycetales*	*Streptomyces* sp. 13*s*	Spore of *Streptomyces* sp. 3022*a*	Chloramphenicol	Aerobic	*p*-nitrophenylserinol, *N*-acetyl-*p*-nitrophenylserinol, *p*-nitrobenzyl alcohol and *p*-nitrobenzoic acid (CAP-2 to 2.3)	[144,203]
		Streptomyces sp.	Soil	Chloramphenicol	Aerobic	*N.d.*	[147]
Gammaproteobacteria	*Enterobacteriales*	*Klebsiella* sp. I1-2-CHL-3 and I1-3-CHL-1 *K. pneumoniae* I-11-CHL-1 and I-7-CHL-1 *Escherichia fergusonii* I-10-CHL	Gut microbiota	Chloramphenicol	Aerobic	2-[(hydroxymethyl)amino]-1-(4-nitrophenyl)ethanol (CAP-3); dichloroacetic acid (CAP-3.1)	[153]
		Salmonella sp.	Culture collection of clinical, non-clinical and food samples	Florfenicol	Aerobic	*N.d.*	[15]
	Pasteurellales	*Haemophilus influenzae Rd KW20*	Model organism	Chloramphenicol	Aerobic	Reduction of *p*-nitro group (CAP-1)	[146]
Clostridia	*Clostridiales*	*Clostridium beijerinckii* NCIMB 8052	Culture collection	Chloramphenicol	Anaerobic	Reduction of *p*-nitro group (CAP-1)	[145]
Flavobacteriia	*Flavobacteriales*	*Flavobacterium* sp. CB 6	Soil	Chloramphenicol	Aerobic	CAP-5 to 5.10	[150,204]
		Flavobacterium sp. CB 60	Private culture collection	Chloramphenicol	Aerobic	3′-*O*-acetyl-chloramphenicol (CAP-6)	[148]

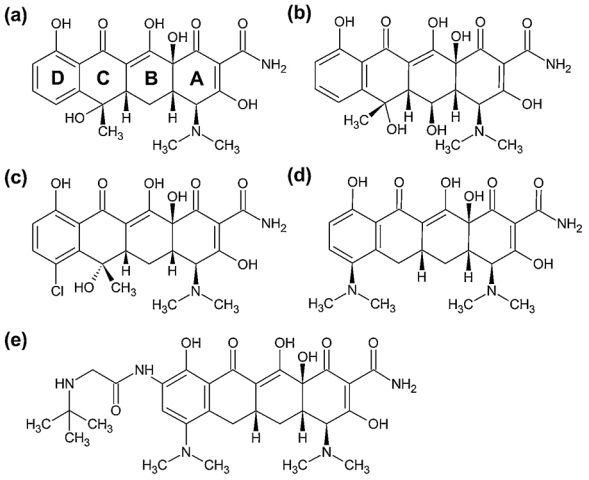


Fig. 9. Tetracycline molecules consist of four aromatic ring nucleus (A to D) to which several functional groups are attached. Four-ring structure of (a) tetracycline with the aromatic rings identified (A to D), (b) oxytetracycline, (c) chlortetracycline, (d) minocycline and (e) tigecycline.

Tetracyclines

Tetracycline antibiotics exist as natural compounds produced by *Streptomyces* sp. [157]. They act as inhibitors of protein synthesis by preventing the binding of aminoacyl-tRNA to the ribosomal acceptor site [158]. The ones described first were chlortetracycline, oxytetracycline and tetracycline, and they are still widely used (Fig. 9). Later, semi-synthetic analogues with increased solubility and oral absorption were introduced into clinical practice. These analogues divide themselves into second-generation or semi-synthetic tetracyclines (e.g., minocycline) and third-generation tetracyclines or glycylcyclines (e.g., tigecycline) [126,158]. Despite their reduced use in human medicine, tetracyclines are currently the most relevant drugs for veterinary applications, specifically for food-producing animals, either for treatment [98,159] or as growth promoters [23].

Tetracyclines are amphoteric and strong chelating agents (see suppl. Table S1 for detailed physicochemical properties). They adsorb to sediments and activated sludge [137,160,161] and thus preferentially contaminate soils [162]. However, residues of tetracyclines have been detected in a broad range of environmental compartments, such as WWTP effluent (μg/L), activated sludge (μg/kg), surface waters (ng/L), and soil (μg/kg) [163–166]. As observed for other antibiotic classes, resistance to tetracycline antibiotics is extremely prevalent [167–169], and to date, more than 30 resistance genes have been described as encoding various efflux pumps and ribosomal protection proteins. Detailed reviews and databases containing information on the structure and resistance to these antibiotics can be found in [76,77,126,158].

Abiotic degradation

Tetracyclines have reduced stability and undergo extensive transformation in waters and soils [170] depending on pH and the presence of cations [171]. Natural tetracyclines are relatively stable under acidic conditions, though very unstable in alkaline solutions. Tetracycline, for instance, generally undergoes epimerization in acidic and neutral conditions (TET-c, suppl. Fig. S3). However, this reaction is easily reversed and is furthermore inhibited in the presence of calcium and magnesium at pH ≥ 6. Also, in acidic conditions, both tetracycline and its epimer can be irreversibly dehydrated to anhydrotetracycline (TET-d, suppl. Fig. S3) and 4-epi-anhydrotetracycline, respectively [172].

Under alkaline conditions, transformation is more extensive and results in the irreversible cleavage of the hydroxyl group in the C ring, thus yielding isotetracycline (TET-e, suppl. Fig. S3) that can be reversibly epimerized to 4-epi-isotetracycline. However, these products still retained some degree of antimicrobial activity and toxicity [171,173], and anhydrotetracycline and its epimer were both more toxic against tetracycline-resistant bacteria, suggesting that this modification may have altered the drug's mode of action [171]. Photolysis was also shown to transform tetracyclines, causing the loss of all antimicrobial activity. These reactions consist mainly of the loss of *N*-methyl-, amino- and hydroxyl- groups (TET-a and -b, suppl. Fig. S3). However, the toxicity of these degradation products against luminescent *Vibrio fischeri* was higher than that of the original compound [174,175].

Biotransformation and biodegradation

Despite their limited stability in aqueous solutions, tetracyclines are often reported as nonbiodegradable under aerobic and anaerobic conditions. They are mainly eliminated through abiotic transformation and adsorption, with biological transformation playing only a minor role [136,176]. This behavior has been observed in several different tests, including CBT, ZWT, and CO_2-evolution tests with activated sludge [116,118,161]. The results were also reported under anaerobic conditions, during which these drugs adsorbed to the sludge with detrimental effects on the microbiota [117,177]. However, it is still unclear whether these compounds become stable when adsorbed or whether they undergo further abiotic or biotic transformations.

The degradation of tetracyclines by individual microorganisms has been reported mostly in fungal species from phyla *Basidiomycota* (classes *Tremellomycetes* and *Agaricomycetes*) and, *Ascomycota* (classes *Sordariomycetes* and *Eurotiomycetes*), and, to a minor extent, in bacterial strains from genera *Stenotrophomonas* and *Sphingobacterium* (Table 5). As far as we are aware, the most extensive transformation within the four-ring nucleus of tetracyclines was described for *Paecilomyces* sp. CMB-MF010, a known producer of a tetracycline-like molecule, and *Fusarium* sp. CMB-MF017 [178]. Both isolates catalyzed a similar reaction by cleaving rings A and B from oxytetracycline and doxycycline, resulting in the accumulation of *hemi*-cyclines (OXY-1, Fig. 10). These products were devoid of antimicrobial activity. It is unclear whether the fungi were able to use tetracyclines partially as a carbon and energy source, and the biodegradability of these metabolites was not further assessed.

Conversely, the bacterial transformation of tetracyclines is quite distinct. This metabolic feat was accidentally discovered [179] while cloning a transposon from the obligate anaerobe *Bacteroides fragilis*. A new resistance gene, named TetX, was shown to inactivate tetracycline enzymatically in transformed *E. coli*, but only when this strain was grown under aerobic conditions. The pathway was subsequently described [180] and TetX was identified as an NADH-dependent flavoprotein that performed O_2-dependent hydroxylation of tetracyclines (TET-3, Fig. 10). This reaction resulted in further abiotic transformation, forming a black pigment thought to be a high molecular weight polymer without antimicrobial activity. The enzyme encoded by TetX was able to transform both natural and semi-synthetic tetracyclines [180]. Nevertheless, due to its O_2 dependency, it did not confer resistance to its original host, *Bacteroides fragilis*. It was only in 2009 that a TetX gene conferring resistance to its host was discovered [181]. The *Sphingobacterium* sp. strain PM2-P1-29 contained a TetX gene and was also flanked by transposon-like elements. It was not possible to prove actual conjugation of this element, but since then, TetX has been detected in samples from oral microbiome [182] and even in human pathogens [183].

Others have recently shown that new tetracycline-modifying enzymes may be discovered using culture-independent approaches [182,184]. Tet37 was discovered by using functional metagenomics with oral microbiome samples from healthy individuals [182]. This enzyme is not a homolog of the previously described TetX, but was also found to catalyze the NADPH-dependent transformation of tetracyclin Furthermore, nine new flavoproteins were discovered (GenBank accession numbers from KR857681–KR857689) while investigating the metagenome of farm and grassland soil as well as the genome of a human pathogen (*Legionella longbeachae*) [184]. These proteins share little amino acid sequence similarity with the original TetX gene (˜20%) and also catalyzed a different reaction from that of the original enzyme

Table 5
Fungi and bacteria able to degrade or transform tetracyclines. N.d. Not determined.

Class	Order	Species	Origin	Antibiotic	Conditions	Identified metabolites	Reference
Tremellomycetes	*Trichosporonales*	*Trichosporon mycotoxinivorans* XPY-10	Wastewater	Tetracycline	Aerobic	Tet-2 to 2.4	[205]
Sordariomycetes	*Hypocreales*	*Trichoderma deliquescens* RA114 *Trichoderma harzianum* RA115	Marine sediment	Oxytetracycline	Aerobic	*N.d.*	[206]
		Fusarium sp. CMB-MF017	Marine environment	Tetracycline Oxytetracycline Minocycline Chlortetracycline Doxycycline	Aerobic	*seco*-cyclines (Tet-1) *hemi*-cyclines (Oxy-1)	[178]
	Xylariales	*Xylaria digitate*	*N.d.*	Tetracycline Demeclocycline Oxytetracycline Chlortetracycline	Aerobic	*N.d.* Possible attack on the B-C moiety	[207]
Eurotiomycetes	*Eurotiales*	*Penicilium crustosum* RA118	Marine sediment	Oxytetracycline	Aerobic	*N.d.*	[206]
		Paecilomyces sp. CMB-MF010	Marine environment	Tetracycline Oxytetracycline Minocycline Chlortetracycline Doxycycline	Aerobic	*seco*-cyclines (Tet-2) *hemi*-cyclines (Oxt-1)	[178]
Agaricomycetes	*Agaricales*	*Pleurotus ostreatus* SMR684	Culture collection	Oxytetracycline	Aerobic	2-acetyl-2-decarboxamidooxytetracycline (Oxy-2)	[208]
Gammaproteobacteria	*Xanthomonadales*	*Stenotrophomonas maltophilia* DT1	Soil	Tetracycline	Aerobic	Cleavage of N-methyl, carbonyl, and amine groups (Tet-5 to 5.4)	[209]
Sphingobacteriia	*Sphingobacteriales*	*Sphingobacterium* sp. PM2-P1-29	Soil	Tetracycline	Aerobic	TET-3	[181,210]

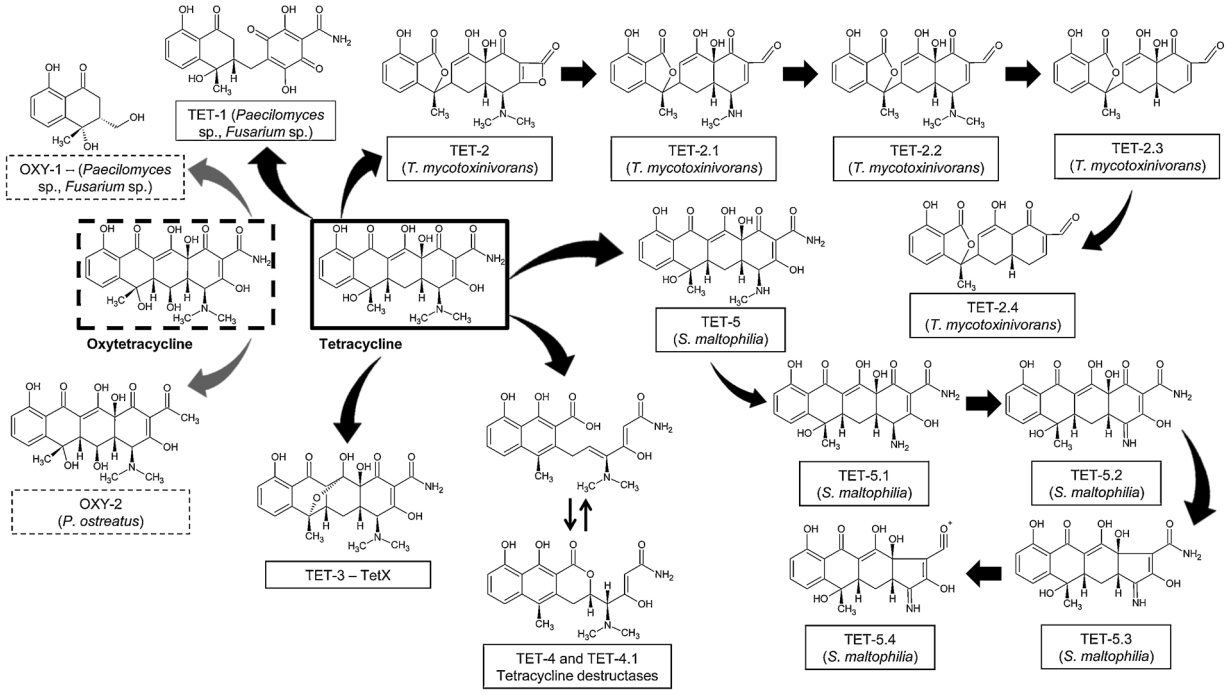


Fig. 10. Main degradation pathways for tetracyclines by fungi, yeast, and bacterial species.

by cleaving the A-ring of tetracycline (TET-4 and -4.1, Fig. 10). Nevertheless, by homology modeling, they were found to be structurally similar to TetX and share its flavin adenine dinucleotide (FAD)-binding and oxidoreductase domains. The low sequence similarity between these structural homologs suggests that they may have arisen from convergent evolution as described for other enzymes [185,186].

Ligninolytic enzymes, namely lignin and manganese peroxidases from white rot fungus *Phanerochaete chrysosporium,* have also been successfully applied to eliminate tetracycline and oxytetracycline in buffer solutions [187,188], but the antibacterial activity and nature of these degradation products have yet to be elucidated. Laccase of *Trametes versicolor* [189] was shown to degrade tetracycline extensively by dehydroxylation, (bi)demethylation and oxidation of the A and C rings. However, no cleavage within the four-ring structure was observed, suggesting that these products might be stable, as shown for other abiotic transformation products.

The impact of these antibiotic degraders was further assessed in [16], which revealed that resistant populations expressing TetX2, an ortholog of TetX, protected sensitive bacteria and allowed them to resume growth after inactivation of the antibiotic. Although no particular cases of treatment failure due to indirect resistance have been reported, some of these enzymes have been recently detected in human pathogens [183,184], strongly suggesting that the use of these strains as biotechnological tools might further aggravate the burden of antibiotic resistance.

Conclusions

Although knowledge on antibiotic degradation and transformation by bacteria and fungi is vast, the underlying metabolic pathways, catabolic enzymes and genes are quite dispersed, and bacteria capable of subsisting on antibiotics appear to be rare. Nonetheless, biodegradation and biotransformation reactions have sometimes been extensively described and can be summarized as follows for the antibiotic classes discussed here:

- **Sulfonamides**: easily degraded, with cleavage of the sulfonamide group by many heterotrophic bacteria isolated from soil and activated sludge. Some bacteria were shown to subsist on these antibiotics by using them as a carbon and energy sources.
- **Trimethoprim**: only partially degraded by AOB and heterotrophic bacteria from NAS.
- **Aminoglycosides:** biotransformation well documented, but to date, only a single bacterial strain from soil has been shown to use streptomycin as a carbon and energy source, although the enzymes involved in this process were not identified.
- **Amphenicols:** many microorganisms were reported to degrade or transform amphenicol antibiotics. Thus far, the degradation of the aromatic moiety has only been described in one species, suggesting that this part of the molecule may be recalcitrant to further degradation.
- **Tetracyclines:** these molecules are degraded primarily by fungi with partial cleavage of the stable four-ring core structure. These degradation mechanisms have not yet been linked to actual mineralization of the molecule or the subsistence phenotype.

Even though many of these results are promising from a biotechnological point of view, the risks of the direct use of these antibiotic degraders for bioremediation and bioaugmentation purposes must be considered. In fact, decades of research on antibiotic resistance have revealed that these phenotypic traits can quickly become fixed in a microbial population by compensatory mutations and co-resistance events [190] resulting in aggravating effects on human health. Despite these concerns, it is regrettable that most studies often focus exclusively on antibiotic disappearance and ignore characterization of the degradation process from a molecular and ecological viewpoint. On the one hand, there are insights into the specific mechanisms of degradation, while on the other hand, the genes involved in these processes are largely unknown, which limits understanding of the role of these degraders in clinical and environmental settings. Enzyme discovery should be intensified by complementing classical approaches with high-throughput approaches, which have proven to be successful e.g. in the

discovery of new tetracycline oxidoreductases [182,184].

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.nbt.2019.08.002.

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