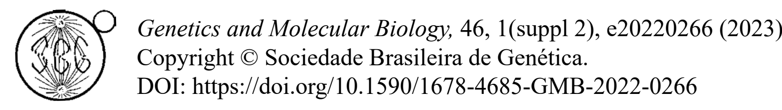
Genetics and Molecular Biology, 46, 1(suppl 2), e20220266 (2023)
Copyright © Sociedade Brasileira de Genética.
DOI: https://doi.org/10.1590/1678-4685-GMB-2022-0266

Review Article
Young Brazilian Geneticists - Special Issue

Intercepting biological messages: Antibacterial molecules targeting nucleic acids during interbacterial conflicts

Julia Takuno Hespanhol[1]*, Lior Karman[1]*, Daniel Enrique Sanchez-Limache[1] and Ethel Bayer-Santos[1]

[1]*Universidade de São Paulo, Instituto de Ciências Biomédicas, Departamento de Microbiologia, São Paulo, SP, Brazil.*

Abstract

Bacteria live in polymicrobial communities and constantly compete for resources. These organisms have evolved an array of antibacterial weapons to inhibit the growth or kill competitors. The arsenal comprises antibiotics, bacteriocins, and contact-dependent effectors that are either secreted in the medium or directly translocated into target cells. During bacterial antagonistic encounters, several cellular components important for life become a weak spot prone to an attack. Nucleic acids and the machinery responsible for their synthesis are well conserved across the tree of life. These molecules are part of the information flow in the central dogma of molecular biology and mediate long- and short-term storage for genetic information. The aim of this review is to summarize the diversity of antibacterial molecules that target nucleic acids during antagonistic interbacterial encounters and discuss their potential to promote the emergence antibiotic resistance.

Keywords: Antibiotics, bacteriocins, effectors, DNase, RNase.

Received: September 21, 2022; Accepted: December 25, 2022.

Introduction

Bacteria live in dense polymicrobial communities constantly competing for resources and use either exploitative competition in which molecules like siderophores can be used to improve the acquisition of micronutrients; or interference competition in which cytotoxic molecules are used to inactive target cells (Granato *et al*., 2019). During evolution, bacteria have evolved a diverse array of weapons to inhibit the growth or kill competitors, which are broadly divided into contact-independent and contact-dependent antagonistic mechanisms (Peterson *et al*., 2020). These weapons specialized for biological conflicts evolved to target many cellular components essential for life, such as the genetic information flow through the central dogma, the cell wall, membranes, and key molecules like NAD^+. As bacteria have been fighting these microscopic battles for millions of years using diverse antimicrobial molecules, it is not a surprise that studies on bacteria preserved in frozen glaciers identified the presence of antibiotic resistance genes that pre-dated human discovery of the first antibiotic (Mindlin and Petrova, 2017). In this review, we will examine molecules such as antibiotics, bacteriocins and effectors produced by bacteria and used during interbacterial conflicts to target DNA and several types of RNAs. We will end by highlighting the underappreciated but important role of these molecules in promoting antimicrobial resistance in natural environments.

Molecules of the central dogma

In molecular biology, the central dogma is an explanation of the flow of genetic information within a biological system. It refers to the information passing from DNA to RNA, and RNA to proteins (Crick, 1970; Morange, 2009). The machinery associated with their synthesis is among the most conserved, and (arguably) important molecules within a living cell. DNA and RNA are polymers of nucleotides, which are composed of a nitrogenous base, a pentose sugar, and a phosphate group (Rich, 1959; Minchin and Lodge, 2019). The bases are either purines (adenine or guanine), or pyrimidines (cytosine and thymine for DNA or uracil for RNA). The nucleotides are connected by phosphodiester bonds between the 5'-phosphate group and the 3'-hydroxyl group, while the bases adenine/thymine (or adenine/uracil for RNA) and guanine/cytosine establish hydrogen bonds (Rich, 1959; Minchin and Lodge, 2019).

DNA replication occurs in a semiconservative manner (Meselson and Stahl, 1958; Hanawalt, 2004). Helicases use energy of ATP hydrolysis to open the double-strand (Abdel-Monem *et al*., 1976; Oakley, 2019), DNA primases synthesizes RNA primers that will be used by DNA polymerases (Scherzinger *et al*., 1977; Oakley, 2019), while topoisomerases help in the unwinding process (Wang, 1971). Preservation of the integrity of the genomic information is fundamental for life and there are many DNA repair mechanisms that can either correct errors originated during replication or fix damages induced by external agents (Schärer, 2003). Damaged nucleotides can be repaired by base excision repair (BER) or nucleotide excision repair (NER) (Uphoff and Sherratt, 2017). BER recognizes abnormal bases in the nucleotides along the DNA molecule, such as uracil that spawn from cytosine deamination. BER includes the hydrolyzation of the abnormal base from the nucleotide, followed by the cleavage of the DNA by endonucleases (Uphoff and Sherratt, 2017). Meanwhile, NER removes an entire nucleotide that causes large distortions in the DNA double-helix, and includes the recognition of the lesion by the enzymes UvrA and UvrB, followed by incision at flanking sites of the distortion by UvrC endonuclease and

Send correspondence to Ethel Bayer-Santos. Universidade de São Paulo, Instituto de Ciências Biomédicas, Departamento de Microbiologia, Av. Prof. Lineu Prestes, 1374, 05508-000, São Paulo, SP, Brazil. E-mail: ebayersantos@usp.br.

* These authors contributed equally to the article.

displacement of the damaged strand by UvrD helicase (Uphoff and Sherratt, 2017). After excision from both BER or NER, DNA polymerase I and DNA ligase resynthesize DNA in the gap (Uphoff and Sherratt, 2017). A double-strand break (DSB) can be repaired by homologous recombination (HR) that preserves the previous genetic information or by non-homologous end-joining (NHEJ), which can lead to the loss or alteration of the original information (Wyman *et al.*, 2004; Shuman and Glickman, 2007).

The information stored in DNA is decoded into RNAs by RNA polymerases (RNAP) (Ebright, 2000). The transcribed RNA could be a transfer RNA (tRNA), a ribosomal RNA (rRNA) or a messenger RNA (mRNA). In bacteria, the 70S ribosome is composed by two subunits: the 30S subunit comprises the 16S rRNA and 21 proteins; while the 50S subunit contains the 23S rRNA, 5S rRNA and 33 proteins (Deutscher, 2009). In several cases, the final step in the expression of the information contained in genes is the synthesis of proteins (Rodnina, 2018), which begins with the association of the ribosome with an mRNA via interaction of the 30S subunit with the Shine-Dalgarno sequence in the mRNA (Shine and Dalgarno, 1974). The elongation follows as the codon in the mRNA is exposed to match the corresponding anti-codon of an aminoacyl-tRNA. The peptidyl transferase center of the ribosome establishes the peptide bond, which is mediated by a catalytic rRNA (Monro, 1967). Overall, fidelity and effectiveness of these steps are required for the maintenance of genetic information and its transfer into molecules that perform work inside living cells.

Bacterial antagonistic mechanisms

Bacteria inhabit complex environments where they interact and compete with other organisms, both prokaryotic and eukaryotic. Several systems specialized in biological conflict, both defensive and offensive, emerged during evolution to combat competitors, predators, and parasites (Figure 1). These systems participate in an arms race in which their genes have a high rate of evolution. Probably the most well-known antibacterial molecules are antibiotics, which are produced by a variety of organisms (Berdy, 2005). Antibiotics are bioactive secondary metabolites not synthesized by ribosomes (Berdy, 2005). They belong to different classes, usually based on their molecular strutures, and target several metabolic processes, including those related to the central dogma (Etebu and Arikekpar, 2016). These molecules are produced and secreted in the extracellular environment by ATP-binding cassette (ABC) transporters (Méndez and Salas, 2001). Producing-bacteria are protected from antibiotics by different mechanisms, including the synthesis of efflux pumps or specific enzymes that degrade/modify the antibiotic or its target (Darby *et al.*, 2022) (Figure 1).

Bacteriocins are another type of biomolecule used in antagonistic encounters that are synthesized by ribosomes and can be divided into colicins and microcins (Cascales *et al.*, 2007). Colicins are larger bacteriocins (>10 kDa) secreted by a diversity of bacteria, and *Escherichia coli* was the first and most extensively studied. Colicins have three domains: an N-terminal translocation domain, a central receptor-binding domain and a toxic C-terminal domain (Cascales *et al.*, 2007). These proteins are released in the medium and are internalized by binding to specific outer membrane receptors. Colicin-producers encode immunity proteins that bind to the toxic domains to neutralize their effect (Cascales *et al.*, 2007). The expression of these proteins is largely regulated by the SOS response to DNA damage (Walker, 1996; Cascales *et al.*, 2007). Microcins consist of smaller polypeptides (<10 kDa)

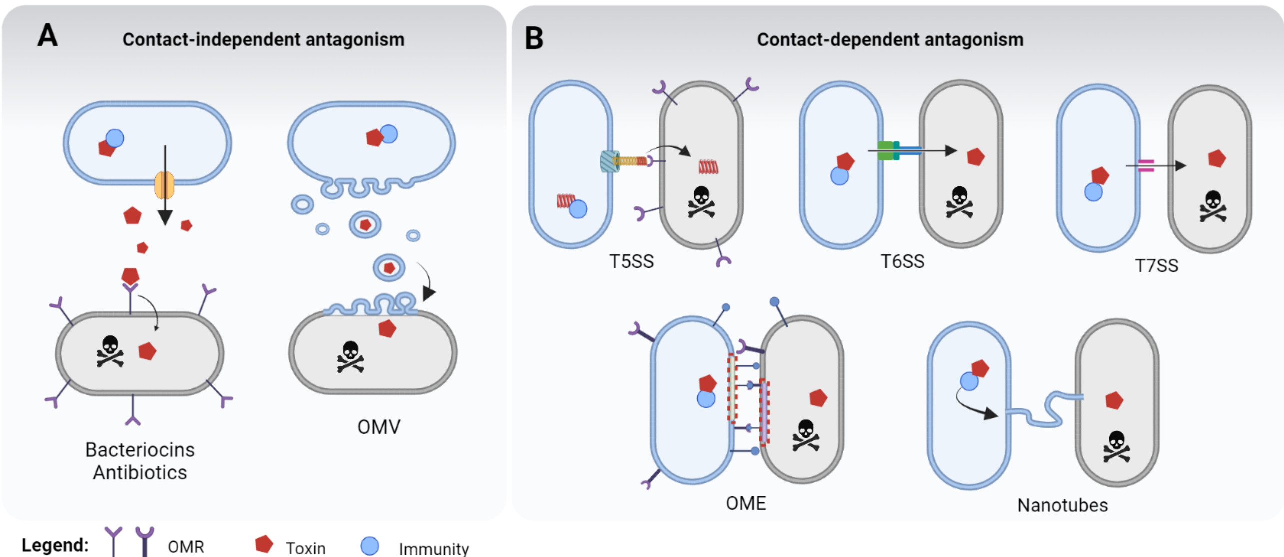

Figure 1 - Antagonistic strategies used by bacteria to counteract competitors. (A) Contact-independent antagonism. Colicins, microcins and antibiotics (red hexagon) reach targets by binding to OMRs (outer membrane receptors) prior to internalization. Autointoxication is prevented by immunity proteins, degrading/modifying proteins or efflux pumps (blue circles). Outer membrane vesicles (OMVs) deliver toxins to competing bacteria by membrane fusion. **(B)** Contact-dependent antagonism. T5SS presents CdiB anchored in cell membrane and CdiA extended. Receptor-binding domain (RBD) of CdiA interacts with OMR of targets to translocate CdiA-CT (red) into competitors. T6SS is anchored in the cell membrane and upon contraction propelled into target cell to deliver toxins (red hexagon). T7SS effectors (red hexagons) secreted into target cells upon contact. Outer membrane exchange (OME) events can transfer toxic proteins (red hexagons) that reach targets. Nanotubes are membrane extensions that connect two bacteria to transport toxins (red hexagons). Cognate immunity proteins produced by attacking bacteria are represented by blue circles. Created with BioRender.com.

that require post-translational modification prior to secretion. Microcins target closely related species via binding to outer membrane receptors, and immunity is conferred either by a specific protein that interacts with the microcin or by efflux pumps (Duquesne *et al*., 2007) (Figure 1).

Many types of macromolecular complexes, named protein secretion systems, are key players in bacterial antagonist interactions (Klein *et al*., 2020). These include the T1SS, T4SS, T5SS and T6SS of Gram-negative bacteria and T7SS of Gram-positives (Figure 1) (Klein *et al*., 2020). The T1SS uses glycine-zipper proteins that form large aggregates in the producer outer membrane and kill target bacteria upon contact (García-Bayona *et al*., 2017). The bacteria killing T4SS apparatus is evolutionarily related to the conjugative machinery and relays on the coupling protein VirD4 for effector selection and translocation into competitors through an extracellular pilus (Souza *et al*., 2015). A subtype of T5SS mediating contact-dependent growth inhibition (CDI) is composed of two proteins, an outer membrane protein CdiB that anchors an exoprotein with a central receptor-binding and a C-terminal toxic domain (CdiA), which interacts with an outer membrane receptor at a target cell to deliver the toxic C-terminus (Aoki *et al*., 2005). The T6SS is a contractile nanomachine evolutionarily related to bacteriophage tails that fire an array of effectors inside target cells at each contraction event (Hood *et al*., 2010; Basler, 2015). The T7SS secretes effectors with an LXG N-terminal and C-terminal toxic domains and participates in bacterial competition in Gram-positives (Cao *et al*., 2016). The vast array of macromolecules specialized in interbacterial conflicts reinforce their importance for bacterial fitness.

The protein complexes described above only mediate the secretion/translocation of the real key players in bacterial antagonism: the toxic molecules used to poison targets cells. In bacteria, there are two main types of toxic molecules: proteinaceous and small molecules (Ruhe *et al*., 2020). Proteinaceous antimicrobials contemplate ribosome-synthesized molecules, such as bacteriocins and effectors (Ruhe *et al*., 2020), while antibiotics are synthetized via the secondary metabolism (Walsh, 2016). Many effector proteins contain multiple domains, usually a conserved N-terminus that engage in protein export that varies according to the secretion system it is associated with (Ruhe *et al*., 2020); and a variable C-terminus that contains the toxic domains (Zhang *et al*., 2012; Ruhe *et al*., 2020). Effectors with this configuration are commonly known as polymorphic toxins (Zhang *et al*., 2012; Ruhe *et al*., 2020). Next, we will discuss these two main types of antibacterial molecules.

Proteinaceous antimicrobials targeting nucleic acids

A large variety of DNase and RNase domains have been predicted by *in silico* analysis of polymorphic toxins (Zhang *et al*., 2012). Most DNase effectors experimentally characterized to date belong to the His-Me finger superfamily (Pfam CL0263) or to the PD-(D/E)xK superfamily (CL0236). On the other hand, RNase effectors are more diverse and belong to the colicin D/E5 (CL0640), Ntox28 (PF15605), EndoU (CL0695) and PD-(D/E)xK (Table 1, Figure 2).

His-Me finger superfamily

The most representative superfamily of DNases is the His-Me finger, also known as HNH superfamily, named after the first characterized enzyme showing the conserved His-Asn-His residues (Wu *et al*., 2020). This superfamily is defined by the compact catalytic conserved ββα-fold, consisting of a β-hairpin followed by an α-helix in which a highly conserved histidine (H) is located at the end of the first β-strand and a metal-binding conserved residue in α-helix (Zn^{2+} or Mg^{2+}) (Wu *et al*., 2020), thus the name His-Me finger. His-Me finger is thought to mediate nonspecific DNA cleavage, with the α-helix fitting into the DNA minor groove, which aligns the β-hairpin with the DNA phosphodiester backbone (Flick *et al*., 1998). For cleavage, the metal ion destabilizes the scissile phosphodiester and neutralize the negatively charged transition state (Maté and Kleanthous, 2004). The conserved H residue then activates a water molecule for a nucleophilic attack on the scissile phosphate to hydrolyze the bond (Yang *et al*., 2011). Even though the amino acid sequences of members of this superfamily are incredible variable, the compact ββα-fold and catalytic mechanism is well conserved (Jablonska *et al*., 2017; Wu *et al*., 2020). This fold is present in all kingdoms of life, and in bacteria the enzymes have variable functions spanning from genome maintenance to host defense and target offense (Wu *et al*., 2020).

All the characterized His-Me finger bacteriocins and effectors described to date that were empirically tested were shown to degrade genomic or plasmid DNA in a nonspecific manner (Table 1, Figure 2). These include several colicins from *E. coli* (Schaller and Nomura, 1976; Males and Stocker, 1980; Cooper and James, 1984; Toba *et al*., 1988; Chak *et al*., 1991, 1996; Garinot-Schneider *et al*., 1996; Pommer *et al*., 1998; Kurazono *et al*., 2000; Hsia *et al*., 2004; Nipič *et al*., 2013; Zaw *et al*., 2013). Other bacteria also encode bacteriocins from the His-Me superfamily, such as *Pseudomonas aeruginosa* (Ohkawa *et al*., 1973; Sano and Kageyama, 1981; Sano *et al*., 1993; Ghequire and De Mot, 2014; Turano *et al*., 2017, 2020), *Klebsiella pneumoniae* (Cooper and James, 1985; James *et al*., 1987; Riley *et al*., 2001) and *Pectobacterium carotovorum* (Roh *et al*., 2010) (Table 1).

Moreover, there are secreted effectors belonging to the His-Me superfamily (Table 1) such as T6SS effectors RhsA (rearrangement hotspot A) and RhsB from *Dickeya dadantii* (Koskiniemi *et al*., 2013). These Rhs effetors were shown to confer competitive advantage to *D. dadantii*, inducing loss of DAPI (4′,6-diamidino-2-phenylindole) staining in target cells and leading to plasmid degradation in overexpressing bacteria (Koskiniemi *et al*., 2013). Other organisms that encode T6SS effectors from the His-Me superfamily are *Vibrio parahaemolyticus* (Salomon *et al*., 2014; Fridman *et al*., 2022), *Serratia marcescens* (Alcoforado-Diniz and Coulthurst, 2015), *Acinetobacter baumannii* (Fitzsimons *et al*., 2018), *E. coli* (Nipič *et al*., 2013; Ma *et al*., 2017), *Aeromonas dhakensis* (Pei *et al*., 2020), and *Pseudomonas spp*. (Hachani *et al*., 2014; Bernal *et al*., 2017; Pissaridou *et al*., 2018; Li *et al*., 2022). In addition, the T7SS effectors EsaD (Ess-associated gene D) from *Staphylococcus aureus* (Cao *et al*., 2016; Ohr *et al*., 2017) and YeeF-CT from *Bacillus subtilis* (Holberger *et al*., 2012; Kaundal *et al*., 2020)

Table 1 – Antibacterial molecules that target nucleic acids.

Name	Activity	Classification (Pfam)		Organism	References
		Superfamily	Family/Clade		
Bacteriocin					
Carocin D	DNase	His-Me_finger (CL0263)	–	*Pectobacterium carotovorum*	Roh *et al*., 2010
Carocin S1	DNase	–	–	*Pectobacterium carotovorum*	Chuang *et al*., 2007
Carocin S3	DNase	–	–	*Pectobacterium carotovorum*	Wang *et al*., 2020
Colicin E2	DNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Escherichia coli W3110*	Schaller and Nomura, 1976
Colicin E7	DNase RNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Escherichia coli K317*	Males and Stocker, 1980; Chak *et al*., 1991; Hsia *et al.,* 2004
Colicin E8	DNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Escherichia coli J*	Cooper and James 1984; Toba *et al*., 1988
Colicin E9	DNase RNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Escherichia coli J*	Cooper and James 1984; Chak *et al.,* 1991; Garinot-Schneider *et al*., 1996; Pommer *et al.,* 1998
Klebicin A	DNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Klebsiella pneumoniae*	Cooper and James, 1985; James *et al*., 1987
Klebicin B	DNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Klebsiella pneumoniae*	Riley *et al*., 2001
Pyocin AP41	DNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Pseudomonas aeruginosa*	Sano and Kageyama, 1981; Sano *et al.,* 1993
Pyocin S1	DNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Pseudomonas aeruginosa*	Sano *et al*., 1993
Pyocin S2	DNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Pseudomonas aeruginosa*	Ohkawa *et al*., 1973; Sano *et al.*, 1993
Pyocin S3	DNase	–	–	*Pseudomonas aeruginosa*	Duport *et al.,* 1995
Pyocin S8	DNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Pseudomonas aeruginosa*	Turano *et al*., 2017; Turano *et al.*, 2020
Pyocin S9	DNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Pseudomonas aeruginosa*	Ghequire and De Mot, 2014
Usp	DNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Escherichia coli*	Kurazono, 2000; Nipic *et al*., 2013; Zaw *et al.,* 2013
Carocin S2	tRNase	Colicin D/E5 (CL0640)	Colicin_D (PF11429)	*Pectobacterium carotovorum*	Chan *et al*., 2011
Colicin E5	tRNase	Colicin D/E5 (CL0640)	Colicin_E5 (PF12106)	*Shigella sonnei 101BM*	Males and Stocker, 1982; Ogawa *et al.,* 1999; Masaki and Ogawa, 2002
Colicin D	tRNase	Colicin D/E5 (CL0640)	Colicin_D (PF11429)	*Escherichia coli K-12 W1485*	Timmis and Hedges, 1972; Tomita *et al*., 2000; Masaki and Ogawa, 2002
Klebicin D	tRNase	Colicin D/E5 (CL0640)	Colicin_D (PF11429)	*Klebsiella pneumoniae*	Chavan *et al*., 2005
Pyocin S4	tRNase	Colicin D/E5 (CL0640)	Colicin_E5 (PF12106)	*Pseudomonas aeruginosa*	Parret and De Mot, 2000
Pyocin S6	rRNase	–	E3 rRNase (PF09000)	*Pseudomonas aeruginosa*	Dingermans *et al*., 2016
Cloacin DF13	rRNase	–	E3 rRNase (PF09000)	*Enterobacter cloacae*	De Graff *et al*., 1973
Colicin E3	rRNase	–	E3 rRNase (PF09000)	*Escherichia coli CA38 Pseudomonas spp.*	Senior and Holland, 1971; Bowman *et al*., 1971; Lasater *et al.,* 1989; Ogawa *et al*., 1999
Colicin E4	rRNase	–	E3 rRNase (PF09000)	*Citrobacter 20-78*	Horak, 1975; Smarda *et al.,* 1988; Smarda *et al.,* 2002; Hirao *et al.,* 2004

Table 1 – Cont.

Name	Activity	Classification (Pfam)		Organism	References
		Superfamily	Family/Clade		
Colicin E6	rRNase	–	E3 rRNase (PF09000)	*Shigella sonnei*	Males and Stocker, 1982; Sharma *et al.,* 2002; Hirao *et al.,* 2004
Klebicin C	rRNase	–	E3 rRNase (PF09000)	*Klebsiella pneumoniae*	Chavan *et al*., 2005
Microcin B17	DNA gyrase	–	–	*Escherichia coli Pseudomonas spp.*	Baquero and Moreno, 1984; Moreno and Baquero 1986; Heddle *et al., 2001*
			T4SS		
Smlt4382	DNAse	His-Me_finger (CL0263)	AHH (PF14412)	*Stenotrophomonas maltophilia*	Bayer-Santos *et al.,* 2019
XAC3266	DNAse	His-Me_finger (CL0263)	AHH (PF14412)	*Xanthomonas citri*	Souza *et al.,* 2015
			T5SS		
CdiA-CT$^{3937\text{-}2}$	DNase	–	–	*Dickeya dadantii*	Aoki *et al*., 2010
CdiA$_2$-CT	DNase	PD-(D/E)XK (CL0236)	Tox-REase 7 (PF15649)	*Acinetobacter baumannii*	Roussin *et al*., 2019
CdiA-CT^{GN05224}	RNase	EndoU (CL0695)	EndoU_bacteria (PF14436)	*Klebsiella aerogenes GN05224*	Michalska et al., 2018
CdiA-CT^{STECO31}	tRNase	EndoU (CL0695)	EndoU_bacteria (PF14436)	*Escherichia coli STEC_O31*	Michalska et al., 2018
CdiA-CT$_{II}^{Bp1026b}$	tRNase	PD-(D/E)XK (CL0236)	CdiA_C (PF18451)	*Burkholderia pseudomallei*	Morse *et al*., 2012
CdiA-CT^{E479}	tRNase	PD-(D/E)XK (CL0236)	CdiA_C_tRNase (PF18664)	*Burkholderia pseudomallei*	Nikolakakis *et al*., 2012
CdiA-CT^{EC869}	tRNase	Colicin D/E5 (CL0640)	–	*Escherichia coli EC869*	Jones *et al*., 2017
CdiA-CT^{EC3006}	tRNase	Colicin D/E5 (CL0640)	Colicin_D (PF11429)	*Escherichia coli EC3006*	Willet *et al*., 2015; Gucinski *et al*., 2019
CdiA-CT^{Kp342}	tRNase	Colicin D/E5 (CL0640)	Colicin_D (PF11429)	*Klebsiella pneumoniae 342*	Gucinski *et al*., 2019
CdiA-CT^{K96243}	tRNase	Colicin D/E5 (CL0640)	Colicin_E5 (PF12106)	*Burkholderia pseudomallei*	Nikolakakis *et al*., 2012
CdiA-CT^{E478}	tRNase	Colicin D/E5 (CL0640)	Colicin_E5 (PF12106)	*Burkholderia pseudomallei*	Nikolakakis *et al*., 2012
CdiA-CT^{UPEC536}	tRNase	–	Ntox28 (PF15605)	*Escherichia coli UPEC536*	Aoki *et al*., 2010; Diner *et al*., 2012
CdiA-CT$_{o1}^{EC93}$	tRNase	–	Ntox28 (PF15605)	*Escherichia coli EC93*	Poole *et al*., 2011
CdiA-CT^{ECL}	rRNase	–	E3 rRNase (PF09000)	*Enterobacter cloacae*	Beck *et al*., 2014
CdiA-CT^{EC16}	rRNase	–	E3 rRNase (PF09000)	*Dickeya chrysanthemi*	Beck *et al*., 2014
CdiA-CT^{49162}	rRNase	–	E3 rRNase (PF09000)	*Enterobacter hormaechei*	Beck *et al*., 2014
CdiA-CT^{0038}	rRNase	–	E3 rRNase (PF09000)	*Pseudomonas viridiflava*	Beck *et al*., 2014
			T6SS		
ET4	DNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Escherichia coli PE086*	Ma *et al*., 2017
Hcp-ET1	DNase	His-Me_finger (CL0263)	HNH (PF01844)	*Escherichia coli STEC004*	Ma *et al*., 2017
RhsA	DNase	His-Me_finger (CL0263)	Endonuclea_NS_2 (PF13930)	*Dickeya dadantii*	Koskiniemi *et al*., 2013
RhsB	DNase	His-Me_finger (CL0263)	HNH (PF01844)	*Dickeya dadantii*	Koskiniemi *et al*., 2013
Rhs2	DNase	His-Me_finger (CL0263)	HNH (PF01844)	*Serratia marcescens*	Alcoforado-Diniz and Coulthurst, 2015

Table 1 – Cont.

Name	Activity	Classification (Pfam)		Organism	References
		Superfamily	Family/Clade		
Rhs2	DNase	His-Me_finger (CL0263)	AHH (PF14412)	*Acinetobacter baumannii*	Fitzsimons *et al*., 2018
TseI	DNase	His-Me_finger (CL0263)	Tox-HNH-EHHH (PF15657)	*Aeromonas dhakensis*	Pei *et al*., 2020
Tse7 (PA0099)	DNase	His-Me_finger (CL0263)	Tox-GHH2 (PF15635)	*Pseudomonas aeruginosa*	Hachani *et al*., 2014; Pissaridou *et al*., 2018
Tke2	DNase RNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Pseudomonas putida*	Bernal *et al*., 2017
Tke4	DNase RNase	His-Me_finger (CL0263)	Tox-SHH (PF15652)	*Pseudomonas putida*	Bernal *et al*., 2017
Txe1	DNase	His-Me_finger (CL0263)	–	*Pseudomonas plecoglossicida*	Li *et al*., 2022
Txe2	DNase	His-Me_finger (CL0263)	AHH (PF14412)	*Pseudomonas plecoglossicida*	Li *et al*., 2022
Txe4	DNase	His-Me_finger (CL0263)	Tox-SHH (PF15652)	*Pseudomonas plecoglossicida*	Li *et al*., 2022
VP1415	DNase	His-Me_finger (CL0263)	AHH (PF14412)	*Vibrio parahaemolyticus*	Salomon *et al*., 2014
Hcp-ET3	DNase	–	–	*Escherichia coli UT189*	Ma *et al*., 2017
VgrG-NucSe1	DNase	His-Me_finger (CL0263)	HNH (PF01844)	*Salmonella arizonae*	Blondel *et al*., 2009; Ho *et al*., 2017
VPA1263	DNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Vibrio parahaemolyticus*	Salomon *et al*., 2014; Fridman *et al*., 2022
PT1	DNase	–	–	*Escherichia marmotae*	Nachmias *et al*., 2022
IdrD	DNase	PD-(D/E)XK (CL0236)	–	*Proteus mirabilis*	Sirias *et al*., 2020
PoNe	DNase	PD-(D/E)XK (CL0236)	–	*Vibrio parahaemolyticus*	Jana *et al*., 2019
RhsB	DNase	PD-(D/E)XK (CL0236)	–	*Acidovorax citrulli*	Pei *et al*., 2022
TseT	DNase	PD-(D/E)XK (CL0236)	Tox-REase-5 (PF15648)	*Pseudomonas aeruginosa*	Burkinshaw *et al*., 2018; Wen *et al*., 2021
TseTBg	DNase RNase	PD-(D/E)XK (CL0236)	Tox-REase-5 (PF15648)	*Burkholderia gladioli*	Yadav *et al*., 2021
TseV	DNase	PD-(D/E)XK (CL0236)	VRR_NUC (PF08774)	*Pseudomonas aeruginosa*	Wang *et al*., 2021
TseV2/TseV3	DNase	PD-(D/E)XK (CL0236)	VRR_NUC (PF08774)	*Salmonella bongori*	Hespanhol *et al*., 2021
Tce1	DNase	–	toxin_43/Ntox15 (PF15604)	*Pseudomonas putida*	Song *et al*., 2021
Tde1/2	DNase	–	toxin_43/Ntox15 (PF15604)	*Agrobacterium tumefaciens*	Ma *et al*., 2014; Bondage *et al*., 2016
SED_RS01930	RNase	–	Ntox47 (PF15540)	*Salmonella enterica* Dublin	Amaya *et al*., 2022
Tre23	ADP-ribosyltranferase	–	Tox-ART-HYD1 (PF15633)	*Photorhabdus laumondii*	Jurenas *et al*., 2021
RhsP2	ADP-ribosyltranferase	–	–	*Pseudomonas aeruginosa*	Bullen *et al*., 2022
DddA	Deamination	Cytidine deaminase-like (CL0109)	DddA-like (PF14428)	*Burkholderia cenocepacia*	Mok *et al*., 2020; Moraes *et al*., 2021
SsdA	Deamination	Cytidine deaminase-like (CL0109)	DYW_deaminase (PF14432)	*Pseudomonas syringae*	Moraes *et al*., 2021
			T7SS		
EsaD/EssD	DNase	His-Me_finger (CL0263)	Endonuclea_NS_2 (PF13930)	*Staphylococcus aureus*	Cao *et al*., 2016; Ohr *et al*., 2016

Table 1 – Cont.

Name	Activity	Classification (Pfam)		Organism	References
		Superfamily	Family/Clade		
YeeF	DNase	His-Me_finger (CL0263)	Endonuclea_NS_2 (PF13930)	*Bacillus subtilis*	Holberger *et al.*, 2012; Kaundal *et al.,* 2020
PT7	DNase	–	–	*Bacillus cereus BAG3X2-1*	Nachmias *et al*., 2022
YobL	rRNase	His-Me_finger (CL0263)	LHH (PF14411)	*Bacillus subtilis*	Holberger *et al*., 2012
YxiD	rRNase	His-Me_finger (CL0263)	–	*Bacillus subtilis*	Holberger *et al*., 2012
YqcG	RNase	His-Me_finger (CL0263)	GH-E (PF14410)	*Bacillus subtilis*	Holberger *et al*., 2012
BC_0920	RNase	EndoU (CL0695)	EndoU_bacteria (PF14436)	*Bacillus cereus*	Holberger *et al*., 2012
OME					
SitA1	DNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Myxococcus xanthus*	Vassallo *et al*., 2017
SitA2	DNase	His-Me_finger (CL0263)	Colicin DNase (PF12639)	*Myxococcus xanthus*	Vassallo *et al*., 2017
SitA3	tRNase	PD-(D/E)XK (CL0236)	CdiA_C (PF18451)	*Myxococcus xanthus*	Vassallo *et al*., 2017
Nanotube					
WapA-CT^{168}	tRNase	unknown	–	*Bacillus subtilis*	Koskiniemi *et al*., 2013
WapA-CT^{natto}	tRNase	unknown	–	*Bacillus subtilis*	Koskiniemi *et al*., 2013
WapA-CT$^{T\text{-}UB\text{-}10}$	tRNase	unknown	–	*Bacillus subtilis*	Koskiniemi *et al*., 2013
WapA-CT ^{PY79}	tRNase	unknown	–	*Bacillus subtilis*	Stempler *et al*., 2017
OMV					
MafB$^{MGI\text{-}1NEM8013}$	RNase	EndoU (CL0695)	EndoU_bacteria (PF14436)	*Neisseria meningitidis*	Jamet *et al*., 2015
Antibiotic					
Bleomycin, Phleomycin, Tallysomycin, Zorbamycin	DNase	Glycopeptides	Bleomycins	*Streptomyces verticillus*	Umezawa *et al*., 1966; Takeshita et al., 1978; Kross *et al*., 1982; Hecht, 2000
Calicheamicin	DNase	–	Enediynes	*Micromonospora echinospora ssp. calichensis*	Zein *et al*., 1988
Daunorubicin	DNase	–	Anthracyclines	*Streptomyces peucetius*	Marco *et al*., 1975
Kibdelomycin	DNA gyrase	–	–	*Kibdelosporangium sp. (MA7385)*	Philips *et al*., 2011
Amycolamicin	DNA gyrase	–	–	*Amycolatopsis sp. (MK575-fF)*	Sawa *et al*., 2012
Coumarin	DNA gyrase	–	–	*Streptomyces spp.*	Maxwell and Lawson, 2003; Oblak *et al.,* 2007
Cyclothialidine	DNA gyrase	–	–	*Streptomyces filipinensis NR0484*	Goetschi *et al*., 1993; Oblak *et al.,* 2007
Rifamycin	RNA polymerase	Macrolides	Ansamycin	*Amycolatopsis rifamycinica*	Sensi *et al*., 1959; Campbell *et al.,* 2001; Floss and Yu, 2005
Fidaxomicin	RNA polymerase	Macrolides	Lipiarmycin	*Dactylosporangium aurantiacum subsp. hamdenensis*	Theriault *et al*, 1987; Artsimovitch *et al*., 2012
Gentamicin, Streptomycin, Hygromycin, Neomycin, Paromomycin, Kanamycin, Spectinomycin, Kasugamycin, Spectinomycin	16S rRNA	Aminoglycoside	–	*Actinomycetes*	Schatz *et al*., 1944; Waksman and Lechevalier, 1949; Umezawa *et al*., 1957; Mann and Bromer, 1958; Mason *et al*., 1961; Weinsten *et al*., 1963; Wilson, 2009

Table 1 – Cont.

Name	Activity	Classification (Pfam)		Organism	References
		Superfamily	Family/Clade		
Tetracycline	16S rRNA	Tetracyclines	–	*Streptomyces aureofaciens*	Putnam *et al.*, 1953; Brodersen *et al.*, 2000; Pioletti *et al.*, 2001
Pactamycin	16S rRNA	–	Aminocyclopentitol	*Streptomyces pactum*	Bhuyan, 1962; Brodersen *et al.*, 2000
Edeine	16S rRNA	–	Edeine	*Brevibacillus brevis*	Kurylo-Borowska, 1959; Pioletti *et al.*, 2001
Erythromycin	23S rRNA	Macrolides	–	*Actinomycetes*	Schlünzen *et al.*, 2001; Reviewed by Vázquez-Laslop and Mankin, 2018;
Lincomycin	23S rRNA	–	Lincosamides	*Streptomyces lincolnensis*	Mason *et al.*, 1962
Blasticidin S	23S rRNA	–	Aminoacyl nucleoside	*Streptomyces griseochromogenes*	Takeuchi *et al.*, 1958; Hansen *et al.*, 2003
Viomycin/ Capreomycin	16S rRNA 23S rRNA	Cyclic peptides	Tuberactinomycins	*Streptomyces puniceus*	Finlay *et al.*, 1951; Herr and Redstone, 1966; Johansen *et al*, 2006

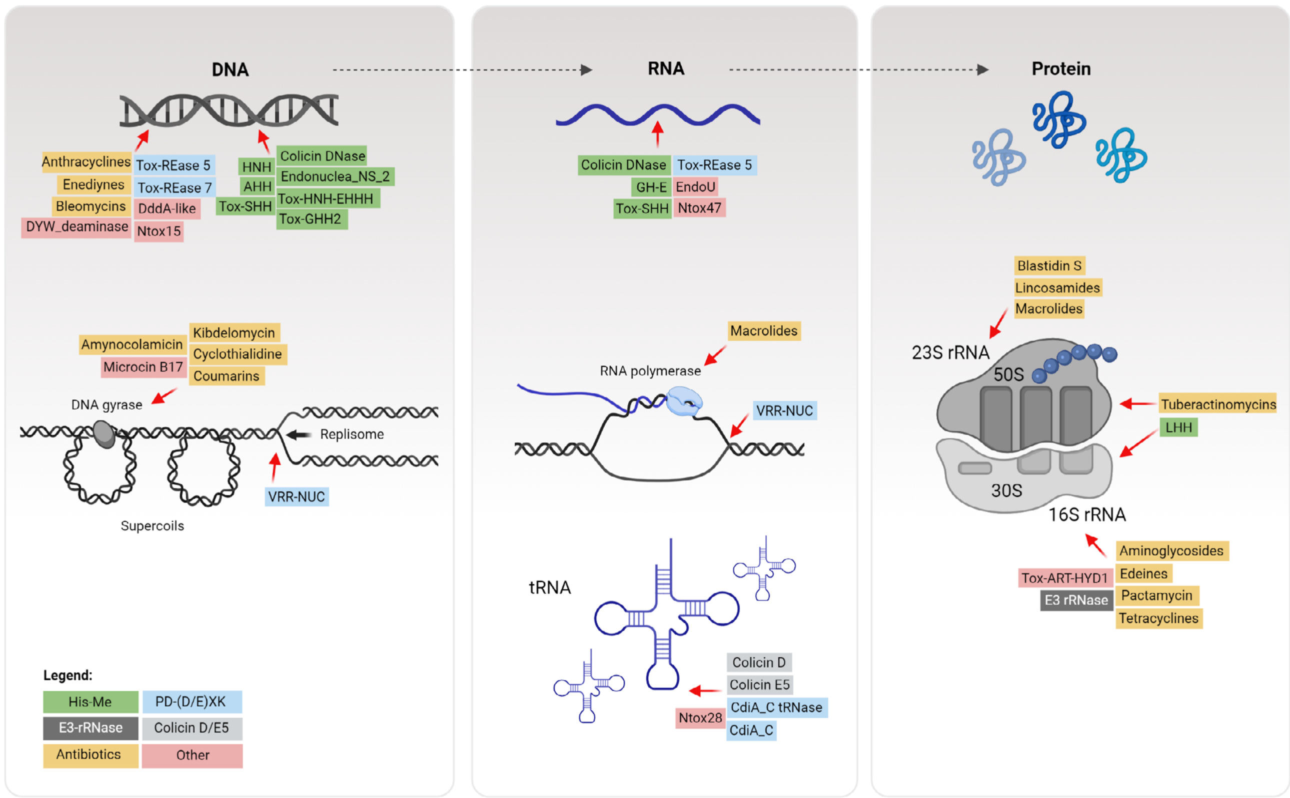

Figure 2 - Antibiotics, bacteriocins, and effectors targeting nucleic acids. Schematic representation of the information flow through the molecules of the central dogma (DNA, RNA and protein). Antibiotics, bacteriocins, and contact-dependent effectors targeting nucleic acids either by binding and inhibition or by enzymatic cleavage are indicated. Molecules were grouped according to their protein domains: His-Me finger (green), PD-(D/E)xK (blue), Colicin D/E5 (light grey), E3-rRNAse (dark grey), antibiotics (orange), others (light red). The complete list of molecules is described in Table 1. Created with BioRender.com.

are representatives from this superfamily. Predicted T4SS effectors also encode nuclease domains, such as Smlt4382 from *Stenotrophomonas maltophilia* (Bayer-Santos *et al.*, 2019) and XAC3266 from *Xanthomonas citri* (Souza *et al.*, 2015), both with AHH domain (PF14412). Besides protein secretion systems, SitA1 and SitA2 toxins from *Myxococcus xanthus* also belong to the His-Me superfamily and are delivered via outer membrane exchange events in which bacteria donate and receive outer membrane material from kin (Vassallo *et al.*, 2017). The extensive list of DNases containing the conserved ββα-fold demonstrate that it is widely distributed weapon used during antagonistic interactions.

Although most toxins belonging to the His-Me finger target DNA, there are a few examples that also target RNA.

Examples include colicin E7, which shows both DNase and RNase activity *in vitro* (Hsia *et al*., 2004), and colicin E9 (Pommer *et al*., 2001). Moreover, there are two effectors that have been shown to exclusively target RNA: YobL and YxiD from the T7SS of *B. subtilis* cleave rRNA *in vivo* (Holberger *et al*., 2012). These demonstrade the versatility of domains with the ββα-fold to cleave different subtrates.

It is worth highlighting that most effectors described above are polymorphic toxins, harboring a translocation N-terminal domain in addition to their toxic C-terminal domains. These include RhsA and RhsB with a N-terminal RHS_repeat (PF05593) and C-terminal Endonuc_NS_2 (PF13930) and HNH domains (PF01844), respectively (Koskiniemi *et al*., 2013); VP1415 with N-terminal PAAR (PF05488) and C-terminal AHH (PF14412) domains (Salomon *et al*., 2014); Hcp-ET1 with N-terminal Hcp (PF05638) and C-terminal HNH domains (Ma *et al*., 2017); SARI_02603 with N-terminal VgrG (PF04717) and C-terminal HNH (PF01844) domains (Blondel *et al*., 2009; Ho *et al*., 2017).

PD-(D/E)xK superfamily

The PD-(D/E)xK superfamily is the second most abundant among bacteriocins and effectors with nuclease activity. Like the His-Me finger, proteins belonging to PD-(D/E)xK share small amino acid sequence similarity, but present conserved secondary structure signatures (Steczkiewicz *et al*., 2012). The conserved fold of this group comprise an α-helix followed by three antiparallel β-strands and a second α-helix followed by a final β-strand (αβββαβ) (Steczkiewicz *et al*., 2012). The catalytic residues are located in the second and third β-strand; the first α-helix has structural role and is related to the formation of the active site, while the second α-helix is involved in substrate binding (Wah *et al*., 1998). Conserved aspartic acid and glutamic acid (D/E) residues coordinate the metal ion (usually Mg^{2+}), while the conserved lysine (K) associates with a water molecule to hydrolyze the phosphodiester bond (Kelly *et al*., 2007). This superfamily includes enzymes related to DNA metabolism (Steczkiewicz *et al*., 2012).

Effectors belonging to the PD-(D/E)xK superfamily degrade both DNA and RNA (Table 1, Figure 2). These include T5SS effectors CdiA-$CT_{II}^{Bp1026b}$ and CdiA-CT^{E479} from *Burkholderia pseudomallei* (Morse *et al*., 2012; Nikolakakis *et al*., 2012), and $CdiA_2$-CT from *A. baumannii* (Roussin *et al*., 2019). Examples of T6SS effectors belonging to the PD-(D/E)xK are TseT from *P. aeruginosa* (Burkinshaw *et al*., 2018; Wen *et al*., 2021), TseTBg from *Burkholderia gladioli* (Yadav *et al*., 2021), PoNe (polymorphic nuclease effector) from *V. parahaemolyticus* (Jana *et al*., 2019), IdrD from *Proteus mirabilis* (Sirias *et al*., 2020), RhsB from *Acidovorax citrulli* (Pei *et al*., 2022), and TseV from *P. aeruginosa* and *Salmonella bongori* (Wang *et al*., 2021; Hespanhol *et al*., 2022). In addition, SitA3 involved in interbacterial antagonism via outer membrane exchange contains the conserved αβββαβ fold (Vassallo *et al*., 2017).

The first PD-(D/E)xK effector was described in *P. aeruginosa* (TseT) and contains a Tox-REase-5 domain (PF15648) (Zhang *et al*., 2012; Burkinshaw *et al*., 2018). Homologs of TseT have been characterized in *B. gladioli* (TseTBg1 and TseTBg2) (Yadav *et al*., 2021), and degrade both DNA and RNA (Yadav *et al*., 2021). Interestingly, the DNase activity of TseTBg was affected by methylation. A DNA methylase (Dam^{BG}) is encoded next to the effector, and plasmids isolated from Dam^{BG}-producing *E. coli* were not degraded by TseTBg1 or TseTBg2 (Yadav *et al*., 2021). In addition, point mutations in conserved aspartic acid (D) and lysine (K) of TseTBg1 and TseTBg2 abrogated DNase activity (Yadav *et al*., 2021). Another curiosity is that these effectors are encoded next to two cognate immunity proteins: one of them neutralizes the enzymatic activity *in vitro* while the second directly binds to the promoter region of the effector, acting as a transcriptional repressor (Yadav *et al*., 2021).

The VRR-Nuc (virus-type replication repair nuclease) domain is found in enzymes involved in interstrand DNA crosslink repair (Kratz *et al*., 2010; Liu *et al*., 2010; MacKay *et al*., 2010; Smogorzewska *et al*., 2010; Gwon *et al*., 2014; Wang *et al*., 2014; Zhao *et al*., 2014), but recent studies identified effectors containing this domain - named TseVs (type VI effector VRR-Nuc) (Wang *et al*., 2021; Hespanhol *et al*., 2022). TseV2 and TseV3 from *S. bongori* were shown to participate in interbacterial competition in a T6SS-dependent manner (Hespanhol *et al*., 2022). TseV3 is a structure-specific nuclease that cleaves DNA substrates with a Y shape (named splayed arm), which resemble replication forks or transcription bubbles (Hespanhol *et al*., 2022). TseV2 and TseV3 induce DNA double-strand breaks and activate the SOS response *in vivo* (Hespanhol et *al*., 2022).

Enzymatic assays also showed the ability of additional PD-(D/E)xK superfamily members to degrade DNA *in vitro*. These include PoNe (Jana *et al*., 2019), RhsB (Pei *et al*., 2022), and IdrD (Sirias *et al*., 2020). Moreover, the T5SS effectors $CdiA_2$-$CT^{Ab30011}$ from *A. baumannii* (Roussin *et al*., 2019) and CdiA-CT^{E479} from *B. pseudomallei* (Nikolakakis *et al*., 2012) were experimentally shown to degrade nucleic acids, leading to cell growth arrest. The first induces target cell DNA damage, while the second is specific to $tRNA^{Arg}$ (Nikolakakis *et al*., 2012; Roussin *et al*., 2019). In summary, similar to the His-Me finger representatives, PD-(D/E)xK members can target both DNA and RNA molecules.

E3-rRNase family

Members of the E3 rRNase family (PF09000) are the most frequent found in bacteriocins and effectors that target ribosomal RNAs (Table 1, Figure 2). Colicin E3 from *E. coli* was the first to be characterized (Bowman *et al*., 1971; Senior and Holland, 1971; Lasater *et al*., 1989; Ogawa *et al*., 1999), hence the name of the group E3-rRNase. Several homologs were later identified, such as colicin E4 and E6 from *E. coli* (Horak, 1975; Males and Stocker, 1982; Šmarda *et al*., 1988; Sharma *et al*., 2002; Hirao *et al*., 2004), cloacin DF13 from *Enterobacter cloacae* (De Graaf *et al*., 1973), pyocin S6 from *P. aeruginosa* (Dingemans *et al*., 2016) and klebicin C from *K. pneumoniae* (Chavan *et al*., 2005). The E3-rRNase domain has a highly specific activity towards the phosphodiester bond between nucleotides $adenine_{1493}$ and $guanine_{1494}$ of the 16S rRNA (Lasater *et al*., 1989). The T5SS effectors CdiA-CT^{ECL} from *E. cloacae* and CdiA-CT^{EC16} from *Erwinia chrysanthemi* contain an E3-rRNase domain and display activity against the

16S rRNA at the same position (Beck *et al*., 2014). CdiA-CT[49162] and CdiA-CT[0038] from *Enterobacter hormaechei* and *Pseudomonas viridiflava* are homologs that contain the E3-rRNase domain; however, their enzymatic activity was not experimentally validated (Beck *et al*., 2014).

Colicin D/E5 superfamily

The first member of the Colicin D/E5 clan (CL0640) was isolated from *E. coli* and named colicin D (Timmis and Hedges, 1972). Later, a second member of this clan was identified in *Shigella sonnei* and called colicin E5 (Males and Stocker, 1982). This protein is homologous to colicin E3 in the receptor-binding and translocation domains but shows a distinct toxic domain (Yajima *et al*., 2006). Both colicin E5 and colicin D were shown to be ribonucleases that target tRNAs and cleave anticodon loops between the 34 and 35 nucleotides of queuine-containing tRNAs, and between the 38 and 39 nucleotides of $tRNAs^{Arg}$, respectively (Ogawa *et al*., 1999; Tomita *et al*., 2000; Masaki and Ogawa, 2002). The catalytic domain found in these colicins were grouped with other metal-independent RNases as part of the BECR-fold (Barnase-EndoU-ColicinD/E5-RelE), which contain a similar structure composed of a α-helix and an anti-parallel β-sheet formed by four strands (Zhang *et al*., 2012). In colicin D, a large positively charged surface promotes tRNA binding and brings the anticodon loop close to a histidine residue located at the α-helix (His_{611}), which carries the catalytic function by acting as a general base (Yajima *et al*., 2004). Colicin E5 possesses a positively charged cleft that promotes RNA docking (Lin *et al*., 2005) and targets $tRNA^{His}$, $tRNA^{Tyr}$, $tRNA^{Asn}$ and $tRNA^{Asp}$ between their modified queuine nucleotide Q34 and U35 (Ogawa *et al*., 1999). The catalytic residues that participate in E5 enzymatic activity do not include a catalytic histidine that usually participate in RNA cleavage (Lin *et al*., 2005; Yajima *et al*., 2006), but instead residues R33 and K25 act as acid-base pairs (Inoue-Ito *et al*., 2012).

Besides colicin D and E5, other bacterial effectors have been described to belong to this clan (Table 1, Figure 2). Pyocin S4 from *P. aeruginosa* (Parret and De Mot, 2000) and klebicin D from *K. pneumoniae* (Chavan *et al*., 2005) have C-terminal domains that belong to the colicin D/E5 superfamily, and carocin S2 from *P. carotovorum* has ribonuclease activity *in vitro* (Chan *et al*., 2011). The CDI system has a variety of effectors that belong to this clan. The CdiA-CT[EC869] and CdiA-CT[EC3006] from *E. coli* are tRNases that have a different cleavage site located at the tRNA acceptor stem (Willett *et al*., 2015; Jones *et al*., 2017; Gucinski *et al*., 2019), the same is observed for CdiA-CT[Kp342] from *K. pneumoniae* (Gucinski *et al*., 2019). CdiA-CT[K96243] and CdiA-CT[E478] from *B. pseudomallei* present the same activity as colicin E5 (Aoki *et al*., 2010; Nikolakakis *et al*., 2012). In summary, members of the colicin D/E5 superfamily target tRNA by cleaving at distinct sites.

EndoU superfamily

EndoU RNases comprise nucleases from eukaryotic and viral RNA-processing enzymes (Zhang *et al*., 2011) and polymorphic bacterial toxins (Zhang *et al*., 2012). As the letter "E" in the BECR fold, EndoU toxins are metal-independent ribonucleases that contain the typical four stranded β-sheet next to a α-helix structure (Zhang *et al*., 2012), and are predicted to have ribonuclease activity carried out by two histidine residues (Zhang *et al*., 2011; Michalska *et al*., 2018). This superfamily has been described to be related to Ribonuclease A (Mushegian *et al*., 2020).

Four EndoU antibacterial toxins were verified experimentally, and the results showed that this fold presents some diversity in its mode of action. The T7SS effector BC_0920 from *Bacillus cereus* has RNase activity (Holberger *et al*., 2012). MafB[MGI-1NEM8013], an outer membrane exported toxin from *Neisseria meningitidis*, is a nonspecific ribonuclease with a preference for urydilates (Jamet *et al*., 2015). CdiA-CT[STECO31], a T5SS secreted toxin from *E. coli* (Michalska *et al*., 2018), presents a specific cleavage site at the anticodon loop of $tRNA^{Glu}$; while CdiA-CT[GN05224] from *Klebsiella aerogenes* shows tRNase activity *in vivo* (Michalska *et al*., 2018).

Even though bioinformatic analysis can broadly predict protein function, the precise mode of action of each nuclease within a superfamily requires empirical biochemical assays to accurately determine activity.

Other nuclease domains

Besides the nuclease groups mentioned above, other domains can be found in bacteriocins and effectors. Tde1 and Tde2 (type VI DNase effectors) from *Agrobacterium tumefaciens* have a Ntox15 domain (Zhang *et al*., 2012; Bondage *et al*., 2016), which is a polymorphic toxic domain characterized by an all α-helical fold and conserved HxxD catalytic residues (Zhang *et al*., 2012). Both effectors display DNase activity (Bondage *et al*., 2016). Several WapA proteins from *B. subtilis* display tRNAse activity, such as WapA-CT[168], WapA-CT[natto] and WapA-CT[T-UB-10]; however, the toxic domains remain undetermined (Koskiniemi *et al*., 2013). In addition, Wap-CT[PY79] was hypothesized to display tRNAse activity based on sequence similarity (Stempler *et al*., 2017).

A recently discovered effector with no detectable domain and DNase activity is Tce1 (T6SS contact-independent antibacterial effector 1) from *Yersinia pseudotuberculosis* (Song *et al*., 2021). Tce1 is a Ca^{2+}- and Mg^{2+}-dependent enzyme that displays an interesting mechanism of target-cell delivery, which can be either dependent or independent of contact (via the outer membrane receptors BtuB and OmpF) (Song *et al*., 2021).

Also recently, new polymorfic toxin C-teminal domains (PTs) were described (Nachmias *et al*., 2022). The toxic domains of PT1 and PT7 were shown to be non-specific DNases that did not show sequence or structural similarity to any known nuclease (Nachmias *et al*., 2022). PT1 is likely secreted by the T6SS, while PT7 is probably secreted via the T7SS (Nachmias *et al*., 2022).

Other toxins with undetectable domains but with experimentally characterized nuclease activities comprise carocin S1 and S3 from *P. carotovorum* (Chuang *et al*., 2007; Wang *et al*., 2020), pyocin S3 from *P. aeruginosa* (Duport *et al*., 1995), and the T6SS effector Hcp-ET3 from *E. coli* (Ma *et al*., 2017). The characterization of these and other new toxic domains is an interesting source of information to the discovery of novel enzymatic activities.

Deaminases

Deaminases are enzymes that induce the deamination of nucleotides and are related to salvage pathways of purines and pyrimidines (Nygaard, 1993). Several deaminase domains have been predicted in polymorphic toxins (Iyer *et al*., 2011; Zhang *et al*., 2012). The first characterized T6SS deaminase effector was DddA (dsDNA deaminase toxin A) from *Burkholderia cenocepacia* (Mok *et al*., 2020). DddA promotes deamination of cytosine and its conversion to uracil in dsDNA, leading to a DNA mismatch during replication that needs to be repaired by the base excision repair (BER) pathway (Uphoff and Sherratt, 2017; de Moraes *et al*., 2021). An example of deaminases targeting ssDNA is the T6SS effector SsdA (ssDNA deaminase toxin A) from *Pseudomonas syringae*, which deaminases cytosine into uracil (de Moraes *et al*., 2021). Sublethal doses of DddA are related to an increase in the frequency of mutations, with a preference for C/G to A/T substitutions (Mok *et al*., 2020; de Moraes *et al*., 2021). The action of these mutagenic effectors can promote antibiotic resistance in natural settings (de Moraes *et al*., 2021).

ADP-ribosyltransferases

ADP-ribosyltranferases (ARTs) are enzymes able of transferring an ADP-ribose from the cofactor β-nicotinamide adenine dinucleotide (NAD^+) into certain targets, which could be either amino acids or nucleotides (Mikolčević *et al*., 2021). In bacteria, many ARTs are virulence factors involved in pathogenesis that modify specific host cell proteins to manipulate cellular functions (Yoshida and Tsuge, 2021). These ARTs can be classified into two families: diphtheria toxin (DTX) with the conserved residues H-Y-E, and cholera toxin (CTX) with the conserved residues R-S-E (Mikolčević *et al*., 2021).

Among the weapons used in interbacterial antagonism, Tre23 (type VI secretion ADP-ribosyltranferase effector 23) from *Photorhabdus laumondii* is an ART from the H-Y-E clade that transfers ADP-ribose to 23S rRNA (Jurėnas *et al*., 2021). This modification occurs at the 23S rRNA GTPase-associated site of the ribosome, which is necessary for elongation during translation, thus stopping protein synthesis (Jurėnas *et al*., 2021) (Figure 2). Another RNA modifying toxin is RhsP2 from *P. aeruginosa* (Bullen *et al*., 2022). Interestingly, this enzyme displays the conserved residues Y-E and E from the two DTX and CTX ART families (Bullen *et al*., 2022). RhsP2 ADP-ribosylates a series of non-coding RNAs in target cells, including 4.5S rRNA, 6S rRNA, tRNAs, hindering multiple essential pathways (Bullen *et al*., 2022). Thus, ART toxins provide another layer of antagonistic strategies that bacteria use to interfere with molecules of the central dogma.

Antibacterial small molecules targeting nucleic acids

Bacteria produce several classes of antibiotics that target nucleic acids, such as aminoglycosides, tetracyclines and macrolides (Table 1, Figure 2). The structural diversity of these molecules provides distinct opportunities for inhibition of the information flow thought the central dogma. Some antibiotics can induce DNA cleavage, inhibit DNA gyrases/ topoisomerases or RNA polymerases, or bind to ribosomal RNAs to interfere with protein synthesis.

Among the antibiotics that induce DNA cleavage there are bleomycins, calicheamicin and daunorubicin. The bleomycin group comprises bleomycins, phleomycins, tallysomycin and zorbamycins (Hecht, 2000). Bleomycins are glycopeptides first isolated from *Streptomyces verticillus* (Umezawa *et al*., 1966) that promote oxidative cleavage of double-strand DNA in a sequence-specific manner (Takeshita *et al*., 1978; Kross *et al*., 1982). These antibiotics rely on the presence of molecular oxygen and a redox active metal like Fe^{2+} or Cu^+ (Burger *et al*., 1981; Hecht, 2000). Bleomycins are composed of four functional domains: metal-binding, DNA-binding, linker region connecting the two previous domains, and a disaccharide moiety that promotes cell selectivity (Boger and Cai, 1999). The metal-binding domain is responsible for the specificity of DNA sequence (Sugiyama *et al*., 1986), which consists mainly of GT dinucleotides but can also be GC and AT (Kross *et al*., 1982). Phleomycins, tallysomycins and zorbamycins have slightly different sequence specificity but cleave DNA in a similar mechanism (Kross *et al*., 1982). Calicheamicin belongs to the enediynes group of antibiotics and was first isolated from *Micromonospora echinospora ssp. calichensis* (Zein *et al*., 1988). It promotes double-strand DNA cleavage in a sequence-specific manner, preferentially at AGGA, TCCT and ACCT (Zein *et al*., 1988). The mechanism of cleavage requires the removal of hydrogen atoms (abstraction) from the DNA backbone (Lee *et al*., 1991). Daunomycin from *Streptomyces peucetius* can intercalate and form complexes with DNA, leading to chromosome fragmentation (Marco *et al*., 1975).

Some antibiotics promote DNA degradation by arresting topoisomerases. Type II topoisomerases function by promoting metal-dependent DNA double-strand breaks, followed by ATP-dependent translocation of DNA segments and rejoining the separated DNA ends (Gentry and Osheroff, 2013). The DNA gyrase and topoisomerase IV (topo IV) are type II topoisomerases found in bacteria and are composed of two domains: GyrA and GyrB, and ParC and ParE, respectively (Levine *et al*., 1998). The GyrA or ParC domains interact with DNA, while GyrB or ParE bind and hydrolyze the ATP necessary for enzymatic function (Levine *et al*., 1998). Some groups of antibiotics bind to the ATP-binding site of GyrB and ParE to inhibit the activity of the topoisomerase complex, thus generating DNA breaks and the collapse of the replication fork (Anderson *et al*., 2000; Maxwell and Lawson, 2003). These antibiotics comprise coumarins and cyclothialidines from *Streptomyces spp*. (Goetschi *et al*., 1993; Oblak *et al*., 2007), kibdelomycin from *Kibdelosporangium sp*. (Phillips *et al*., 2011), and amycolamicin from *Amycolatopsis sp*. (Sawa *et al*., 2012).

Transcription is another seductive target for antibacterial natural products. Rifamycin from *Amycolatopsis rifamycinica* (Sensi, 1959) is a macrolide antibiotic that blocks transcription by binding to the β-subunit of the RNA polymerase, thus stopping DNA-dependent RNA synthesis via transcript elongation arrest (Campbell *et al*., 2001; Floss and Yu, 2005).

Fidaxomicin isolated from *Dactylosporangium aurantiacum* (Theriault *et al*., 1987) prevents RNA transcription by blocking DNA double-strand opening in promotor regions, thus inhibiting transcription initiation by the RNA polymerase (Artsimovitch *et al*., 2012).

The ribosome is the center of protein synthesis. It is a large ribonucleoprotein complex composed of two subunits (30S and 50S) forming the 70S bacterial ribosome. The 30S subunit contain the 16S rRNA, while the 50S subunit contain the 23S rRNA and 5S rRNA (Deutscher, 2009). These nanomachines are one of the favorite targets when it comes to bacterial growth inhibition by antibiotics. Most of these antibacterial molecules inhibit ribosome activity by binding directly to the rRNAs and arresting translation by acting as allosteric inhibitors. Here we focused only on antibiotics produced by bacteria that interfere with protein synthesis by binding to rRNAs.

The 30S ribosomal subunit is the target of aminoglycosides, tetracyclines, pactamycin and edeine, which bind at different sites of the 16S rRNA. Aminoglycosides gentamicin from *Micromonospora spp*. (Weinstein *et al*., 1963), hygromycin B from *Streptomyces hygroscopicus* (Mann and Bromer, 1958), neomycin from *Streptomyces fradiae* (Waksman and Lechevalier, 1949), paromomycin from *Streptomyces krestomuceticus*, kanamycin from *Streptomyces kanamyceticus* (Umezawa *et al*., 1957) and streptomycin from *Streptomyces griseus* (Schatz *et al*., 1944) can target the helix 44 of 16S rRNA (Wilson, 2009). Meanwhile, aminoglycoside spectinomycin from *Streptomyces spectabilis* (Mason *et al*., 1961) targets the helix 34 of 16S rRNA (Wilson, 2009). Lastly, aminoglycoside kasugamycin from *Streptomyces kasugaensis* (Umezawa *et al*., 1965) binds to 16S rRNA at the messenger RNA channel (Schuwirth *et al*., 2006). Tetracycline from *Streptomyces aureofaciens* (Putnam *et al*., 1953) binds to helixes 31 and 34 (Brodersen *et al*., 2000; Pioletti *et al*., 2001). Pactamycins from *Streptomyces pactum* (Bhuyan, 1962) binds at the central domain of 16S rRNA (Brodersen *et al*., 2000), while edeine from *Brevibacillus brevis* (Kurylo-borowska, 1959) binds to helixes 44 and 45 (Pioletti *et al*., 2001).

The 50S subunit is also widely affected by antibiotics. Erythromycin, lincomyicin, blasticidin, viomycin and capreomycin target the 23S rRNA. Antibiotics from the macrolide class are produced by diverse Actinomycetes (Dinos, 2017) and can bind to the 23S rRNA at the nascent peptide exit tunnel (Schlünzen *et al*., 2001; Vázquez-Laslop and Mankin, 2018). Lincomycin from *Streptomyces lincolnensis* (Mason *et al*., 1962) binds to the peptidyl transferase cavity at the ribosomal A site (Douthwaite, 1992). Blasticidin S from *Streptomyces griseochromogenes* (Takeuchi *et al*., 1958) and sparsomycin from *Streptomyces sparsogenes* (Owen *et al*., 1962) bind to the 23S rRNA at the ribosomal P site (Johnston *et al*., 2002; Hansen *et al*., 2003). Tuberactinomycins, such as capreomycin from *Streptomyces capreolus* (Herr Jr and Redstone, 1966) and viomycin from *Streptomyces puniceus* (Finlay *et al*., 1951), can interact with both 30S and 50S ribosomal subunits by binding to 16S rRNA at helix 44 and to 23S rRNA at helix 69 (Johansen *et al*., 2006). In summary, antibiotics collectively work in several steps to prevent the information flow through the central dogma.

Contribution to the development of antibiotic resistance

During the evolutionary arms race in which bacteria developed several weapons to inactivate or kill competitors, immunity mechanisms to prevent self-intoxication and protect sister-cells evolved concomitantly. For proteinaceous antibacterial molecules like effectors and bacteriocins, the expression of a specific immunity protein is usually the most common mechanism of defense (Zhang *et al*., 2012; Ruhe *et al*., 2020). For small molecules like antibiotics, there are several mechanisms that could render a cell resistant: (1) target modification by specific enzymes; (2) target bypass via mutations in the targets that lead to reduced affinity; (3) degrading or modifying proteins that act on the molecules; (4) reduced intake via altered membrane permeability; (5) efflux pumps that export the molecules (Darby *et al*., 2022).

During interbacterial competitions, effectors and bacteriocins that target the DNA contribute to the emergence of antibiotic resistance by increasing the rate of mutagenesis in cells that receive a sublethal dose. The deaminase T6SS effector DddA has been shown to increase the rate of C/G to T/A mutation, leading to emergence of rifamycin resistance by introducing point mutations in the *rpoB* gene, which encodes the β-subunit of RNA polymerase (de Moraes *et al*., 2021). In addition, cleavage of the 16S rRNA by colicin E3 promotes faster tRNA-mRNA translocation in ribosomes, thus making it less sensitive to inhibition by the antibiotic viomycin (Lancaster *et al*., 2008).

In general, DNA damage induced by bacteriocins or effectors activate the SOS response, which can induce the activation of the translesion DNA repair pathway and promote mutations (Patel *et al*., 2010). The mutagenesis can also be responsible for altering gene expression or characteristics of membrane channels important for antibiotic internalization (Livermore, 1990). Mutations in the promoter region of OmpF (outer membrane protein F) leads to its downregulation, thus conferring β-lactam resistance in *E. coli* (Delcour, 2009). Similarly, point mutations in OmpF in *Enterobacter aerogenes* reduce outer membrane permeability and promote resistance to β-lactam antibiotics, which act by inhibiting peptidoglycan synthesis (Dé *et al*., 2001).

In addition to contributing to an increase in the mutation rate of target cells, antibacterial molecules (e.g., lipases and peptidoglycan hydrolases) can promote the lysis of target cells and the release of extracellular DNA, which could be uptaken by the attacker bacterium and incorporated into its genome, thus stimulating horizontal gene transfer and the spread of genes encoding antibiotic resistance. Examples of this include the T6SSs of *Vibrio cholerae* and *Acinetobacter baylyi* (Borgeaud *et al*., 2015; Cooper *et al*., 2017; Ringel *et al*., 2017). Curiously, *V. cholerae* have its T6SS gene cluster under the control of competence regulators (Borgeaud *et al*., 2015), demonstrating the relationship between the bacterial competition and horizontal gene transfer events.

Perspectives

Nucleases are possibly the most ancient biological weapons and likely used in periods prior to the development of individual cells surrounded by membranes. Their activities

are among the chemical armaments used in biological conflicts across all organizational levels. For example, endonuclease domains of the His-Me superfamily are found in nucleic acid–degrading snake toxins, bacterial polymorphic toxins, bacterial restriction-modification systems conferring antiviral immunity, and eukaryotic apoptosis systems (Zhang *et al*., 2012; Trummal *et al*., 2014; Jablonska *et al*., 2017). There is still a wide array of predicted nucleic acids-targeting enzymes that require further empiral characterization. While it is possible to extropolate the possible activities of predicted groups based on similarities to known enzymes, such as Ntox18, Ntox19, Ntox22 and Ntox30 that are expected to be metal-independent RNases (Zhang *et al*., 2012), there are Ntox groups for which the nature of catalysis could not be predicted (Zhang *et al*., 2012).

The large number of antibacterial molecules targeting the central dogma and the number of resistance mechanisms promoting immunity to these molecules, call our attention to the fact that antibiotic resistance is an ancient and naturally occurring phenomenon widespread in the environment. It is important to note that these molecules attacking the central dogma act as part of a miscellaneous arsenal of toxins that damage other cellular components and their combined effect dictates the aftermath of antagonistic interactions. Experimental data confirmed that antibiotic resistance can arise solely by competitive interactions between bacteria without previous antibiotic exposure (Koch *et al*., 2014). Bacteria joined an arms race millions of years prior to the discovery of antibiotics and studying the mechanisms and outcomes of antagonistic interaction might help us anticipate the emergence of antibiotic resistance in different settings.

Acknowledgements

This work was supported by Sao Paulo Research Foundation grant 2017/02178-2 to E.B.-S, and FAPESP fellowships to J.T.H (2022/01364-5), L.K. (2022/01444-9), D.E.S.-L. (2019/22715-8) and E.B.-S. (2018/04553-8). J.T.H. and D.E.S.-L. were supported by the CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

EB-S conceptualized the study; JTH, LK, DESL and EB-S conducted the literature revision; JTH, LK, DESL and EB-S wrote the manuscript; JTH, LK and EB-S elaborated figures. All authors read and approved the final version.

References

Abdel-Monem M, Dürwald H and Hoofman-Berling H (1976) Enzymic unwinding of DNA: 2. Chain Separation by an ATP-Dependent DNA unwinding enzyme. Eur J Biochem 65:441-449.

Alcoforado-Diniz J, Coulthurst SJ (2015) Intraspecies competition in *Serratia marcescens* is mediated by type VI-secreted Rhs effectors and a conserved effector-associated accessory protein. J Bacteriol 197:2350-2360.

Anderson VE, Zaniewski RP, Kaczmarek FS, Gootz TD and Osheroff N (2000) Action of quinolones against *Staphylococcus aureus* topoisomerase IV: basis for DNA cleavage enhancement. Biochemistry 39:2726-2732.

Aoki SK, Pamma R, Hernday AD, Bickham JE, Braaten BA and Low DA (2005) Contact-dependent inhibition of growth in *Escherichia coli*. Science 309:1245-1248.

Aoki SK, Diner EJ, de Roodenbeke CtK, Burgess BR, Poole SJ, Braaten BA, Jones AM, Webb JS, Hayes CS and Cotter PA (2010) A widespread family of polymorphic contact-dependent toxin delivery systems in bacteria. Nature 468:439-442.

Artsimovitch I, Seddon J and Sears P (2012) Fidaxomicin is an inhibitor of the initiation of bacterial RNA synthesis. Clin Infect Dis 55:S127-S131.

Basler M (2015) Type VI secretion system: secretion by a contractile nanomachine. Philos Trans R Soc Lond B Biol Sci 370:20150021.

Bayer-Santos E, Cenens W, Matsuyama BY, Oka GU, Di Sessa G, Mininel IDV, Alves TL and Farah CS (2019) The opportunistic pathogen *Stenotrophomonas maltophilia* utilizes a type IV secretion system for interbacterial killing. PLoS Pathogens 15:e1007651.

Beck CM, Morse RP, Cunningham DA, Iniguez A, Low DA, Goulding CW and Hayes CS (2014) CdiA from *Enterobacter cloacae* delivers a toxic ribosomal RNase into target bacteria. Structure 22:707-718.

Berdy J (2005) Bioactive microbial metabolites. J Antibiot (Tokyo) 58:1-26.

Bernal P, Allsopp LP, Filloux A and Llamas MA (2017) The *Pseudomonas putida* T6SS is a plant warden against phytopathogens. ISME J 11:972-987.

Bhuyan B (1962) Pactamycin production by *Streptomyces pactum*. Appl Microbiol 10:302-304.

Blondel CJ, Jiménez JC, Contreras I and Santiviago CA (2009) Comparative genomic analysis uncovers 3 novel loci encoding type six secretion systems differentially distributed in *Salmonella* serotypes. BMC Genomics 10:354.

Boger DL and Cai H (1999) Bleomycin: Synthetic and mechanistic studies. Angew Chem Int Ed 38: 448-476.

Bondage DD, Lin J-S, Ma L-S, Kuo C-H and Lai E-M (2016) VgrG C terminus confers the type VI effector transport specificity and is required for binding with PAAR and adaptor–effector complex. Proc Natl Acad Sci U S A 113:E3931-E3940.

Borgeaud S, Metzger LC, Scrignari T and Blokesch M (2015) The type VI secretion system of *Vibrio cholerae* fosters horizontal gene transfer. Science 347:63-67.

Bowman C, Dahlberg J, Ikemura T, Konisky J and Nomura M (1971) Specific inactivation of 16S ribosomal RNA induced by colicin E3 *in vivo*. Proc Natl Acad Sci U S A 68:964-968.

Brodersen DE, Clemons Jr WM, Carter AP, Morgan-Warren RJ, Wimberly BT and Ramakrishnan V (2000) The structural basis for the action of the antibiotics tetracycline, pactamycin, and hygromycin B on the 30S ribosomal subunit. Cell 103:1143-1154.

Bullen NP, Sychantha D, Thang SS, Culviner PH, Rudzite M, Ahmad S, Shah VS, Filloux A, Prehna G and Whitney JC (2022) An ADP-ribosyltransferase toxin kills bacterial cells by modifying structured non-coding RNAs. Mol Cell 82:3484-3498.e3411.

Burger RM, Peisach J and Horwitz SB (1981) Activated bleomycin. A transient complex of drug, iron, and oxygen that degrades DNA. J Biol Chem 256:11636-11644.

Burkinshaw BJ, Liang X, Wong M, Le AN, Lam L and Dong TG (2018) A type VI secretion system effector delivery mechanism dependent on PAAR and a chaperone–co-chaperone complex. Nat Microbiol 3:632-640.

Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A and Darst SA (2001) Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell 104:901-912.

Cao Z, Casabona MG, Kneuper H, Chalmers JD and Palmer T (2016) The type VII secretion system of *Staphylococcus aureus* secretes a nuclease toxin that targets competitor bacteria. Nat Microbiol 2:16183.

Cascales E, Buchanan SK, Duché D, Kleanthous C, Lloubes R, Postle K, Riley M, Slatin S and Cavard D (2007) Colicin biology. Microbiol Mol Biol Rev 71:158-229.

Chak KF, Kuo W-S and James R (1991) Cloning and characterization of the ColE7 plasmid. Microbiology 137:91-100.

Chak KF, Safo MK, Ku W-Y, Hsieh S-Y and Yuan HS (1996) The crystal structure of the immunity protein of colicin E7 suggests a possible colicin-interacting surface. Proc Natl Acad Sci U S A 93:6437-6442.

Chan Y-C, Wu J-L, Wu H-P, Tzeng K-C and Chuang D-Y (2011) Cloning, purification, and functional characterization of Carocin S2, a ribonuclease bacteriocin produced by *Pectobacterium carotovorum*. BMC Microbiol 11:99.

Chavan M, Rafi H, Wertz J, Goldstone C and Riley MA (2005) Phage associated bacteriocins reveal a novel mechanism for bacteriocin diversification in *Klebsiella*. J Mol Evol 60:546-556.

Chuang D-y, Chien Y-c and Wu H-P (2007) Cloning and expression of the *Erwinia carotovora* subsp. *carotovora* gene encoding the low-molecular-weight bacteriocin carocin S1. J Bacteriol 189:620-626.

Cooper PC and James R (1984) Two new E colicins, E8 and E9, produced by a strain of *Escherichia coli*. Microbiology 130:209-215.

Cooper PC and James R (1985) Three immunity types of klebicins which use the cloacin DF13 receptor of *Klebsiella pneumoniae*. Microbiology 131:2313-2318.

Cooper RM, Tsimring L and Hasty J (2017) Inter-species population dynamics enhance microbial horizontal gene transfer and spread of antibiotic resistance. Elife 6:e25950.

Crick F (1970) Central dogma of molecular biology. Nature 227:561-563.

Darby EM, Trampari E, Siasat P, Gaya MS, Alav I, Webber MA and Blair J (2022) Molecular mechanisms of antibiotic resistance revisited. Nat Rev Microbiol. DOI: 10.1038/s41579-022-00820-y

Dé E, Baslé A, Jaquinod M, Saint N, Malléa M, Molle G and Pagès JM (2001) A new mechanism of antibiotic resistance in Enterobacteriaceae induced by a structural modification of the major porin. Mol Microbiol 41:189-198.

De Graaf F, Niekus H and Klootwijk J (1973) Inactivation of bacterial ribosome in vivo and in vitro by cloacin DF13. FEBS Lett 35:161-165.

de Moraes MH, Hsu F, Huang D, Bosch DE, Zeng J, Radey MC, Simon N, Ledvina HE, Frick JP and Wiggins PA (2021) An interbacterial DNA deaminase toxin directly mutagenizes surviving target populations. Elife 10:e62967.

Delcour AH (2009) Outer membrane permeability and antibiotic resistance. Biochim Biophys Acta Proteins Proteom 1794:808-816.

Deutscher MP (2009) Maturation and degradation of ribosomal RNA in bacteria. Prog Mol Biol Transl Sci 85:369-391.

Dingemans J, Ghequire MG, Craggs M, De Mot R and Cornelis P (2016) Identification and functional analysis of a bacteriocin, pyocin S6, with ribonuclease activity from a *Pseudomonas aeruginosa* cystic fibrosis clinical isolate. Microbiologyopen 5:413-423.

Dinos GP (2017) The macrolide antibiotic renaissance. Br J Pharmacol 174:2967-2983.

Douthwaite S (1992) Functional interactions within 23S rRNA involving the peptidyltransferase center. J Bacteriol 174:1333-1338.

Duport C, Baysse C and Michel-Briand Y (1995) Molecular characterization of pyocin S3, a novel S-type pyocin from *Pseudomonas aeruginosa*. J Biol Chem 270:8920-8927.

Duquesne S, Destoumieux-Garzón D, Peduzzi J and Rebuffat S (2007) Microcins, gene-encoded antibacterial peptides from enterobacteria. Nat Prod Rep 24:708-734.

Ebright RH (2000) RNA polymerase: structural similarities between bacterial RNA polymerase and eukaryotic RNA polymerase II. J Mol Biol 304:687-698.

Etebu E and Arikekpar I (2016) Antibiotics: classification and mechanisms of action with emphasis on molecular perspectives. Int J Appl Microbiol Biotechnol Res 4:90-101.

Finlay A, Hobby G, Hochstein F, Lees T, Lenert T, Means J, P'an S, Regna P, Routien J and Sobin B (1951) Viomycin, a new antibiotic active against mycobacteria. Am Rev Tuberc 63:1-3.

Fitzsimons TC, Lewis JM, Wright A, Kleifeld O, Schittenhelm RB, Powell D, Harper M and Boyce JD (2018) Identification of novel *Acinetobacter baumannii* type VI secretion system antibacterial effector and immunity pairs. Infect Immun 86:e00297-18.

Flick KE, Jurica MS, Monnat RJ and Stoddard BL (1998) DNA binding and cleavage by the nuclear intron-encoded homing endonuclease I-PpoI. Nature 394:96-101.

Floss HG and Yu T-W (2005) Rifamycin mode of action, resistance, and biosynthesis. Chem Rev 105:621-632.

Fridman CM, Jana B, Ben-Yaakov R, Bosis E and Salomon D (2022) A DNase type VI secretion system effector requires its MIX domain for secretion. Microbiol Spectr:e02465-02422.

García-Bayona L, Guo MS and Laub MT (2017) Contact-dependent killing by *Caulobacter crescentus* via cell surface-associated, glycine zipper proteins. Elife 6:e24869.

Garinot-Schneider C, Pommer AJ, Moore GR, Kleanthous C and James R (1996) Identification of putative active-site residues in the DNase domain of colicin E9 by random mutagenesis. J Mol Biol 260:731-742.

Gentry A and Osheroff N (2013) DNA topoisomerases: type II. In: Lennarz WJ and Lane MD (eds) Encyclopedia of Biological Chemistry. 2nd edition. Academic Press, London, pp 163-168.

Ghequire MGK and De Mot R (2014) Ribosomally encoded antibacterial proteins and peptides from *Pseudomonas*. FEMS Microbiol Rev 38:523-568.

Goetschi E, Angehrn P, Gmuender H, Hebeisen P, Link H, Masciadri R and Nielsen J (1993) Cyclothialidine and its congeners: A new class of DNA gyrase inhibitors. Pharmacol Ther 60:367-380.

Granato ET, Meiller-Legrand TA and Foster KR (2019) The evolution and ecology of bacterial warfare. Curr Biol 29:R521-R537.

Gucinski GC, Michalska K, Garza-Sánchez F, Eschenfeldt WH, Stols L, Nguyen JY, Goulding CW, Joachimiak A and Hayes CS (2019) Convergent evolution of the Barnase/EndoU/Colicin/RelE (BECR) fold in antibacterial tRNase toxins. Structure 27:1660-1674.e1665.

Gwon GH, Kim Y, Liu Y, Watson AT, Jo A, Etheridge TJ, Yuan F, Zhang Y, Kim Y and Carr AM (2014) Crystal structure of a Fanconi anemia-associated nuclease homolog bound to 5′ flap DNA: basis of interstrand cross-link repair by FAN1. Genes Dev 28:2276-2290.

Hachani A, Allsopp LP, Oduko Y and Filloux A (2014) The VgrG proteins are "a la carte" delivery systems for bacterial type VI effectors. J Biol Chem 289:17872-17884.

Hanawalt PC (2004) Density matters: the semiconservative replication of DNA. Proc Natl Acad Sci U S A 101:17889-17894.

Hansen JL, Moore PB and Steitz TA (2003) Structures of five antibiotics bound at the peptidyl transferase center of the large ribosomal subunit. J Mol Biol 330:1061-1075.

Hecht SM (2000) Bleomycin: New perspectives on the mechanism of action. J Nat Prod 63:158-168.

Herr Jr EB and Redstone MO (1966) Chemical and physical characterization of capreomycin. Ann N Y Acad Sci 135:940-946.

Hespanhol JT, Sanchez-Limache DE, Nicastro GG, Mead L, Llontop EE, Chagas-Santos G, Farah CS, de Souza RF, da Silva Galhardo R, Lovering A *et al*. (2022) Antibacterial T6SS effectors with a VRR-Nuc domain are structure-specific nucleases. Elife 11:e82437.

Hirao I, Harada Y, Nojima T, Osawa Y, Masaki H and Yokoyama S (2004) *In vitro* selection of RNA aptamers that bind to colicin E3 and structurally resemble the decoding site of 16S ribosomal RNA. Biochemistry 43:3214-3221.

Ho BT, Fu Y, Dong TG and Mekalanos JJ (2017) *Vibrio cholerae* type 6 secretion system effector trafficking in target bacterial cells. Proc Natl Acad Sci U S A 114:9427-9432.

Holberger LE, Garza-Sánchez F, Lamoureux J, Low DA and Hayes CS (2012) A novel family of toxin/antitoxin proteins in *Bacillus* species. FEBS Lett 586:132-136.

Hood RD, Singh P, Hsu F, Güvener T, Carl MA, Trinidad RR, Silverman JM, Ohlson BB, Hicks KG and Plemel RL (2010) A type VI secretion system of *Pseudomonas aeruginosa* targets a toxin to bacteria. Cell Host Microbe 7:25-37.

Horak V (1975) Typing of *Shigella sonnei* colicins by means of specific indicators. Zentralbl Bakteriol Orig A 233:58-63.

Hsia K-C, Chak K-F, Liang P-H, Cheng Y-S, Ku W-Y and Yuan HS (2004) DNA binding and degradation by the HNH protein ColE7. Structure 12:205-214.

Inoue-Ito S, Yajima S, Fushinobu S, Nakamura S, Ogawa T, Hidaka M and Masaki H (2012) Identification of the catalytic residues of sequence-specific and histidine-free ribonuclease colicin E5. J Biochem 152:365-372.

Iyer LM, Zhang D, Rogozin IB and Aravind L (2011) Evolution of the deaminase fold and multiple origins of eukaryotic editing and mutagenic nucleic acid deaminases from bacterial toxin systems. Nucleic Acids Res 39:9473-9497.

Jablonska J, Matelska D, Steczkiewicz K and Ginalski K (2017) Systematic classification of the His-Me finger superfamily. Nucleic Acids Res 45:11479-11494.

James R, Schneider J and Cooper PC (1987) Characterization of three group A klebicin plasmids: Localization of their E colicin immunity genes. Microbiology 133:2253-2262.

Jamet A, Jousset AB, Euphrasie D, Mukorako P, Boucharlat A, Ducousso A, Charbit A and Nassif X (2015) A new family of secreted toxins in pathogenic *Neisseria* species. PLoS Pathogens 11:e1004592.

Jana B, Fridman CM, Bosis E and Salomon D (2019) A modular effector with a DNase domain and a marker for T6SS substrates. Nat Commun 10:3595.

Johansen SK, Maus CE, Plikaytis BB and Douthwaite S (2006) Capreomycin binds across the ribosomal subunit interface using tlyA-encoded 2′-O-methylations in 16S and 23S rRNAs. Mol Cell 23:173-182.

Johnston NJ, Mukhtar TA and Wright GD (2002) Streptogramin antibiotics: mode of action and resistance. Curr Drug Targets 3:335-344.

Jones AM, Garza-Sánchez F, So J, Hayes CS and Low DA (2017) Activation of contact-dependent antibacterial tRNase toxins by translation elongation factors. Proc Natl Acad Sci U S A 114:E1951-E1957.

Jurėnas D, Payelleville A, Roghanian M, Turnbull KJ, Givaudan A, Brillard J, Hauryliuk V and Cascales E (2021) *Photorhabdus* antibacterial Rhs polymorphic toxin inhibits translation through ADP-ribosylation of 23S ribosomal RNA. Nucleic Acids Res 49:8384-8395.

Kaundal S, Deep A, Kaur G and Thakur KG (2020) Molecular and biochemical characterization of YeeF/YezG, a polymorphic toxin-immunity protein pair from *Bacillus subtilis*. Front Microbiol 11:95.

Kelly SJ, Li J, Setlow P and Jedrzejas MJ (2007) Structure, flexibility, and mechanism of the *Bacillus stearothermophilus* RecU Holliday junction resolvase. Proteins 68:961-971.

Klein TA, Ahmad S and Whitney JC (2020) Contact-dependent interbacterial antagonism mediated by protein secretion machines. Trends Microbiol 28:387-400.

Koch G, Yepes A, Förstner KU, Wermser C, Stengel ST, Modamio J, Ohlsen K, Foster KR and Lopez D (2014) Evolution of resistance to a last-resort antibiotic in *Staphylococcus aureus* via bacterial competition. Cell 158:1060-1071.

Koskiniemi S, Lamoureux JG, Nikolakakis KC, de Roodenbeke CtK, Kaplan MD, Low DA and Hayes CS (2013) Rhs proteins from diverse bacteria mediate intercellular competition. Proc Natl Acad Sci U S A 110:7032-7037.

Kratz K, Schöpf B, Kaden S, Sendoel A, Eberhard R, Lademann C, Cannavó E, Sartori AA, Hengartner MO and Jiricny J (2010) Deficiency of FANCD2-associated nuclease KIAA1018/FAN1 sensitizes cells to interstrand crosslinking agents. Cell 142:77-88.

Kross J, Henner WD, Hecht SM and Haseltine WA (1982) Specificity of deoxyribonucleic acid cleavage by bleomycin, phleomycin, and tallysomycin. Biochemistry 21:4310-4318.

Kurazono H, Yamamoto S, Nakano M, Nair G, Terai A, Chaicumpa W and Hayashi H (2000) Characterization of a putative virulence island in the chromosome of uropathogenic *Escherichia coli* possessing a gene encoding a uropathogenic-specific protein. Microb Pathog 28:183-189.

Kurylo-Borowska Z (1959) Isolation and properties of pure edeine, an antibiotic of the strain. *Bacillus brevis* Vm4 Bull Inst Marine Med Gdansk 10:151-163.

Lancaster LE, Savelsbergh A, Kleanthous C, Wintermeyer W and Rodnina MV (2008) Colicin E3 cleavage of 16S rRNA impairs decoding and accelerates tRNA translocation on *Escherichia coli* ribosomes. Mol Microbiol 69:390-401.

Lasater L, Cann P and Glitz DG (1989) Localization of the site of cleavage of ribosomal RNA by colicin E3: Placement on the small ribosomal subunit by electron microscopy of antibody-complementary oligodeoxynucleotide complexes. J Biol Chem 264:21798-21805.

Lee MD, Ellestad GA and Borders DB (1991) Calicheamicins: Discovery, structure, chemistry, and interaction with DNA. Acc Chem Res 24:235-243.

Levine C, Hiasa H and Marians KJ (1998) DNA gyrase and topoisomerase IV: Biochemical activities, physiological roles during chromosome replication, and drug sensitivities. Biochim Biophys Acta 1400:29-43.

Li Y, Yan X and Tao Z (2022) Two type VI secretion DNase effectors are utilized for interbacterial competition in the fish pathogen *Pseudomonas plecoglossicida*. Front Microbiol 13:869278.

Lin Y-L, Elias Y and Huang RH (2005) Structural and mutational studies of the catalytic domain of colicin E5: A tRNA-specific ribonuclease. Biochemistry 44:10494-10500.

Liu T, Ghosal G, Yuan J, Chen J and Huang J (2010) FAN1 acts with FANCI-FANCD2 to promote DNA interstrand cross-link repair. Science 329:693-696.

Livermore DM (1990) Antibiotic uptake and transport by bacteria. Scand J Infect Dis Suppl 74:15-22.

Ma J, Pan Z, Huang J, Sun M, Lu C and Yao H (2017) The Hcp proteins fused with diverse extended-toxin domains represent a novel pattern of antibacterial effectors in type VI secretion systems. Virulence 8:1189-1202.

MacKay C, Déclais A-C, Lundin C, Agostinho A, Deans AJ, MacArtney TJ, Hofmann K, Gartner A, West SC and Helleday T (2010) Identification of KIAA1018/FAN1, a DNA repair nuclease recruited to DNA damage by monoubiquitinated FANCD2. Cell 142:65-76.

Males BM and Stocker B (1980) *Escherichia coli* K317, formerly used to define colicin group E2, produces colicin E7, is immune to colicin E2, and carries a bacteriophage-restricting conjugative plasmid. J Bacteriol 144:524-531.

Males B and Stocker B (1982) Colicins E4, E5, E6 and A and properties of btuB+ colicinogenic transconjugants. Microbiology 128:95-106.

Mann RL and Bromer W (1958) The isolation of a second antibiotic from *Streptomyces hygroscopicus*. J Am Chem Soc 80:2714-2716.

Marco AD, Arcamone F and Zunino F (1975) Daunomycin (daunorubicin) and adriamycin and structural analogues: biological activity and mechanism of action. In: Corcoran JW, Hahn FE, Snell JF and Arora KL (eds) Mechanism of action of antimicrobial and antitumor agents. Springer, Berlin, pp 101-128.

Masaki H and Ogawa T (2002) The modes of action of colicins E5 and D, and related cytotoxic tRNases. Biochimie 84:433-438.

Mason D, Dietz A and Smith R (1961) Actino-spectacin, a new antibiotic. I. Discovery and biological properties. Antibiot Chemother (Northfield) 11:118-122.

Mason D, Dietz A and DeBoer C (1962) Lincomycin, a new antibiotic. I. Discovery and biological properties. Antimicrob Agents Ch 1962:554-559.

Maté MaJ and Kleanthous C (2004) Structure-based analysis of the metal-dependent mechanism of HNH endonucleases. J Biol Chem 279:34763-34769.

Maxwell A and Lawson DM (2003) The ATP-binding site of type II topoisomerases as a target for antibacterial drugs. Curr Top Med Chem 3:283-303.

Méndez C and Salas JA (2001) The role of ABC transporters in antibiotic-producing organisms: drug secretion and resistance mechanisms. Res Microbiol 152:341-350.

Meselson M and Stahl FW (1958) The replication of DNA in *Escherichia coli*. Proc Natl Acad Sci U S A 44:671-682.

Michalska K, Quan Nhan D, Willett JL, Stols LM, Eschenfeldt WH, Jones AM, Nguyen JY, Koskiniemi S, Low DA and Goulding CW (2018) Functional plasticity of antibacterial EndoU toxins. Mol Microbiol 109:509-527.

Mikolčević P, Hloušek-Kasun A and Ahel I, Mikoč A (2021) ADP-ribosylation systems in bacteria and viruses. Comput Struct Biotechnol J 19:2366-2383.

Minchin S and Lodge J (2019) Understanding biochemistry: structure and function of nucleic acids. Essays Biochem 63:433-456.

Mindlin S and Petrova M (2017) On the origin and distribution of antibiotic resistance: permafrost bacteria studies. Mol Gen Microbiol Virol 32:169-179.

Mok BY, de Moraes MH, Zeng J, Bosch DE, Kotrys AV, Raguram A, Hsu F, Radey MC, Peterson SB and Mootha VK (2020) A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing. Nature 583:631-637.

Monro RE (1967) Catalysis of peptide bond formation by 50 S ribosomal subunits from *Escherichia coli*. J Mol Biol 26:147-151.

Morange M (2009) The Central Dogma of molecular biology. Resonance 14:236-247.

Morse RP, Nikolakakis KC, Willett JL, Gerrick E, Low DA, Hayes CS and Goulding CW (2012) Structural basis of toxicity and immunity in contact-dependent growth inhibition (CDI) systems. Proc Natl Acad Sci U S A 109:21480-21485.

Mushegian A, Sorokina I, Eroshkin A and Dlakić M (2020) An ancient evolutionary connection between Ribonuclease A and EndoU families. RNA 26:803-813.

Nachmias N, Dotan N, Fraenkel R, Rocha MC, Kluzek M, Shalom M, Rivitz A, Shamash-Halevy N, Cahana I and Deouell N *et al*. (2022) Systematic discovery of antibacterial and antifungal bacterial toxins. bioRxiv:2021.2010.2019.465003.

Nikolakakis K, Amber S, Wilbur JS, Diner EJ, Aoki SK, Poole SJ, Tuanyok A, Keim PS, Peacock S and Hayes CS (2012) The toxin/immunity network of *Burkholderia pseudomallei* contact-dependent growth inhibition (CDI) systems. Mol Microbiol 84:516-529.

Nipič D, Podlesek Z, Budič M, Črnigoj M and Žgur-Bertok D (2013) *Escherichia coli* uropathogenic-specific protein, Usp, is a bacteriocin-like genotoxin. J Infect Dis 208:1545-1552.

Nygaard P (1993) Purine and pyrimidine salvage pathways. In: Sonenshein AL, Hoch JA and Losick R (eds) *Bacillus subtilis* and other gram-positive bacteria: biochemistry, physiology, and molecular genetics. American Society for Microbiology, Washington, pp 359-378.

Oakley AJ (2019) A structural view of bacterial DNA replication. Protein Sci 28:990-1004.

Oblak M, Kotnik M and Solmajer T (2007) Discovery and development of ATPase inhibitors of DNA gyrase as antibacterial agents. Curr Med Chem 14:2033-2047.

Ogawa T, Tomita K, Ueda T, Watanabe K, Uozumi T and Masaki H (1999) A cytotoxic ribonuclease targeting specific transfer RNA anticodons. Science 283:2097-2100.

Ohkawa I, Kageyama M and Egami F (1973) Purification and properties of pyocin S2. J Biochem 73:281-289.

Ohr RJ, Anderson M, Shi M, Schneewind O and Missiakas D (2017) EssD, a nuclease effector of the *Staphylococcus aureus* ESS pathway. J Bacteriol 199:e00528-00516.

Owen S, Dietz A and Camiener G (1962) Sparsomycin, a new antitumor antibiotic. I. Discovery and biological properties. Antimicrob Agents Ch 1962:772–779.

Parret A and De Mot R (2000) Novel bacteriocins with predicted tRNase and pore-forming activities in *Pseudomonas aeruginosa* PAO1. Mol Microbiol 35:472-473.

Patel M, Jiang Q, Woodgate R, Cox MM and Goodman MF (2010) A new model for SOS-induced mutagenesis: how RecA protein activates DNA polymerase V. Crit Rev Biochem Mol Biol 45:171-184.

Pei T-T, Li H, Liang X, Wang Z-H, Liu G, Wu L-L, Kim H, Xie Z, Yu M and Lin S (2020) Intramolecular chaperone-mediated secretion of an Rhs effector toxin by a type VI secretion system. Nat Commun 11:1865.

Pei T-T, Kan Y, Wang ZH, Tang MX, Li H, Yan S, Cui Y, Zheng HY, Luo H and Liang X (2022) Delivery of an Rhs-family nuclease effector reveals direct penetration of the gram-positive cell envelope by a type VI secretion system in *Acidovorax citrulli*. mLife 1:66-78.

Peterson SB, Bertolli SK and Mougous JD (2020) The central role of interbacterial antagonism in bacterial life. Curr Biol 30:R1203-R1214.

Phillips JW, Goetz MA, Smith SK, Zink DL, Polishook J, Onishi R, Salowe S, Wiltsie J, Allocco J and Sigmund J (2011) Discovery of kibdelomycin, a potent new class of bacterial type II topoisomerase inhibitor by chemical-genetic profiling in *Staphylococcus aureus*. Chem Biol 18:955-965.

Pioletti M, Schlünzen F, Harms J, Zarivach R, Glühmann M, Avila H, Bashan A, Bartels H, Auerbach T and Jacobi C (2001) Crystal structures of complexes of the small ribosomal subunit with tetracycline, edeine and IF3. EMBO J 20:1829-1839.

Pissaridou P, Allsopp LP, Wettstadt S, Howard SA, Mavridou DA and Filloux A (2018) The *Pseudomonas aeruginosa* T6SS-VgrG1b spike is topped by a PAAR protein eliciting DNA damage to bacterial competitors. Proc Natl Acad Sci U S A 115:12519-12524.

Pommer AJ, Cal S, Keeble AH, Walker D, Evans SJ, Kühlmann UC, Cooper A, Connolly BA, Hemmings AM and Moore GR (2001) Mechanism and cleavage specificity of the HNH endonuclease colicin E9. J Mol Biol 314:735-749.

Pommer AJ, Wallis R, Moore GR, James R and Kleanthous C (1998) Enzymological characterization of the nuclease domain from the bacterial toxin colicin E9 from *Escherichia coli*. Biochem J 334:387-392.

Putnam L, Hendricks F and Welch H (1953) Tetracycline, a new antibiotic. Antibiot Chemother 3:1183-1186.

Rich A (1959) Molecular structure of the nucleic acids. Rev Mod Phys 31:191.

Riley MA, Pinou T, Wertz JE, Tan Y and Valletta CM (2001) Molecular characterization of the klebicin B plasmid of *Klebsiella pneumoniae*. Plasmid 45:209-221.

Ringel PD, Hu D and Basler M (2017) The role of type VI secretion system effectors in target cell lysis and subsequent horizontal gene transfer. Cell Rep 21:3927-3940.

Rodnina MV (2018) Translation in prokaryotes. Cold Spring Harb Perspect Biol 10:a032664.

Roh E, Park T-H, Kim M-i, Lee S, Ryu S, Oh C-S, Rhee S, Kim D-H, Park B-S and Heu S (2010) Characterization of a new bacteriocin, Carocin D, from *Pectobacterium carotovorum* subsp. *carotovorum* Pcc21. Appl Environ Microbiol 76:7541-7549.

Roussin M, Rabarioelina S, Cluzeau L, Cayron J, Lesterlin C, Salcedo SP and Bigot S (2019) Identification of a contact-dependent growth inhibition (CDI) system that reduces biofilm formation and host cell adhesion of *Acinetobacter baumannii* DSM30011 strain. Front Microbiol 10:2450.

Ruhe ZC, Low DA and Hayes CS (2020) Polymorphic toxins and their immunity proteins: diversity, evolution, and mechanisms of delivery. Annu Rev Microbiol 74:497-520.

Salomon D, Kinch LN, Trudgian DC, Guo X, Klimko JA, Grishin NV, Mirzaei H and Orth K (2014) Marker for type VI secretion system effectors. Proc Natl Acad Sci U S A 111:9271-9276.

Sano Y and Kageyama M (1981) Purification and properties of an S-type pyocin, pyocin AP41. J Bacteriol 146:733-739.

Sano Y, Matsui H, Kobayashi M and Kageyama M (1993) Molecular structures and functions of pyocins S1 and S2 in *Pseudomonas aeruginosa*. J Bacteriol 175:2907-2916.

Sawa R, Takahashi Y, Hashizume H, Sasaki K, Ishizaki Y, Umekita M, Hatano M, Abe H, Watanabe T and Kinoshita N (2012) Amycolamicin: a novel broad-spectrum antibiotic inhibiting bacterial topoisomerase. Chemistry 18: 15772-15781.

Schaller K and Nomura M (1976) Colicin E2 is DNA endonuclease. Proc Natl Acad Sci U S A 73:3989-3993.

Schärer OD (2003) Chemistry and biology of DNA repair. Angew Chem Int Ed 42:2946-2974.

Schatz A, Bugle E and Waksman SA (1944) Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. Clin Orthop Relat Res 437:3-6.

Scherzinger E, Lanka E, Morelli G, Seiffert D and Yuki A (1977) Bacteriophage-T7-induced DNA-priming protein: A novel enzyme involved in DNA replication. Eur J Biochem 72:543-558.

Schlünzen F, Zarivach R, Harms J, Bashan A, Tocilj A, Albrecht R, Yonath A and Franceschi F (2001) Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. Nature 413:814-821.

Schuwirth BS, Day JM, Hau CW, Janssen GR, Dahlberg AE, Cate JHD and Vila-Sanjurjo A (2006) Structural analysis of kasugamycin inhibition of translation. Nat Struct Mol Biol 13:879-886.

Senior B and Holland I (1971) Effect of colicin E3 upon the 30S ribosomal subunit of *Escherichia coli*. Proc Natl Acad Sci U S A 68:959-963.

Sensi P (1959) Rifamycin, a new antibiotic, preliminary report. Farmaco Ed Sci 14:146-147.

Sharma S, Waterfield N, Bowen D, Rocheleau T, Holland L, James R and Ffrench-Constant R (2002) The lumicins: Novel bacteriocins from *Photorhabdus luminescens* with similarity to the uropathogenic-specific protein (USP) from uropathogenic *Escherichia coli*. FEMS Microbiol Lett 214:241-249.

Shine J and Dalgarno L (1974) The 3′-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. Proc Natl Acad Sci U S A 71:1342-1346.

Shuman S and Glickman MS (2007) Bacterial DNA repair by non-homologous end joining. Nat Rev Microbiol 5:852-861.

Sirias D, Utter DR and Gibbs KA (2020) A family of contact-dependent nuclease effectors contain an exchangeable, species-identifying domain. bioRxiv:2020.02.20.956912.

Šmarda J, Uher P, Osecký P and Šmarda J (1988) Modes of action of colicins E4–E7: Rates of basic biosyntheses inhibition. Zentralbl Bakteriol Mikrobiol Hyg A 269:7-14.

Smogorzewska A, Desetty R, Saito TT, Schlabach M, Lach FP, Sowa ME, Clark AB, Kunkel TA, Harper JW and Colaiácovo MP (2010) A genetic screen identifies FAN1, a Fanconi anemia-associated nuclease necessary for DNA interstrand crosslink repair. Mol Cell 39:36-47.

Song L, Pan J, Yang Y, Zhang Z, Cui R, Jia S, Wang Z, Yang C, Xu L and Dong TG (2021) Contact-independent killing mediated by a T6SS effector with intrinsic cell-entry properties. Nat Commun 12:423.

Souza DP, Oka GU, Alvarez-Martinez CE, Bisson-Filho AW, Dunger G, Hobeika L, Cavalcante NS, Alegria MC, Barbosa LR and Salinas RK (2015) Bacterial killing via a type IV secretion system. Nat Commun 6:6453.

Steczkiewicz K, Muszewska A, Knizewski L, Rychlewski L and Ginalski K (2012) Sequence, structure and functional diversity of PD-(D/E) XK phosphodiesterase superfamily. Nucleic Acids Res 40:7016-7045.

Stempler O, Baidya AK, Bhattacharya S, Malli Mohan GB, Tzipilevich E, Sinai L, Mamou G and Ben-Yehuda S (2017) Interspecies nutrient extraction and toxin delivery between bacteria. Nat Commun 8:315.

Sugiyama H, Kilkuskie RE, Chang LH, Ma LT, Hecht SM, Van der Marel GA and Van Boom JH (1986) DNA strand scission by bleomycin: Catalytic cleavage and strand selectivity. J Am Chem Soc 108:3852-3854.

Takeshita M, Grollman AP, Ohtsubo E and Ohtsubo H (1978) Interaction of bleomycin with DNA. Proc Natl Acad Sci U S A 75:5983-5987.

Takeuchi S, Hirayama K, Ueda K, Sakai H and Yonehara H (1958) Blasticidin S, a new antibiotic. J Antibiot (Tokyo) 11:1-5.

Theriault RJ, Karwowski JP, Jackson M, Girolami RL, Sunga GN, Vojtko CM and Coen LJ (1987) Tiacumicins, a novel complex of 18-membered macrolide antibiotics I. Taxonomy, fermentation and antibacterial activity. J Antibiot (Tokyo) 40:567-574.

Timmis K and Hedges AJ (1972) The killing of sensitive cells by colicin D. Biochim Biophys Acta 262:200-207.

Toba M, Masaki H and Ohta T (1988) Colicin E8, a DNase which indicates an evolutionary relationship between colicins E2 and E3. J Bacteriol 170:3237-3242.

Tomita K, Ogawa T, Uozumi T, Watanabe K and Masaki H (2000) A cytotoxic ribonuclease which specifically cleaves four isoaccepting arginine tRNAs at their anticodon loops. Proc Natl Acad Sci U S A 97: 8278-8283.

Trummal K, Aaspõllu A, Tõnismägi K, Samel M, Subbi J, Siigur J and Siigur E (2014) Phosphodiesterase from *Vipera lebetina* venom–structure and characterization. Biochimie 106: 48-55.

Turano H, Gomes F, Barros-Carvalho GA, Lopes R, Cerdeira L, Soares Netto LE, Gales AC and Lincopan N (2017) Tn 6350, a novel transposon carrying pyocin S8 genes encoding a bacteriocin with activity against carbapenemase-producing *Pseudomonas aeruginosa*. Antimicrob Agents Ch 61:e00100-00117.

Turano H, Gomes F, Domingos RM, Degenhardt MF, Oliveira CL, Garratt RC, Lincopan N and Soares Netto LE (2020) Molecular structure and functional analysis of pyocin S8 from *Pseudomonas aeruginosa* reveals the essential requirement of a glutamate residue in the HNH motif for DNase activity. J Bacteriol 202:e00346-00320.

Umezawa H, Ueda M, Maeda K, Yagishita K, Kondō S, Okami Y, Utahara R, Ōsato Y, Nitta K and Takeuchi T (1957) Production and isolation of a new antibiotic, kanamycin. J Antibiot (Tokyo) 10:181-188.

Umezawa H, Okami Y, Hashimoto T, Suhara Y, Hamada M and Takeuchi T (1965) A new antibiotic, kasugamycin. J Antibiot (Tokyo) 18:101-103.

Umezawa H, Maeda K, Takeuchi T and Okami Y (1966) New antibiotics, bleomycin A and B. J Antibiot (Tokyo) 19:200-209.

Uphoff S and Sherratt DJ (2017) Single-molecule analysis of bacterial DNA repair and mutagenesis. Annu Rev Biophys 46:411-432.

Vassallo CN, Cao P, Conklin A, Finkelstein H, Hayes CS and Wall D (2017) Infectious polymorphic toxins delivered by outer membrane exchange discriminate kin in myxobacteria. Elife 6:e29397.

Vázquez-Laslop N and Mankin AS (2018) How macrolide antibiotics work. Trends in biochemical sciences 43:668-684.

Wah DA, Bitinaite J, Schildkraut I and Aggarwal AK (1998) Structure of Fok I has implications for DNA cleavage. Proc Natl Acad Sci U S A 95:10564-10569.

Waksman SA and Lechevalier HA (1949) Neomycin, a new antibiotic active against streptomycin-resistant bacteria, including tuberculosis organisms. Science 109:305-307.

Walker GC (1996) The SOS response of *Escherichia coli*. In: Neidhardt F, Curtiss III R, Ingraham J, Lin E, Low K, Magasanik B, Reznikoff W, Riley M, Schaechter M and Umbarger H (eds) *Escherichia coli* and *Salmonella*: Cellular and molecular biology. 2nd edition. ASM Press, Washington, pp 1400-1416.

Walsh CT (2016) Insights into the chemical logic and enzymatic machinery of NRPS assembly lines. Nat Prod Rep 33:127-135.

Wang J-W, Derilo RC, Lagitnay RBJS, Wu H-P, Chen K-I and Chuang D-Y (2020) Identification and characterization of the bacteriocin Carocin S3 from the multiple bacteriocin producing strain of *Pectobacterium carotovorum* subsp. *carotovorum*. BMC Microbiol 20:273.

Wang JC (1971) Interaction between DNA and an *Escherichia coli* protein ω. J Mol Biol 55:523-533.

Wang R, Persky NS, Yoo B, Ouerfelli O, Smogorzewska A, Elledge SJ and Pavletich NP (2014) Mechanism of DNA interstrand cross-link processing by repair nuclease FAN1. Science 346:1127-1130.

Wang S, Geng Z, Zhang H, She Z and Dong Y (2021) The *Pseudomonas aeruginosa* PAAR2 cluster encodes a putative VRR-NUC domain-containing effector. FEBS J 288:5755-5767.

Weinstein M, Luedemann G, Oden E, Wagman G, Rosselet J, Marquez J, Coniglio C and Herzog H (1963) Gentamicin, a new antibiotic complex from *Micromonospora*. J Med Chem 6:463-464.

Wen H, Liu G, Geng Z, Zhang H, Li Y, She Z and Dong Y (2021) Structure and SAXS studies unveiled a novel inhibition mechanism of the *Pseudomonas aeruginosa* T6SS TseT-TsiT complex. Int J Biol Macromol 188:450-459.

Willett JL, Gucinski GC, Fatherree JP, Low DA and Hayes CS (2015) Contact-dependent growth inhibition toxins exploit multiple independent cell-entry pathways. Proc Natl Acad Sci U S A 112:11341-11346.

Wilson DN (2009) The A–Z of bacterial translation inhibitors. Crit Rev Biochem Mol Biol 44:393-433.

Wu C-C, Lin JL and Yuan HS (2020) Structures, mechanisms, and functions of His-Me finger nucleases. Trends Biochem Sci 45:935-946.

Wyman C, Ristic D and Kanaar R (2004) Homologous recombination-mediated double-strand break repair. DNA Repair 3:827-833.

Yadav SK, Magotra A, Ghosh S, Krishnan A, Pradhan A, Kumar R, Das J, Sharma M and Jha G (2021) Immunity proteins of dual nuclease T6SS effectors function as transcriptional repressors. EMBO Rep 22:e51857.

Yajima S, Nakanishi K, Takahashi K, Ogawa T, Hidaka M, Kezuka Y, Nonaka T, Ohsawa K and Masaki H (2004) Relation between tRNase activity and the structure of colicin D according to X-ray crystallography. Biochem Biophys Res Commun 322:966-973.

Yajima S, Inoue S, Ogawa T, Nonaka T, Ohsawa K and Masaki H (2006) Structural basis for sequence-dependent recognition of colicin E5 tRNase by mimicking the mRNA–tRNA interaction. Nucleic Acids Res 34:6074-6082.

Yang W, Chen W-y, Wang H, Ho JW, Huang J-D, Woo PC, Lau SK, Yuen K-Y, Zhang Q and Zhou W (2011) Structural and functional insight into the mechanism of an alkaline exonuclease from *Laribacter hongkongensis*. Nucleic Acids Res 39: 9803-9819.

Yoshida T and Tsuge H (2021) Common mechanism for target specificity of protein-and DNA-targeting ADP-ribosyltransferases. Toxins (Basel) 13:40.

Zaw MT, Yamasaki E, Yamamoto S, Nair GB, Kawamoto K and Kurazono H (2013) Uropathogenic specific protein gene, highly distributed in extraintestinal uropathogenic *Escherichia coli*, encodes a new member of HNH nuclease superfamily. Gut Pathog 5:13.

Zein N, Sinha AM, McGahren WJ and Ellestad GA (1988) Calicheamicin γ1I: an antitumor antibiotic that cleaves double-stranded DNA site specifically. Science 240:1198-1201.

Zhang D, Iyer LM and Aravind L (2011) A novel immunity system for bacterial nucleic acid degrading toxins and its recruitment in various eukaryotic and DNA viral systems. Nucleic Acids Res 39:4532-4552.

Zhang D, de Souza RF, Anantharaman V, Iyer LM and Aravind L (2012) Polymorphic toxin systems: comprehensive characterization of trafficking modes, processing, mechanisms of action, immunity and ecology using comparative genomics. Biol Direct 7:18.

Zhao Q, Xue X, Longerich S, Sung P and Xiong Y (2014) Structural insights into 5′ flap DNA unwinding and incision by the human FAN1 dimer. Nat Commun 5:5726.

Associate Editor: Carlos F. M. Menck

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License (type CC-BY), which permits unrestricted use, distribution and reproduction in any medium, provided the original article is properly cited.