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Evaluation of antibiotic resistance profiles of Halobacteria isolated from the food chain

Coordinator: Ch.mo Prof. Marco Trevisan

Candidate: Irene Falasconi
Matriculation n.: 4212109

Tutor: **Prof. Lorenzo Morelli**
Co-tutor: **Prof. Edoardo Puglisi**

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Abstract

Antimicrobial resistance is now widely acknowledged as a major global public health challenge. There are many environments through which the transmission and diffusion of antibiotic resistance could happen, but one of the main routes of transmission is the food chain. As a matter of fact, antibiotic use is widely spread in animal husbandry and in agriculture. In particular, in animal husbandry antimicrobials have been used both for therapeutic reasons and as growth promoters. As a consequence, a selective pressure on pathogenic and commensal bacteria of animal origin has been exerted during the time, leading to the onset of microorganisms resistant to such compounds. A pivotal role in the spread in the food chain of antibiotic resistance has been played by non-pathogenic bacteria present in food. These microorganisms are not harmful for humans, but they could represent a *reservoir* of antibiotic resistance for foodborne pathogenic bacteria. Usually lactic acid bacteria play this role, since they are present in all fermented food. For this reason, the antibiotic resistance profile of lactic acid bacteria has been assessed.

In recent years, another class of microorganisms, called halophilic archaea, has raised an increasing scientific interest, since they have been found in the human intestinal mucosa as well as in foods such as salted codfish and fermented Asiatic seafood.

As a few papers have studied the antibiotic resistance profiles of halophilic archaea, and the only present do not consider a statistically significant number of microorganisms belonging to the same species, the aim of the present work is to define the antibiotic resistance profile of the major exponent of halophilic archaea, named *Halobacterium salinarum,* and consequently to verify if some strains present antibiotic resistances and if they can transfer these resistances to bacteria present in the food chain.

Chapter 1

General Introduction

Antibiotic resistance problem

Since their discovery, antibiotics and antimicrobial drugs have led to an important decrease in deaths from infectious diseases, and have contributed to the extension in life expectancy experienced during the latter part of the last century (Armstrong et al., 1999).

The use of antibiotics for any purpose – in people, animals, plants – anywhere in the world affects everyone. As a consequence, the widespread use of antimicrobial substances has led to resistant populations of microorganisms in several ecosystems. In animal husbandry, for instance, antimicrobials have been used both for therapeutic reasons and as growth promoters, but both of these uses will provide a selective pressure on pathogenic and commensal bacteria of animal origin (Mayrhofer et al., 2007; Teale, 2002). As a matter of fact antibiotic use in one individual can promote the local survival of resistant strains that can subsequently spread from that individual to others and potentially, over time, to any community worldwide.

In recent decades, the emergence and spread of antibiotic-resistant microbes have challenged clinicians and researchers. At present, antibiotic resistance is a global public health threat that involves all major microbial pathogens and antimicrobial drugs, affecting current and future generations (Capita and Alonso-Calleja, 2013).

At word level, the World Health Organisation (WHO) has the mission of building a better, healthier future for people all over the world. For this reason, tackling antibiotic resistance is becoming a high priority for the WHO. A proof of this interest is that at the World Health Assembly in May 2015 a global action plan on antimicrobial resistance, including antibiotic resistance, was endorsed. The principal aim of this plan is to ensure prevention and treatment of infectious diseases with safe and effective medicines. This plan contemplates five strategic objectives:

1. To improve awareness and understanding of antimicrobial resistance.
2. To strengthen surveillance and research.
3. To reduce the incidence of infection.
4. To optimize the use of antimicrobial medicines.
5. To ensure sustainable investment in countering antimicrobial resistance.

Furthermore at the United Nations General Assembly in New York in September 2016 the Heads of State committed to address the fundamental causes of antimicrobial resistance across multiple sectors, especially human health, animal health and agriculture by taking a broad coordinated approach. Countries, supported by the WHO, reaffirmed their dedication to

develop national action plans on antimicrobial resistance, based on the global action plan (http://www.who.int/en/).

In America the Centers for Disease Control and Prevention (CDC) is monitoring the situation of the antibiotic resistance threats in the United States. Recent reports state that each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die each year as a direct result of these infections. Many more people die from other conditions that were complicated by an antibiotic-resistant infection.

Moreover, data show that antibiotic-resistant infections can happen anywhere. In particular, the majority happen in the general community; however, most deaths related to antibiotic resistance can be found in healthcare settings such as hospitals and nursing homes (https://www.cdc.gov/).

Antimicrobials of human and veterinary importance: the European Union approach

The European Food Safety Authority (EFSA) is the European Union risk assessor and the provider of independent scientific advice and communication on risks associated with the food chain. One of its tasks is to provide a method to identify resistance to antimicrobials of human and veterinary importance in bacterial strains intended for use as feed additives (Efsa and Efsa FEEDAP, 2012). All these antibiotics with some resistance genes are listed in Table 1.

Table 1. Classes of antimicrobials, examples of substances used in human and veterinary medicine and examples of resistance genes (Andreoletti et al., 2008).

Class	Examples of substances used in:		Examples of resistance genes	Comments
	Human medicine	**Veterinary medicine; food production animals in EU**		
Aminoglycosides	amikacin, gentamicin, netilmicin, tobramycin	apramycin, gentamicin, streptomycin	*aac*, *aad (ant)*, *aph*, *armA*, *rpsL (strA)* *rpsD*, *rpsE*, *strB*	No general cross-resistance within class, but some types of resistance will involve cross-resistance between some aminoglycosides
	kanamycin, spectinomycin, streptomycin	neomycin, spectinomycin		
Amphenicols	chloramphenicol, tiamphenicol	chloramphenicol, florfenicol, tiamphenicol	*cat*, *cfr*, *cml*, *flo*	cfr confers cross-resistance to amphenicols, lincosamindes, pleuromutilins, streptogramins, linezolid
Beta-lactam antibiotics				Cross resistance within subclasses but also, depending on mechanism, between subclasses
Penicillins	benzyl-penicillin, ampicillin, amoxicillin (with clavulanic acid)	benzyl-penicillin, ampicillin, amoxicillin (with clavulanic acid)	bla_Z (*bla*-$_{PC}$), *bla*-$_{TEM}$, *bla*-$_{SHV}$, *bla*-$_{OXA}$	
Beta-lactamase resistant penicillins	cloxacillin, dicloxacillin (meticillin)	cloxacillin, dicloxacillin	*bla*-$_{OXA}$, *mecA*	
Cephalosporins, first generation	cephalexin, cefazolin, cephalotin	cefazolin, cephalexin	*bla*-$_{TEM}$, *Bla*-$_{SHV}$, *bla*-$_{CTX}$, *bla*-$_{CMY}$, some *bla*-$_{OXA}$	
Cephalosporins, second generation	cefuroxime, loracarbef	-		
Cephalosporins, third generation	ceftazidime, ceftriaxone	ceftiofur		
Cephalosporins, fourth generation	cefepime, cefpirome	cefepime, cefquinome		
Cephamycins	cefoxitin		*bla*-$_{CMY}$, *bla*-$_{AAC}$	
Carbapenems	ertapenem, imipenem, meropenem	-	*bla*-$_{IMP}$, *bla*-$_{VIM}$, *bla*-$_{KPC}$ some *bla*-$_{OXA}$	

Class	Examples of substances used in:		Examples of resistance genes	Comments
	Human medicine	**Veterinary medicine; food production animals in EU**		
Cyclic polypeptides	bacitracin	(bacitracin)	*bcrABD*	Formerly used as feed additive in EU
Glycopeptides	teicoplanin, vancomycin	(avoparcin)	*van (A-E)*	Formerly avoparcin was used as feed additive in EU
Ionophores	-	monensin, salinomycin		Used as coccidiostats
Lincosamides	clindamycin, lincomycin	clindamycin, lincomycin	*cfr, erm*	Cross-resistance also to macrolides and streptogramin B for certain resistance genotypes
Lipopeptides	daptomycin	-		
Macrolides & ketolides	erythromycin, spiramycin, azithromycin, clarithromycin	spiramycin, tylosin, tulathromycin	*erm, ere, mef, msr*	Cross-resistance also to lincosamides and streptograminB for certain resistance genotypes
Nitrofurans	furazolidone, nitrofurantoin	-		Used formerly as veterinary medicine
Nitroimidazoles	metronidazole, tinidazole	-		Dimetridazole and ronidazole used formerly as veterinary medicine
Orthosomycins	-	avilamycin	*emtA*	Used formerly as feed additive
Oxazolidones	linezolid	-	*cfr*	
Pleuromutilins	-	tiamulin, valnemulin	*cfr*	
Polymixins	colistin, polymixin B	colistin, polymixin B		
Quinolones	nalidixic acid, ciprofloxacin, norfloxacin, moxifloxacin	danofloxacin, enrofloxacin,	*aac(6')*-Ib-cr, *gyrA. parC, qepA, qnr*,	Incomplete cross-resistance. *aac(6')*-Ib-cr also confers resistance to kanamycin
Quinoxalines	-	carbadox, olaquindox	*oqxAB*	Olaquindox used formerly as feed additive in the EU
Streptogramins	pristinamycin, quinpristin/dalfopristin	(virginiamycin)	*cfr, erm, vga , vgb*	Formerly virginiamycin was used as feed additive in EU Cross-resistance between streptogramin B, lincosamides and macrolides for certain resistance genotypes
Sulphonamides & trimethoprim	sulfadiazine, sulfamethoxazole, trimethoprim	sulfadiazine, sulfadoxine, sulfamethoxazole, trimethoprim	*dfr, sul*	
Tetracyclines	chlortetracycline, doxycycline, oxytetracycline	chlortetracycline, doxycycline, oxytetracycline	*tet*	

Class	Examples of substances used in:		Examples of resistance genes	Comments
	Human medicine	**Veterinary medicine; food production animals in EU**		
Miscellaneous	-	flavophospholipol (bambermycin)		Used formerly as feed additive
	fosfomycin	-	*fosAB*	
	fusidic acid	fusidic acid	*fusB*	
	mupirocin	-	*mup*A	Used in human medicine topically for MRSA decontamination
	rifampicin	(rifampicin)	*rpoB*	Use in vet. med limited to foals

Antibiotic use in food production

As mentioned above, antibiotics are used, as regards the agri-food sector, in animals for therapy, disease prevention and, even if not in Europe, also for growth promotion. The classes of antibiotics used in animals are the same of those used in people. However, because of the large number of animals and the industrialized production of food animals, the quantity of antibiotics used in food production in many countries seems to exceed the amounts used medicinally in people infections (World Health Organisation, 2011).

Food animals are treated with antibiotics when affected by respiratory and enteric infections and especially during the early part of an animal's life. Antibiotics are also used to treat infections in individual animals caused by a variety of bacterial pathogens. In particular, antibiotics are often used in dairy cows for treating mastitis and in cows with a high milk output to handle common infections (World Health Organisation, 2011).

Furthermore, antibiotics are used also in fish farming. The intensification in this sector has been accompanied by bacterial infections that are usually treated with antibiotics added to fish foodstuffs. Similar to other industrialized food animal production, the usage of antibiotics in aquaculture can be significant.

Another field where antibiotics are used is agriculture. The number of antibiotics used in plant agriculture is modest relative to applications in human and veterinary medicine and in animal production. The most used are streptomycin, gentamycin, oxytetracycline, oxolinic acid. As a consequence, bacteria affecting plants have become resistant to streptomycin and tetracycline. How much the antibiotic use in agriculture contributes to the development of antibiotic resistance in human pathogens is still a debated issue (McManus et al., 2002).

Potential routes of transmission of antimicrobial resistance in the food chain

Generally, undercooked and raw consumed vegetables and fruit are the most prone food to contain high levels of bacteria, including antimicrobial-resistant bacteria, derived from primary production (Faour-Klingbeil et al., 2016; Losio et al., 2015; Rosenquist et al., 2005). Cooking or other microbicidal treatments (such as high pressure processing) reduce bacteria loads, but if intact DNA coding for resistance is present, it may persist in such treated food (Andreoletti et al., 2008). In addition to primary production, there are a number of processing steps as storage, preparation and serving, which can contaminate the final product (Figure 1).

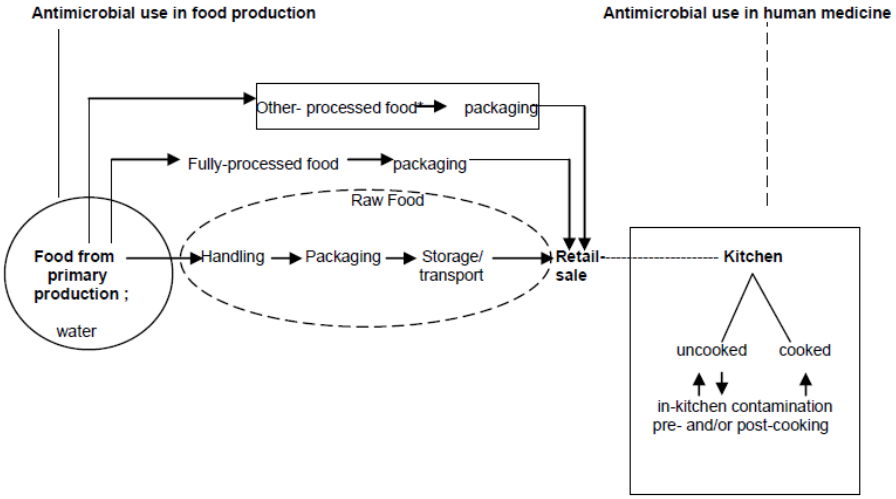


Figure 1. Schematic view of possible routes of transmission of antibiotic resistance via food (Andreoletti et al., 2008).

The role of non-pathogenic food related bacteria

It is estimated that approximately one-quarter of food production involves microbial fermentation processes, primarily lactic acid bacteria (LAB). Since it is known that horizontal transfer of antibiotic resistance genes may take place in the environment, including food and the gastrointestinal tract, there has been the necessity of assessing the safety of bacteria use in food production to antibiotics of human and veterinary importance.

Whereas microorganisms may theoretically transfer antimicrobial resistant genes to pathogens, antimicrobial resistance might be considered as one criterion to assess the safety of strains used in food and feed (Borriello et al., 2003).

In this scenario, a pivotal role was played by the project "Assessment and Critical Evaluation of Antibiotic Resistance Transferability in Food Chain" (ACE-ART) that was aimed at providing a critical evaluation of the impact of antibiotic use in agriculture and in the prophylaxis and treatment of disease in humans on non-pathogenic, food-related bacteria.

The ACE-ART consortium implemented a diffused promotion, targeting both the European authorities and the greater public, and at the same time encouraged cooperation between scientists and industries. Thanks to ACE-ART, some non-pathogenic, food related bacteria, mainly lactic acid bacteria, have been identified as reservoir of drug resistances, which can be transferred to pathogens with important consequences for the food industry and for future preventive and management strategies. Moreover, in the frame of this project the methodology for assessing minimal inhibitory concentrations in bifidobacteria and non-enterococcal lactic acid bacteria was developed (ISO 10932/IDF 223).

As a consequence, the food chain has been considered one of the major routes of antibiotic resistance spread.

Antibiotic resistance mechanisms among bacteria

There are two kinds of antibiotic resistance in a microorganism: when resistance to an antimicrobial is inherent to a bacterial species, it is generally referred to as "intrinsic resistance" (sometimes called "natural resistance") and is typical of all the strains of that species. In other cases, susceptible bacteria have become resistant over the course of the last several decades: in this case we can talk about "acquired resistance" (World Health Organisation, 2011). The major mechanisms of antimicrobial resistance that have been described are three: direct inactivation of the active molecule, loss of bacterial susceptibility to the antimicrobial by modification of the target of action, and decreased amount of drug that reaches the target molecule without modification of the compound itself (Bories et al., 2008).

Intrinsic resistance

As suggested by the name, intrinsic resistance is due to a natural feature of a microorganism. The antibiotic defence mechanisms of intrinsic resistance are, in most cases, related to the presence of low affinity targets, the absence of targets, decreased uptake or efflux of drugs (Bories et al., 2008).

Acquired resistance

Acquired resistance can be achieved by the mutation of genes already present in the bacterial genome or by the acquisition of genes via gain of exogenous DNA.

Generally a simple mutation causes the modification of the target of the antibiotic, resulting in a less affinity of the compound (Flensburg and Skold, 1984) or the overproduction of intrinsic resistance determinants, such as efflux pumps (Beinlich et al., 2001). When a bacterium becomes resistant to an antibiotic through a novel mutation in its DNA, the only way of spreading this resistance is proliferation of the bacterium itself (vertical transfer).

On the other hand, the three routes of acquiring exogenous DNA are: transformation, transduction and conjugation (Figure 2).

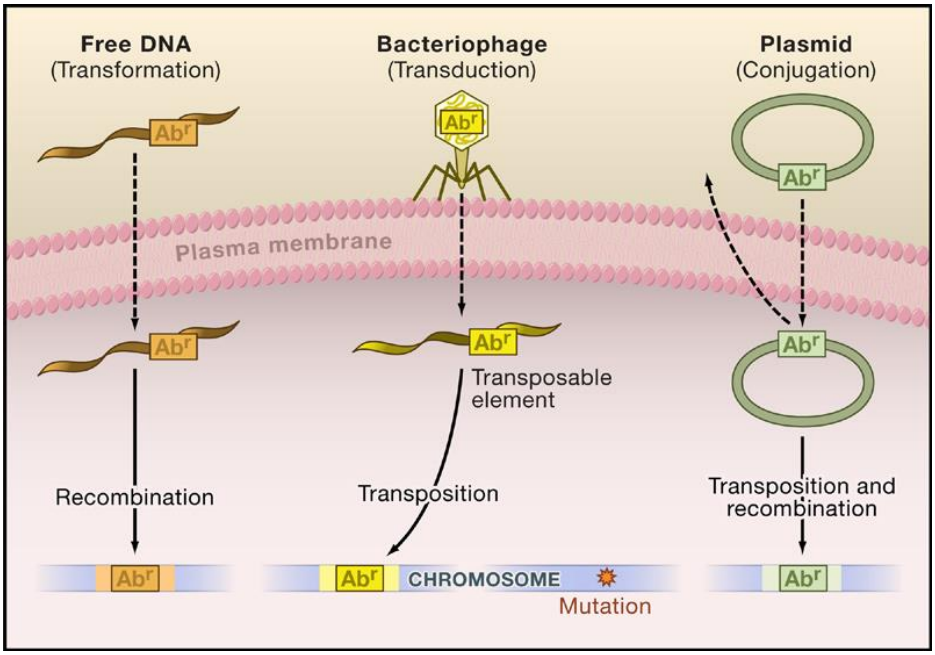


Figure 2. Acquisition of antibiotic resistance by bacteria. Ab^r stands for antibiotic resistance (Alekshun and Levy, 2007).

Transformation is the direct uptake and incorporation of exogenous genetic material from the environment through the cell membrane. Conjugation is the process through which bacteria exchange their genetic material. During transduction foreign DNA is introduced into a cell by a virus or viral vector (Alberts et al., 2002). Both transformation and conjugation can be ascribed as horizontal gene transfer.

Horizontal gene transfer can lead to the acquisition of new genes as a result of gene exchange between bacteria. These newly acquired genes can code for enzymes that modify the drug,

inactivating it, such as β-lactamases or acetyl transferases (Poole, 2002), or can code for mechanisms of decreased susceptibility to antimicrobials due to target modification.

The consequences of horizontal gene transfer are more serious than the ones of vertical transfer. The former mechanism can often promote the simultaneous spread of resistance to several unrelated classes of antibiotics, particularly if the genes for such resistance are co-located on the transmissible genetic element.

To make matters worse, sometimes genes for resistance and virulence can be transferred together, leading to the emergence of new resistance threats of greater virulence and pathogenicity than seen in past generations.

Alteration of the target

Alteration of target sites of antibiotics is a common mechanism of resistance. This is because generally the targets of antibiotics have vital roles in the life of a microorganisms, and the interference with them leads to cell death or growth inhibition. The targets preferred by antibiotics are: protein synthesis (aminoglycosides, tetracyclines, macrolides, chloramphenicol, fusidic acid, mupirocin, streptogramins, oxazolidinones), transcription via RNA polymerase (the rifamycins); chromosome segregation (quinolones) and integrity (metronidazole); and folic acid metabolism (trimethoprim, pyrimethamine) (Lambert, 2005). Generally target alteration is the result of a point mutation in the nucleotide sequence that codifies for it, which results in the transcription of a different mRNA sequence that is translated in a different protein that shows a less affinity with the antibiotic which binds to.

Drug inactivating enzymes

Antibiotic inactivation includes hydrolysis, group transfer, and redox mechanisms. The most widespread modifications are listed in Table 2.

Table 2. Enzymatic strategies of antibiotic inactivation (Wright, 2005).

Strategy	Type	Antibiotics affected
Hydrolysis		β-Lactams Macrolides
Group transfer	Acyl	Aminoglycoside Chloramphenicol Type A Streptogramin
	Phosphoryl	Aminoglycoside Macrolide Rifamycin Peptide
	Thiol	Fosfomycin
	Nucleotidyl	Aminoglycoside Lincosamide
	ADP-ribosyl	Rifamycin
	Glycosyl	Macrolide Rifamycin
Other	Redox	Tetracycline Rifamycin Type A streptogramin
	Lyase	Type B streptogramin

Hydrolysis

Many antibiotics present chemical bonds that are sensible to hydrolysis, such as esters and amides. For this reason bacteria have evolved a mechanism of resistance that involves the cleavage of these bonds.

Among the enzymes that hydrolyse drugs there are: β-lactamases, macrolide esterases and epoxidases.

β-lactamases have one common property of catalysing the hydrolysis of the β-lactam ring of penicillins and cephalosporins. It is interesting that although β-lactam antibiotics present various killing targets (penicillin binding proteins), they are inactivated by only one enzyme.

However, a wide range of bacteria produces this enzyme, presenting different chemical, physical and enzymological properties (Ogawara, 1981).

The macrolide esterases EreA (Ounissi and Courvalin, 1985) and EreB (Arthur et al., 1986) inactivate drugs through the hydrolysis of the macrolactone ring. EreA has been found in Gram-negative bacteria while EreB is thought to originate from Gram-positive organisms on the basis of GC content analysis (Morar et al., 2012). Albeit the most common macrolide resistance mechanism is constituted by modifications of the ribosome, resistance via esterase activity has been increased among clinical isolates worldwide (Nakamura et al., 2000; Yong et al., 2009).

One of the most known epoxidases that act against an epoxide antibiotic is FosX. In particular, it inhibits the antibiotic fosfomycin, which interacts with MurA, the enzyme that catalyses the first committed step in peptidoglycan biosynthesis. FosX action consists on the destruction of the reactive epoxide by ring opening by either a thiol-containing co-substrate (Wright, 2005).

Group transfer

A very large family of resistance enzymes is the group of transferases. These enzymes covalently modify antibiotics resulting in structural alterations that impair target binding. Each of these enzymes takes the name from the group they transfer (Wright, 2005).

One of the most common mechanisms of antibiotic inactivation employed by bacteria is acyltransfer, and specifically acetyltransfer. It consists on the covalent modification of vulnerable hydroxyl and/or amine groups on antibiotics, which results in compounds that lose their ability to bind target and, therefore, become inactive. Among this class the most well characterized are: aminoglycoside acetyltransferases (Wybenga-Groot et al., 1999), chloramphenicol acetyltransferases (Leslie et al., 1988) and streptogramin acetyltransferases (Sugantino and Roderick, 2002).

Kinases are a group of enzymes that catalyses the transfer of phosphate groups from high-energy, phosphate-donating molecules (usually ATP) to specific substrates. The known antibiotic kinases involved in resistance are exclusively O-phospho- transferases, and many share structural and mechanistic details with other kinases such as the protein kinases. Antibiotics that are known to present kinases specific for them are: aminoglycosides (Ramirez and Tolmasky, 2010), macrolides (Noguchi et al., 1995), rifamycins (Morisaki et al., 1993) and peptide antibiotics (Skinner and Cundliffe, 1980).

FosB is a Mg^{2+}-dependent cysteine thiol transferase. It acts on fosfomycin like the aforementioned FosX, by adding l-cysteine to this antibiotic, with a resulted inhibition (Cao et al., 2001).

There are two major classes of nucleotidyltransferases: the ANTs that modify aminoglycosides, and the Lin proteins that inactivate the lincosamide antibiotics such as lincomycin and clindamycin. Their action is to transfer the nucleoside monophosphate group of a nucleotide to the target antibiotic (Bacot-Davis et al., 2016).

ADP-ribosyl transfer is a common mechanism of protein modification in both eukaryotes and prokaryotes. These modifications occur on amino acid residues and require nicotinamide adenine dinucleotide (NAD) as the ADP-ribosyl donor. Although well established as a protein modification mechanism of significant importance, small molecule ADP-ribosylation is unknown with one exception: a reported ADP-ribosylation of the antibiotic rifampin that results in resistance in bacterial pathogens (Quan et al., 1997).

In the group of transferases, glycosylases are the less widespread. They play a role in the protection of microorganisms producing antibiotic, in particular against macrolides and rifamycin (Wright, 2005).

Redox enzymes and lyases

Other mechanisms of resistance are less common than the previously described. These include redox and lyase enzymes. TetX is a well-studied example among the redox enzymes. It is a flavin-dependent monooxygenase that regioselectively hydroxylates the tetracycline substrate, resulting in an unstable compound that undergoes non-enzymatic decomposition (Yang et al., 2004). Whereas Vgb is a lyase that inactivate type B streptogramins. This enzyme linearizes the antibiotic by an elimination reaction (Mukhtar et al., 2001).

Efflux mechanisms

Proteins involved in drug transport

Almost all bacteria species present efflux pumps, and genes encoding for these proteins are located on chromosomes or plasmids (Piddock, 2006; Poole, 2004). Bacterial efflux pumps can be classified into five families, which differ in composition, number of transmembrane spanning regions, energy source and substrate. The families are the following: the resistance-nodulation-division (RND) family, the major facilitator superfamily (MFS), the ATP (adenosine triphosphate)-binding cassette (ABC) superfamily, the small multidrug resistance (SMR) family, which is a member of the much larger drug/metabolite transporter (DMT)

superfamily, and the multidrug and toxic compound extrusion (MATE) family (Piddock, 2006; Poole, 2004; Putman et al., 2000) (Figure 3).

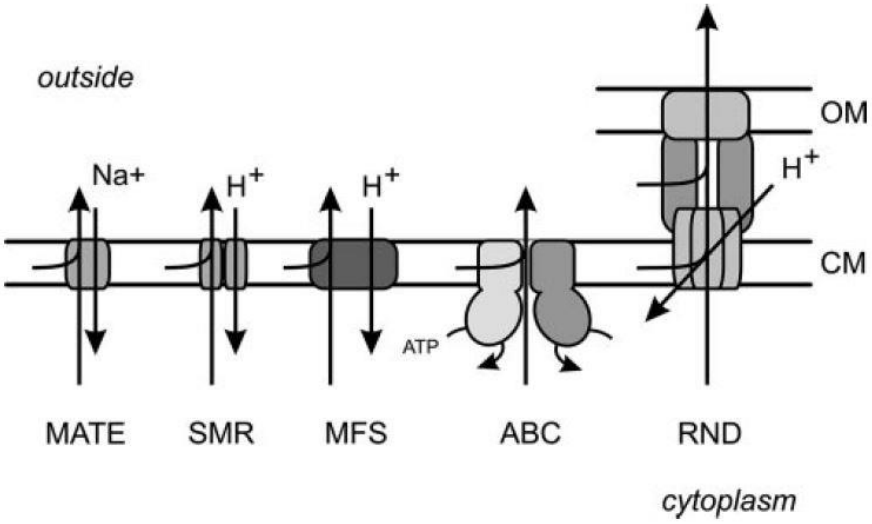


Figure 3. Schematic representation of the major families of Multi-Drug Resistance (MDR) transporters in prokaryotes. OM is outer membrane and CM is cytoplasmic membrane (Lubelski et al., 2007).

ABC and MFS superfamilies are very large and ancient (Higgins, 2001; Pao et al., 1998), while MATE, SMR and RND are smaller families (Brown et al., 1999; Paulsen et al., 1996). All these families are widely distributed in Gram positive and negative bacteria, except for RND superfamily that is only found in Gram negative bacteria (Handzlik et al., 2013).
According to the specific class they belong to, efflux pumps can be constituted by a single-component transporter or multiple-component systems containing not only an inner membrane transporter, but also an outer membrane channel and a periplasmic adaptor protein, such as the RND type efflux pumps (Li and Nikaido, 2004). In the following paragraphs we will see the peculiarity of each of these families.

RND family

The resistance-nodulation-cell-division **(**RND) superfamily of solute transporters consists of a large periplasmic domain that assembles with periplasmic fusion proteins and an outer membrane pore to form a complete tripartite channel from the inner cytoplasm past the outer membrane in Gram-negative bacteria (Li and Nikaido, 2004). This family of membrane proteins uses a proton antiport for transporting substances and it is capable of extruding completely harmful substances from the bacterial cell (Nikaido and Takatsuka, 2009). Multidrug recognition is based on a multisite drug-binding mechanism, in which two

voluminous multidrug-binding pockets in cell membrane exporters recognize a wide range of substrates as a result of permutations at numerous binding sites that are specific for the partial structures of substrate molecules. The voluminous multidrug-binding pocket may have numerous binding sites, seven for a single substrate, suggesting that substrates may move between binding sites during transport, an idea named as multisite-drug-oscillation hypothesis (Yamaguchi et al., 2015). The most well-known RND efflux pump is the AcrAB-TolC system of *E.coli* (Nikaido and Takatsuka, 2009).

MFS family

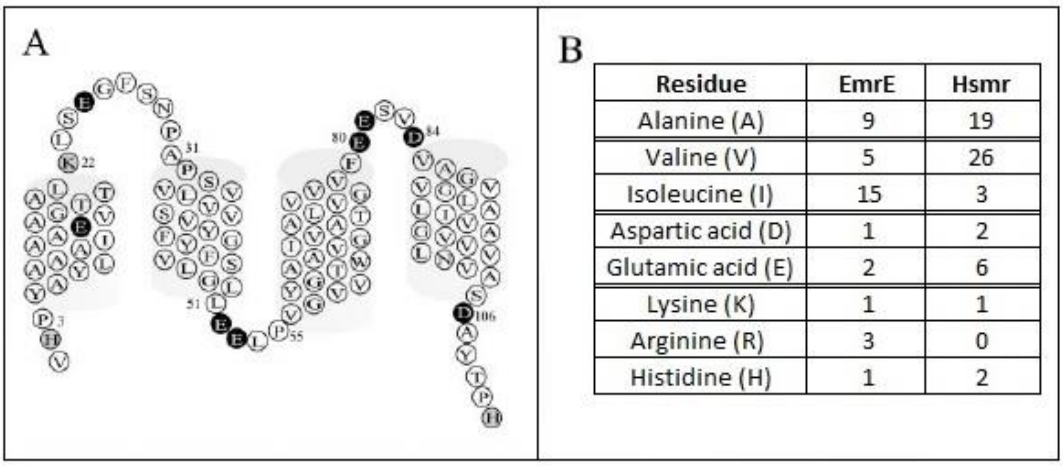
The MFS was originally believed to function primarily in the uptake of sugars (Henderson and Maiden, 1990). Subsequent studies revealed that the MFS family includes uptake or efflux systems for drugs, neurotransmitters, carboxylates, amino acids, osmolites, iron-siderophores, and nucleosides. Several sub-families have been implicated in drug efflux, in particular, the DHA12 and DHA14 families, which consist of proton-driven drug and multidrug efflux proteins (Paulsen et al., 2001). Members of these two families possess either twelve or fourteen transmembrane segments, respectively (Paulsen and Skurray, 1993), and include well characterized members such as Bmr from *Bacillus subtilis* and QacA from *Staphylococcus aureus*. A member of MFS family called Metabolite-H+-
Symporter (MHS) was found in *Haloarcula marismortui* and successfully expressed in *E. coli* (Ma et al., 2013).

ABC family

The number of ABC transporters differs widely between species. For example, almost 70 ABC proteins are codified in *Escherichia coli*, while other species present a few types (Higgins, 2001). It is remarkable to know that there is an ABC transporter for almost all compounds that must pass the cellular membrane, that leads to a variety of different structures of these transporters depending on the substrate they bind to. As a consequence, the substrate specificity depends on the physiological role of each ABC protein. However, the minimal structural organization of an ABC transporter is the presence of four domains, i.e., two nucleotide binding domains (NBDs) and two transmembrane permease domains (TMDs) (Lubelski et al., 2007). ABC transporters are present also in halophilic archaea (Konings et al., 2002), and in particular a transporter for corrinoids belonging to this family was found in *Halobacterium salinarum* NRC-1 (Woodson et al., 2005).

SMR family

The SMR family consists of a number of small, homologous proteins, ranging in size from 104 to 115 amino acyl residues. SMRs facilitate the removal of a broad variety of cationic sanitizing agents, dyes, and antibiotics from the bacterial cell through use of the proton motive force (Poulsen et al., 2011). The best characterized members of this family are the SMR protein of *S. aureus*, known as QacC, Smr and Ebr together with the *E. coli* one called Ebr, MvrC or EmrE (Paulsen et al., 1996). Interestingly, the first Smr in the archaeal kingdom, named Hsmr, has been found in *Halobacterium salinarum.* The Hsmr protein (shown in Figure 4A) presents many of the signature sequence elements of the SMR family. Moreover, it contains a high percentage of negative residues in the loops, characteristic of extreme halophiles. The gene encoding for Hsmr is very rich in GC (68% GC), whereas the percentage of coding GC in the *H. salinarum* genome is slightly lower (65%). At the amino acid level Hsmr shares 54% similarity and 43% identity with the EmrE protein. A simple comparison of the amino acid composition of Hsmr and EmrE reveals some striking differences, which are summarized in Figure 4B (Ninio and Schuldiner, 2003).



Residue	EmrE	Hsmr
Alanine (A)	9	19
Valine (V)	5	26
Isoleucine (I)	15	3
Aspartic acid (D)	1	2
Glutamic acid (E)	2	6
Lysine (K)	1	1
Arginine (R)	3	0
Histidine (H)	1	2

Figure 4. Secondary structure and amino acid composition of Hsmr. A, model of secondary structure of Hsmr based on hydropathy calculations and on analogy to EmrE. Putative transmembrane helices are shown as grey cylinders. Positive charged residues are highlighted, and negative charged are marked in black circles. B, the number of selected residues is given for EmrE (of a total of 110) and Hsmr (112) (Ninio and Schuldiner, 2003).

MATE family

The drug efflux pumps of the MATE family represent an important bacterial antimicrobial agent resistance mechanism. Although the length of proteins in the MATE family ranges from 400 to 700 amino acids, most members consist of 400–550 residues with 12 transmembrane helices. No apparent consensus sequence is conserved in all MATE proteins; however, these transporters are conserved in the three primary domains of life (Archaea, Bacteria and Eukarya), (Tanaka et al., 2013) and all MATE proteins share 40% sequence similarity (Omote et al., 2006). In general, almost all MATE family transporters can recognize fluoroquinolones as transport substrates, such as norfloxacin. Among cationic dyes, MATE transporters pump out acriflavine and ethidium bromide. It should be noted that aminoglycosides (especially kanamycin and streptomycin) might be good substrates (Kuroda and Tsuchiya, 2009). As a consequence, MATE-type transporters confer drug resistance for cationic drugs such as ethidium bromide (EtBr), tetraphenylphosphonium, berberine, acriflavine and norfloxacin (Omote et al., 2006). As concern the Archaea domain, the most well characterized member of the MATE family belongs to *Pyrococcus furiosus*, a member of the Euryarcheota phylum (Tanaka et al., 2013).

Methodologies for studying antibiotic resistance in bacteria

Basically the antibiotic resistance profile of a microorganism can be evaluated using two kinds of methods: phenotypic and genetic methods.

Conventional methods involve the isolation of the bacterium of interest from the various environments taken into consideration. Then the microorganism is exposed to different concentrations of the selected antimicrobial substance and the Minimal Inhibitory Concentration (MIC), that is the lowest concentration of a chemical that prevents visible growth of a bacterium, is defined. Methods that are frequently used for testing cultivated bacteria and yeasts include disk diffusion, broth dilution, agar dilution, and gradient diffusion (Epsilometer test). American approved standards are provided by the Clinical and Laboratory Standards Institute (CLSI).

Also genetic methods are very widespread. They assess the genotype of the microorganism, while with conventional methods the phenotype is determined. Secondly, they are more rapid than conventional ones because they don’t need the cultivation of the microorganism and they can be applied also to microorganisms difficult to cultivate or not cultivable. Finally they are less dangerous if the microorganism that has to be tested is a human pathogen.

The other side of the coin is that different assays have to be performed for each compound tested. Then, while with conventional methods all the mechanisms involved in the resistance against an antibiotic are checked at the same time, genetic methods detect only one specific mechanism at a time. This is the reason why culture based methods are useful for detecting emerging or new forms of antimicrobial resistance. Moreover, for some antibiotics the specific resistance mechanism is not clearly known, so it is no possible to detect this with genetic methods. Finally, unlike for conventional culture-based susceptibility test methods, no standards exist for performing genetic testing methods (Cockerill, 1999).

Halophilic archaea along the food chain

Halophilic archaea are a class of microorganisms belonging to the domain of Archaea. They require NaCl concentration of at least 1.5 M for proliferation, with optimal growth observed at 3.5–4.5 M (Dyall-Smith, 2008; Oren et al., 1997). Its members are found ubiquitously in environments containing sodium chloride concentrations up to saturation (Pfeifer, 2015), such as solar salterns (Manikandan et al., 2009; Pasić et al., 2005) and soda lakes (Corral et al., 2015).

In the last decade, these microorganisms have raised an increasing scientific interest among food microbiologists, since they have been found along the food chain both with culture dependent and independent methods (Lee, 2013). In particular they have been found in salt-fermented seafood (Roh et al., 2010, 2009, 2007a, 2007b; Roh and Bae, 2009), fish sauce (Tapingkae et al., 2008) and commercial salt (Henriet et al., 2014; Shimane et al., 2011; Shimoshige et al., 2013).

Archaea occurrence in human gastrointestinal tract

Archaea are naturally occurring components of the human and animal gut microbiota. The most abundant are methanogens and in particular *Methanobrevibacter smithii* and *Methanosphaera stadmanae* (Chaudhary et al., 2015). For this reason, it is not a coincidence that *M. smithii* was the first methanogen isolated from human stool (Nottingham and Hungate, 1968), even if it was identified as archaeon only 15 years later (Lurie-Weinberger and Gophna, 2015). In the gastrointestinal tract, methanogens are the main actors in the process of methanogenesis (Vanderhaeghen et al., 2015), but they seem to take part also in pathogenic

processes (Pimentel et al., 2012), and in particular they may be involved in inflammatory bowel diseases (Blais Lecours et al., 2014; Ge et al., 2015).

Another class found in the human gastrointestinal tract are halophilic archaea. Indeed they have been detected in human intestinal mucosa (Nkamga et al., 2017; Oxley et al., 2010) and in human stools (Khelaifia and Raoult, 2016) (Figure 5). In addition to metagenomic evidences, a recent study succeeded in isolating two halophilic archaea, *Haloferax alexandrinus* and *Haloferax massiliensis*, from human stool samples, confirming the effective presence of these microorganisms in the human gut (Lagier et al., 2016). However, their possible role in this environment has not yet been deeply investigated.

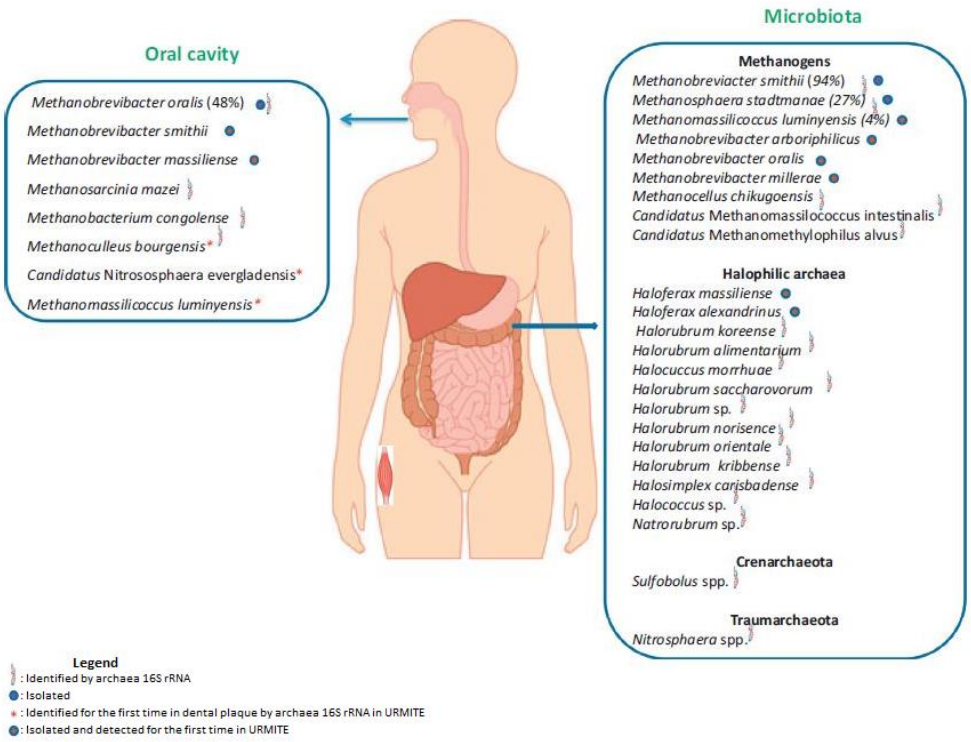


Figure 5. Overview of the Archaea detected and isolated in the human gastrointestinal tract (Nkamga et al., 2017). URMITE stands for Unité de recherche sur les maladies infectieuses et tropicales émergentes.

Antibiotic resistance studies in halophilic archaea

When firstly isolated, halophilic archaea were studied particularly for their exceptional ability to cope with high salt concentrations. For this reason the initial interest of scientists was aimed at understanding the mechanisms regulating such ability (Rodriguez-Valera, 1991). However, since the earlies 1980s, antibiotic resistance studies on these microorganisms started mainly for obtaining antibiotic-resistant mutants serving as genetic markers (Bonelo et al., 1984). The 1980s-1990s was the period when the majority of the studies were conducted: Pecher and Böck (1981) showed the insensitivity of *H. salinarum* to many protein synthesis inhibitors both of 70S and 80S ribosomes; Hilpert and collegues (1981) investigated nine strains of halophilic archaea assessing their insensitivity to the majority of antibiotics acting against Eubacteria and Eukaryotes; Forterre and collegues (1984) demonstrated the inhibition of halophilic archaea growth by aphidicolin, an antibiotic targeting eukaryotic α DNA polymerase; Hummel and Bock (1987) studied some mutations in *H. salinarum* 23S ribosomal RNA conferring resistance to anisomycin, Mankin and Garrett (1991) the *H. salinarum* (formerly *H. halobium*) mutations in the same gene conferring resistance to chloramphenicol while Holmes and Dyall-Smith (1991) the mutations in the DNA gyrase of two *Haloferax* strains conferring resistance to novobiocin; Nieto and collegues (1993) took in consideration 24 extremely halophilic archaea tested against 22 antimicrobial compounds in order to find antimicrobial resistance to use as genetic marker; Oren (1996) assessed the sensitivity of selected members of the family *Halobacteriaceae* to quinolone antibiotics.

In the following years the interest was not completely vanished (Ghosh et al., 2010; Shinde and Thombre, 2016) but sensibly decreased.

However, since up to now there is not a standardized method to assess the minimal inhibitory concentrations (MIC) in halophilic archaea, different methodologies were used, so MIC values of all these works cannot be compared. In addition, none of the works present in literature take in consideration at least 50 different strains of the same species of halophilic archaea, so the found values cannot be considered statistically significant.

References

ACE-ART: Assessment and Critical Evaluation of Antibiotic Resistance Transferability in Food Chain, EU Project. (CT-2004-506214). (http://www.aceart.net/).

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P., 2002. Molecular Biology of the Cell, 4th ed. Garland Science, New York.

Alekshun, M.N., Levy, S.B., 2007. Molecular Mechanisms of Antibacterial Multidrug Resistance. Cell 128, 1037–1050. doi:10.1016/j.cell.2007.03.004

Andreoletti, O., Budka, H., Buncic, S., Colin, P., Collins, J.D., De, A., Griffin, J., Havelaar, A., Hope, J., Klein, G., Kruse, H., Magnino, S., López, A.M., Mclauchlin, J., Nguyen-thé, C., Noeckler, K., Noerrung, B., Maradona, M.P., Roberts, T., Vågsholm, I., Vanopdenbosch, E., 2008. Foodborne antimicrobial resistance as a biological hazard Draft Scientific Opinion of the Panel on Biological Hazards Draft endorsed on 6 March 2008 1–91.

Armstrong, G.L., Conn, L.A., Pinner, R.., 1999. Trends in infectious disease mortality in the United States during the 20th century. J. Am. Med. Assoc. 281, 61–66.

Arthur, M., Autissier, D., Courvalin, P., 1986. Analysis of the nucleotide sequence of the ereB gene encoding the erythromydn esterase type II. Nucleic Acids Res. 14, 4987–4999. doi:10.1093/nar/14.12.4987

Bacot-Davis, V.R., Bassenden, A. V, Berghuis, A.M., 2016. Drug-target networks in aminoglycoside resistance: Hierarchy of priority in structural drug design. Medchemcomm 7, 103–113. doi:10.1039/c5md00384a

Beinlich, K.L., Chuanchuen, R., Schweizer, H.P., 2001. Contribution of multidrug efflux pumps to multiple antibiotic resistance in veterinary clinical isolates of Pseudomonas aeruginosa. FEMS Microbiol. Lett. 198, 129–134.

Blais Lecours, P., Marsolais, D., Cormier, Y., Berberi, M., Haché, C., Bourdages, R., Duchaine, C., 2014. Increased prevalence of Methanosphaera stadtmanae in inflammatory bowel diseases. PLoS One 9, e87734. doi:10.1371/journal.pone.0087734

Bonelo, G., Ventosa, A., Megás, M., Ruiz-Berraquero, F., 1984. The sensitivity of halobacteria to antibiotics. FEMS Microbiol. Lett. 21, 341–345. doi:10.1016/0378-1097(84)90095-8

Bories, G., Brantom, P., de Barberà, J.B., Chesson, A., Sandro, P., Debski, B., Dierick, N., Franklin, A., Gropp, J., Halle, I., Mantovani, A., Mézes, M., Nebbia, C., Rambeck, W., Rychen, G., Von, A., 2008. Technical guidance prepared by the Panel on Additives and

Products or Substances used in Animal Feed (FEEDAP) on the update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. EFSA J. 732, 1–15. doi:10.2903/j.efsa.2008.732

Borriello, S.P., Hammes, W.P., Holzapfel, W., Marteau, P., Schrezenmeir, J., M., V., Valtonen, V., 2003. Safety or of Probiotics That Contain Lactobacilli Bifidobactena. Clin. Infect. Dis. 36, 775–780.

Brown, M., Paulsen, I., Skurray RA, 1999. The multidrug efflux protein NorM is a prototype of a new family of transporters. Mol. Microbiol. 31, 394–395.

Cao, M., Bernat, B.A., Wang, Z., Armstrong, R.N., Helmann, J.D., 2001. FosB, a cysteine-dependent fosfomycin resistance protein under the control of σw, an extracytoplasmic-function σ factor in Bacillus subtilis. J. Bacteriol. 183, 2380–2383. doi:10.1128/JB.183.7.2380-2383.2001

Capita, R., Alonso-Calleja, C., 2013. Antibiotic-Resistant Bacteria: A Challenge for the Food Industry. Crit. Rev. Food Sci. Nutr. 53, 11–48. doi:10.1080/10408398.2010.519837

Chaudhary, P.P., Gaci, N., Borrel, G., O'Toole, P.W., Brugère, J.F., 2015. Molecular methods for studying methanogens of the human gastrointestinal tract: current status and future directions. Appl. Microbiol. Biotechnol. doi:10.1007/s00253-015-6739-2

Cockerill, F.R., 1999. Genetic methods for assessing antimicrobial resistance. Antimicrob. Agents Chemother. 43, 199–212.

Corral, P., Corcelli, A., Ventosa, A., 2015. Halostagnicola bangensis sp. nov., an alkaliphilic haloarchaeon from a soda lake. Int. J. Syst. Evol. Microbiol. 65, 754–9. doi:10.1099/ijs.0.000006

Dyall-Smith, M., 2008. The Halohandbook: protocols for halobacterial genetics. Mark Dyall-Smith, Martinsried, Ger. 1–144.

Efsa, Efsa FEEDAP, 2012. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. Efsa 10, 1–10. doi:10.2903/j.efsa.2012.2740.

Faour-Klingbeil, D., Todd, E.C.D., Kuri, V., 2016. Microbiological quality of ready-to-eat fresh vegetables and their link to food safety environment and handling practices in restaurants. LWT - Food Sci. Technol. 74, 224–233. doi:10.1016/j.lwt.2016.07.051

Flensburg, J., Skold, O., 1984. Regulatory changes in the formation of chromosomal dihydrofolate reductase causing resistance to trimethoprim. J. Bacteriol. 159, 184–190.

Forterre, P., Elie, C., Kohiyama, M., 1984. Aphidicolin inhibits growth and DNA synthesis in halophilic archaebacteria. J. Bacteriol. 159, 800–802.

Ge, T., Mu, C., Zhu, W., 2015. Methanogen and human gut health--A review. Wei Sheng Wu Xue Bao 55, 661–666.

Ghosh, R., Chattopadhyay, P.K., Chattopadhyay, B., Pal, D., 2010. Antibiotic resistance profile of halophilic microorganisms isolated from tannery effluent. Indian J. Biotechnol. 9, 80–86.

Handzlik, J., Matys, A., Kieć-Kononowicz, K., 2013. Recent Advances in Multi-Drug Resistance (MDR) Efflux Pump Inhibitors of Gram-Positive Bacteria S. aureus. Antibiotics 2, 28–45. doi:10.3390/antibiotics2010028

Henderson, P.J., Maiden, M.C., 1990. Homologous sugar transport proteins in Escherichia coli and their relatives in both prokaryotes and eukaryotes. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 326, 391–410. doi:10.1098/rstb.1990.0020

Henriet, O., Fourmentin, J., Delincé, B., Mahillon, J., 2014. Exploring the diversity of extremely halophilic archaea in food-grade salts. Int. J. Food Microbiol. 191C, 36–44. doi:10.1016/j.ijfoodmicro.2014.08.019

Higgins, C.F., 2001. ABC transporters: physiology, structure and mechanism--an overview. Res. Microbiol. 152, 205–210. doi:10.1016/S0923-2508(01)01193-7

Hilpert, R., Winter, J., Hammes, W., Kandler, O., 1981. The sensitivity of archaebacteria to antibiotics. Zentralblatt fur Bakteriol. Angew. und Okol. Microbiol. Abt.1 Orig.C Hyg.

Holmes, M.L., Dyall-Smith, M.L., 1991. Mutations in DNA gyrase result in novobiocin resistance in halophilic archaebacteria. J. Bacteriol. 173, 642–648.

Hummel, H., Bock, A., 1987. 23S Ribosomal RNA mutations in halobacteria conferring resistance to the anti-80S ribosome targeted antibiotic anisomycin. Nucleic Acids Res. 15, 2431–2443.

International Organization for Standardization and International Dairy Federation, 2010. Milk and milk products—determination of the minimal inhibitory concentration (MIC) of antibiotics applicable to bifidobacteria and non-enterococcal lactic acid bacteria (LAB). ISO 10932/IDF 223. International Organization for Standardization and International Dairy Federation, Geneva.

Khelaifia, S., Raoult, D., 2016. Haloferax massiliensis sp. nov., the first human-associated halophilic archaea. New Microbes New Infect. 12, 96–98. doi:10.1016/j.nmni.2016.05.007

Konings, W.N., Albers, S. V, Koning, S., Driessen, A.J.M., 2002. The cell membrane plays a crucial role in survival of bacteria and archaea in extreme environments. Antonie van Leeuwen 81, 61–72.

doi:10.1023/A:1020573408652

Kuroda, T., Tsuchiya, T., 2009. Multidrug efflux transporters in the MATE family. Biochim. Biophys. Acta - Proteins Proteomics 1794, 763–768. doi:10.1016/j.bbapap.2008.11.012

Lagier, J.C., Khelaifia, S., Alou, M.T., Ndongo, S., Dione, N., Hugon, P., Caputo, A., Cadoret, F., Traore, S.I., Seck, E.H., Dubourg, G., Durand, G., Mourembou, G., Guilhot, E., Togo, A., Bellali, S., Bachar, D., Cassir, N., Bittar, F., Delerce, J., Mailhe, M., Ricaboni, D., Bilen, M., Dangui Nieko, N.P., Dia Badiane, N.M., Valles, C., Mouelhi, D., Diop, K., Million, M., Musso, D., Abrahao, J., Azhar, E.I., Bibi, F., Yasir, M., Diallo, A., Sokhna, C., Djossou, F., Vitton, V., Robert, C., Rolain, J.M., La Scola, B., Fournier, P.E., Levasseur, A., Raoult, D., 2016. Culture of previously uncultured members of the human gut microbiota by culturomics. Nat Microbiol 1, 16203. doi:10.1038/nmicrobiol.2016.203

Lambert, P.A., 2005. Bacterial resistance to antibiotics: Modified target sites. Adv. Drug Deliv. Rev. 57, 1471–1485. doi:10.1016/j.addr.2005.04.003

Lee, H.-S., 2013. Diversity of halophilic archaea in fermented foods and human intestines and their application. J. Microbiol. Biotechnol. 23, 1645–53.

Leslie, A.G., Moody, P.C., Shaw, W. V, 1988. Structure of chloramphenicol acetyltransferase at 1.75-A resolution. Proc Natl Acad Sci U S A 85, 4133–4137. doi:10.1073/pnas.85.12.4133

Li, X.-Z., Nikaido, H., 2004. Efflux-mediated drug resistance in bacteria. Drugs 64, 159–204. doi:10.2165/11317030-000000000-00000

Losio, M.N., Pavoni, E., Bilei, S., Bertasi, B., Bove, D., Capuano, F., Farneti, S., Blasi, G., Comin, D., Cardamone, C., Decastelli, L., Delibato, E., De Santis, P., Di Pasquale, S., Gattuso, A., Goffredo, E., Fadda, A., Pisanu, M., De Medici, D., 2015. Microbiological survey of raw and ready-to-eat leafy green vegetables marketed in Italy. Int. J. Food Microbiol. 210, 88–91. doi:10.1016/j.ijfoodmicro.2015.05.026

Lubelski, J., Konings, W.N., Driessen, A.J.M., 2007. Distribution and physiology of ABC-type transporters contributing to multidrug resistance in bacteria. Microbiol. Mol. Biol. Rev. 71, 463–76. doi:10.1128/MMBR.00001-07

Lurie-Weinberger, M.N., Gophna, U., 2015. Archaea in and on the Human Body: Health Implications and Future Directions. PLoS Pathog. 11, 1–8.

Ma, P., Varela, F., Magoch, M., Silva, A.R., Rosário, A.L., Brito, J., Oliveira, T.F., Nogly, P., Pessanha, M., Stelter, M., Kletzin, A., Henderson, P.J.F., Archer, M., 2013. An Efficient Strategy for Small-Scale Screening and Production of Archaeal Membrane Transport Proteins in Escherichia coli. PLoS One 8, 1–14. doi:10.1371/journal.pone.0076913

Manikandan, M., Kannan, V., Pašić, L., 2009. Diversity of microorganisms in solar salterns of Tamil Nadu, India. World J. Microbiol. Biotechnol. 25, 1007–1017. doi:10.1007/s11274-009-9980-y

Mankin, A.S., Garrett, R.A., 1991. Chloramphenicol Resistance Mutations in the Single 23S rRNA Gene of the Archaeon Halobacterium halobium. J. Bacteriol. 173, 3559–3563.

Mayrhofer, S., Domig, K.J., Amtmann, E., Van Hoek, A.H. a M., Petersson, A., Mair, C., Mayer, H.K., Kneifel, W., 2007. Antibiotic susceptibility of Bifidobacterium thermophilum and Bifidobacterium pseudolongum isolates from animal sources. J. Food Prot. 70, 119–24.

McManus, P.S., Stockwell, V.O., Sundin, G.W., Jones, A.L., 2002. Antibiotic use in plant agriculture. Annu. Rev. Phytopathol. 40, 443–465. doi:10.1146/annurev.phyto.40.120301.093927

Morar, M., Pengelly, K., Koteva, K., Wright, G.D., 2012. Mechanism and diversity of the erythromycin esterase family of enzymes. Biochemistry 51, 1740–1751. doi:10.1021/bi201790u

Morisaki, N., Iwasaki, S., Yazawa, K., Mikami, Y., Maeda, A., 1993. Inactivated products of rifampicin by pathogenic Nocardia spp.: structures of glycosylated and phosphorylated metabolites of rifampicin and 3-formylrifamycin SV. J. Antibiot. (Tokyo). 46, 1605–10.

Mukhtar, T.A., Koteva, K.P., Hughes, D.W., Wright, G.D., 2001. Vgb from Staphylococcus aureus inactivates streptogramin B antibiotics by an elimination mechanism not hydrolysis. Biochemistry 40, 8877–8886. doi:10.1021/bi0106787

Nakamura, A., Nakazawa, K., Miyakozawa, I., Mizukoshi, S., Tsurubuchi, K., Nakagawa, M., O'Hara, K., Sawai, T., 2000. Macrolide esterase-producing Escherichia coli clinically isolated in Japan. J. Antibiot. (Tokyo). 53, 516–524.

Nieto, J.J., Fernandez-Castillo, R., Garcia, M.T., Mellado, E., Ventosa, A., 1993. Survey of antimicrobial susceptibility of moderately halophilic eubacteria and extremely halophilic aerobic archaeobacteria: Utilization of antimicrobial resistance as a genetic marker. Syst. Appl. Microbiol. 16, 352–360.

Nikaido, H., Takatsuka, Y., 2009. Mechanisms of RND multidrug efflux pumps. Biochim. Biophys. Acta - Proteins Proteomics 1794, 769–781. doi:10.1016/j.bbapap.2008.10.004

Ninio, S., Schuldiner, S., 2003. Characterization of an archaeal multidrug transporter with a unique amino acid composition. J. Biol. Chem. 278, 12000–12005. doi:10.1074/jbc.M213119200

Nkamga, V.D., Henrissat, B., Drancourt, M., 2017. Archaea: Essential inhabitants of the

human digestive microbiota. Hum. Microbiome J. 3, 1–8. doi:10.1016/j.humic.2016.11.005

Noguchi, N., Emura, A., Matsuyama, H., O'Hara, K., Sasatsu, M., Kono, M., 1995. Nucleotide sequence and characterization of erythromycin resistance determinant that encodes macrolide 2'-phosphotransferase I in Escherichia coli. Antimicrob. Agents Chemother. 39, 2359–2363. doi:10.1128/AAC.39.10.2359

Nottingham, P.M., Hungate, R.E., 1968. Isolation of methanogenic bacteria from feces of man. J. Bacteriol. 96, 2178–2179.

Ogawara, H., 1981. Antibiotic resistance in pathogenic and producing bacteria, with special reference to β-lactam antibiotics. Microbiol. Rev. 45, 591–619.

Omote, H., Hiasa, M., Matsumoto, T., Otsuka, M., Moriyama, Y., 2006. The MATE proteins as fundamental transporters of metabolic and xenobiotic organic cations. Trends Pharmacol. Sci. 27, 587–593. doi:10.1016/j.tips.2006.09.001

Oren, A., 1996. Sensitivity of selected members of the family Halobacteriaceae to quinolone antimicrobial compounds. Arch. Microbiol. 165, 354–358. doi:10.1007/s002030050338

Oren, A., Ventosa, A., Grant, W.D., 1997. Proposed Minimal Standards for Description of New Taxa in the Order Halobacteriales. Int. J. Syst. Bacteriol. 47, 233–238. doi:10.1099/00207713-47-1-233

Ounissi, H., Courvalin, P., 1985. Nucleotide sequence of the gene ereA encoding the erythromycin esterase in Escherichia coli. Gene 35, 271–278. doi:10.1016/0378-1119(85)90005-8

Oxley, A.P. a, Lanfranconi, M.P., Würdemann, D., Ott, S., Schreiber, S., McGenity, T.J., Timmis, K.N., Nogales, B., 2010. Halophilic archaea in the human intestinal mucosa. Environ. Microbiol. 12, 2398–2410. doi:10.1111/j.1462-2920.2010.02212.x

Pao, S.S., Paulsen, I.T., Saier, M.H., 1998. Major facilitator superfamily. Microbiol. Mol. Biol. Rev. 62, 1–34.

Pasić, L., Bartual, S.G., Ulrih, N.P., Grabnar, M., Velikonja, B.H., 2005. Diversity of halophilic archaea in the crystallizers of an Adriatic solar saltern. FEMS Microbiol. Ecol. 54, 491–8. doi:10.1016/j.femsec.2005.06.004

Paulsen, I.T., Chen, J., Nelson, K.E., Saier, M.H., 2001. Comparative genomics of microbial drug efflux systems. J. Mol. Microbiol. Biotechnol. 3, 145–150.

Paulsen, I.T., Skurray, R.A., 1993. Topology, structure and evolution of two families of proteins involved in antibiotic and antiseptic resistance in eukaryotes and prokaryotes — an analysis. Gene 124, 1–11. doi:10.1016/0378-1119(93)90755-R

Paulsen, I.T., Skurray, R.A., Tam, R., Saier, M.H., Turner, R.J., Weiner, J.H., Goldberg, E.B., Grinius, L.L., 1996. The SMR family: a novel family of multidrug efflux proteins involved with the efflux of lipophilic drugs. Mol. Microbiol. 19, 1167–1175. doi:10.1111/j.1365-2958.1996.tb02462.x

Pecher, T., Böck, A., 1981. In vivo susceptibility of halophilic and methanogenic organisms to protein synthesis inhibitors. FEMS Microbiol. Lett. 10, 295–297.

Pfeifer, F., 2015. Haloarchaea and the Formation of Gas Vesicles. Life 5, 385–402. doi:10.3390/life5010385

Piddock, L.J. V, 2006. Multidrug-resistance efflux pumps - not just for resistance. Nat. Rev. Microbiol. 4, 629–636. doi:10.1038/nrmicro1464

Pimentel, M., Gunsalus, R.P., Rao, S.S., Zhang, H., 2012. Methanogens in Human Health and Disease. Am. J. Gastroenterol. Suppl. 1, 28–33. doi:10.1038/ajgsup.2012.6

Poole, K., 2004. Efflux-mediated multiresistance in Gram-negative bacteria. Clin. Microbiol. Infect. 10, 12–26. doi:10.1111/j.1469-0691.2004.00763.x

Poole, K., 2002. Mechanisms of bacterial biocide and antibiotic resistance. J. Appl. Microbiol. Symp. Suppl. 92, 55S–64S. doi:1627 [pii]

Poulsen, B.E., Cunningham, F., Lee, K.K.Y., Deber, C.M., 2011. Modulation of substrate efflux in bacterial small multidrug resistance proteins by mutations at the dimer interface. J. Bacteriol. 193, 5929–5935. doi:10.1128/JB.05846-11

Putman, M., van Veen, H.W., Konings, W.N., 2000. Molecular properties of bacterial multidrug transporters. Microbiol. Mol. Biol. Rev. 64, 672–93. doi:10.1128/MMBR.64.4.672-693.2000

Quan, S., Venter, H., Dabbs, E.R., 1997. Ribosylative inactivation of rifampin by Mycobacterium smegmatis is a principal contributor to its low susceptibility to this antibiotic. Antimicrob. Agents Chemother. 41, 2456–2460.

Ramirez, M.S., Tolmasky, M.E., 2010. Aminoglycoside modifying enzymes. Drug Resist. Updat. 13, 151–171. doi:10.1016/j.drup.2010.08.003

Rodriguez-Valera, F., 1991. General and Applied Aspects of Halophilic Microorganisms. Plenum Press, New York. doi:10.1007/978-1-4615-3730-4

Roh, S.W., Bae, J.W., 2009. Halorubrum cibi sp. nov., an extremely halophilic archaeon from salt-fermented seafood. J. Microbiol. 47, 162–166. doi:10.1007/s12275-009-0016-y

Roh, S.W., Lee, M.L., Bae, J.W., 2010. Haladaptatus cibarius sp. nov., an extremely halophilic archaeon from seafood, and emended description of the genus Haladaptatus. Int. J. Syst. Evol. Microbiol. 60, 1187–1190. doi:10.1099/ijs.0.013037-0

Roh, S.W., Nam, Y. Do, Chang, H.W., Kim, K.H., Sung, Y., Kim, M.S., Oh, H.M., Bae, J.W., 2009. Haloterrigena jeotgali sp. nov., an extremely halophilic archaeon from salt-fermented food. Int. J. Syst. Evol. Microbiol. 59, 2359–2363. doi:10.1099/ijs.0.008243-0

Roh, S.W., Nam, Y. Do, Chang, H.W., Sung, Y., Kim, K.H., Oh, H.M., Bae, J.W., 2007a. Halalkalicoccus jeotgali sp. nov., a halophilic archaeon from shrimp jeotgal, a traditional Korean fermented seafood. Int. J. Syst. Evol. Microbiol. 57, 2296–2298. doi:10.1099/ijs.0.65121-0

Roh, S.W., Nam, Y. Do, Chang, H.W., Sung, Y., Kim, K.H., Oh, H.M., Bae, J.W., 2007b. Natronococcus jeotgali sp. nov., a halophilic archaeon isolated from shrimp jeotgal, a traditional fermented seafood from Korea. Int. J. Syst. Evol. Microbiol. 57, 2129–2131. doi:10.1099/ijs.0.65121-0

Rosenquist, H., Smidt, L., Andersen, S.R., Jensen, G.B., Wilcks, A., 2005. Occurrence and significance of Bacillus cereus and Bacillus thuringiensis in ready-to-eat food. FEMS Microbiol. Lett. 250, 129–136. doi:10.1016/j.femsle.2005.06.054

Shimane, Y., Hatada, Y., Minegishi, H., Echigo, A., Nagaoka, S., Miyazaki, M., Ohta, Y., Maruyama, T., Usami, R., Grant, W.D., Horikoshi, K., 2011. Salarchaeum japonicum gen. nov., sp. nov., an aerobic, extremely halophilic member of the Archaea isolated from commercial salt. Int. J. Syst. Evol. Microbiol. 61, 2266–2270. doi:10.1099/ijs.0.025064-0

Shimoshige, H., Yamada, T., Minegishi, H., Echigo, A., Shimane, Y., Kamekura, M., Itoh, T., Usami, R., 2013. Halobaculum magnesiiphilum sp. nov., a magnesium-dependent haloarchaeon isolated from commercial salt. Int. J. Syst. Evol. Microbiol. 63, 861–866. doi:10.1099/ijs.0.037432-0

Shinde, V., Thombre, R., 2016. Antibiotic resistance profiling of marine halophilic bacteria and haloarchaea. J. Appl. Pharm. Sci. 6, 132–137. doi:10.7324/JAPS.2016.601018

Skinner, R.H., Cundliffe, E., 1980. Resistance to the Antibiotics Viomycin and Capreomycin in the Streptomyces Species Which Produce Them. J. Gen. Microbiol. 120, 95–104. doi:10.1099/00221287-120-1-95

Sugantino, M., Roderick, S.L., 2002. Crystal structure of Vat(D): An acetyltransferase that inactivates streptogramin group A antibiotics. Biochemistry 41, 2209–2216. doi:10.1021/bi011991b

Tanaka, Y., Hipolito, C.J., Maturana, A.D., Ito, K., Kuroda, T., Higuchi, T., Katoh, T., Kato, H.E., Hattori, M., Kumazaki, K., Tsukazaki, T., Ishitani, R., Suga, H., Nureki, O., 2013. Structural basis for the drug extrusion mechanism by a MATE multidrug transporter. Nature 496, 247–51. doi:10.1038/nature12014

Tapingkae, W., Tanasupawat, S., Itoh, T., Parkin, K.L., Benjakul, S., Visessanguan, W., Valyasevi, R., 2008. Natrinema gari sp. nov., a halophilic archaeon isolated from fish sauce in Thailand. Int. J. Syst. Evol. Microbiol. 58, 2378–2383. doi:10.1099/ijs.0.65644-0

Teale, C.J., 2002. Antimicrobial resistance and the food chain. Symp. Ser. Soc. Appl. Microbiol. 85S–89S. doi:10.1046/j.1365-2672.92.5s1.20.x

Vanderhaeghen, S., Lacroix, C., Schwab, C., 2015. Methanogen communities in stools of humans of different age and health status and co-occurrence with bacteria. FEMS Microbiol. Lett. 362. doi:10.1093/femsle/fnv092

Woodson, J.D., Reynolds, A.A., Escalante-Semerena, J.C., 2005. ABC transporter for corrinoids in Halobacterium sp. strain NRC-1. J. Bacteriol. 187, 5901–5909. doi:10.1128/JB.187.17.5901-5909.2005

World Health Organisation, 2011. Tackling antibiotic resistance from a food safety perspective in Europe. World Heal. Organ. 1–88. doi:www.euro.who.int/en/publications/abstracts/tackling-antibiotic-resistance-from-a-food-safety-perspective-in-europe

Wright, G.D., 2005. Bacterial resistance to antibiotics: Enzymatic degradation and modification. Adv. Drug Deliv. Rev. 57, 1451–1470. doi:10.1016/j.addr.2005.04.002

Wybenga-Groot, L.E., Draker, K. ann, Wright, G.D., Berghuis, A.M., 1999. Crystal structure of an aminoglycoside 6'-N-acetyltransferase: Defining the GCN5-related N-acetyltransferase superfamily fold. Structure 7, 497–507. doi:10.1016/S0969-2126(99)80066-5

Yamaguchi, A., Nakashima, R., Sakurai, K., 2015. Structural basis of RND-type multidrug exporters. Front. Microbiol. 6, 1–19. doi:10.3389/fmicb.2015.00327

Yang, W., Moore, I.F., Koteva, K.P., Bareich, D.C., Hughes, D.W., Wright, G.D., 2004. TetX is a flavin-dependent monooxygenase conferring resistance to tetracycline antibiotics. J. Biol. Chem. 279, 52346–52352. doi:10.1074/jbc.M409573200

Yong, D., Toleman, M.A., Giske, C.G., Cho, H.S., Sundman, K., Lee, K., Walsh, T.R., 2009. Characterization of a new metallo-β-lactamase gene, bla NDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrob. Agents Chemother. 53, 5046–5054. doi:10.1128/AAC.00774-09