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REVIEW

Emergence of antibiotic-resistant extremophiles (AREs)

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Abstract Excessive use of antibiotics in recent years has produced bacteria that are resistant to a wide array of antibiotics. Several genetic and non-genetic elements allow microorganisms to adapt and thrive under harsh environmental conditions such as lethal doses of antibiotics. We attempt to classify these microorganisms as antibiotic-resistant extremophiles (AREs). AREs develop strategies to gain greater resistance to antibiotics via accumulation of multiple genes or plasmids that harbor genes for multiple drug resistance (MDR). In addition to their altered expression of multiple genes, AREs also survive by producing enzymes such as penicillinase that inactivate antibiotics. It is of interest to identify the underlying molecular mechanisms by which the AREs are able to survive in the presence of wide arrays of high-dosage antibiotics. Technologically, "omics"-based approaches such as genomics have revealed a wide array of genes differentially expressed in AREs. Proteomics studies with 2DE, MALDI-TOF, and MS/MS have identified specific proteins, enzymes, and pumps that function in the adaptation mechanisms of AREs. This article discusses the molecular mechanisms by which microorganisms develop into AREs and how "omics" approaches can identify the genetic elements of these adaptation mechanisms. These objectives will assist the development of strategies and potential therapeutics to treat outbreaks of pathogenic microorganisms in the future.

Keywords Antibiotic-resistant extremophiles · AREs · Antibiotics · Genomics · Proteomics

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Introduction

Antibacterial resistance has been defined as the ability of microorganisms to remain viable and multiply in the presence of antibiotics. Resistance to antimicrobial agents is a global health concern. Increasing usage and the remains of antibiotics in the environment lead to the development of bacteria into superbugs, capable of adapting and surviving in the presence of multiple classes of antibiotics due to gradual modifications in their antibiotic resistance genes. Because these organisms are resistant to multiple and high-dose antibiotics, we attempt to classify them as antibiotic-resistant extremophiles (AREs). The closely related term *extremophiles* applies to organisms, including microbes, that can sustain life in extreme environmental conditions such as hot springs, volcanic areas, the deep sea, extreme high and low temperatures (>45 °C, <15 °C), high pressure, high salt concentration, radiation, etc. (Mesbah and Wiegel 2008; Kumar et al. 2010; Singh and Gabani 2011; Copeland et al. 2012), and now high levels of antibiotics. In response to the damage caused by antibiotics, AREs are able to induce genes that modify certain proteins and enzymes toward adaptations that lead to their resistance and survival in the presence of a broad range of antibiotics (Rossolini and Thaller 2010). These organisms even show normal growth as would be observed when grown in the presence of antibiotics. For example, treatment of *Stenotrophomonas maltophilia* with ciprofloxacin and gentamicin showed that

the bacterial counts at the end of 24 h exceeded the starting inoculum in a normal growth fashion (Garrison et al. 1996). In another study, a treatment of planktonic bacteria with 25 and 59 mg/kg of linezolid resulted in no significant difference when compared to a treatment with saline (Baldoni et al. 2009).

The common usage of antibiotics for human therapies as well as in agriculture, aquaculture, and livestocks has resulted in adaptations among pathogenic bacteria and their development of resistance to multiple drugs. A well-known case is the methicillin-resistant *Staphylococcus aureus* (MRSA), which is resistant to aminoglycosides, macrolides, tetracycline, chloramphenicol, and linocosamides (Okesola 2011). Other multidrug-resistant (MDR) pathogens currently being investigated include *Acinetobacter baumannii*, *Mycobacterium tuberculosis*, and *Pseudomonas aeruginosa* (Carattoli 2009). MDR *M. tuberculosis*, which is resistant to first- and second-line antibiotic drugs, has recently been identified in Nepal, China, and India and is causing widespread health care crises in these countries (Engstrom et al. 2012; Poudel et al. 2012). Almost 23 % of the currently known strains of *P. aeruginosa* are MDR (Gill et al. 2011). An increase in MDR *P. aeruginosa* has been linked to an increase in septic shock, leading to an increase in patient mortality (Taccone et al. 2012). The widespread use of antibiotics will continue to develop more species of bacteria into MDR organisms. Others in line to become threats to the human population due to their multidrug resistance include *P. aeruginosa* and *A. baumannii*.

Through microbial evolution, bacteria have developed various types of mechanisms for antibiotic resistance. The classes of drugs against which bacteria have developed resistance include β-lactams, glycopeptides, and fluroquinolones (Woodford and Livermore 2009; Rice 2012). Microorganisms can be turned into AREs through gene mutations (Breidenstein et al. 2011; Canton and Morosini 2011) or via exogenous sources such as transferable plasmids that contain a variety of resistance genes (Rice 2012). Multiple AREs have shown different adaptation strategies, such as acquisition of highly efficient efflux pumps, which are actively and efficiently able to exocytose a wide variety of antibiotics (Higgins 2007). Another important evolutionary strategy, spontaneous mutations, allows bacteria to acquire genes, proteins, and enzymes that they then use to combat the deadly effects of antibiotics (Erill et al. 2006). These mechanisms of mutation, transferability, and evolution of antibiotic resistance genes evolve the new generation of antibiotic-resistant bacteria. This article aims to define AREs, their environment and the mechanisms by which microbes turn into AREs coping with high levels of antibiotics. A brief description of genomics and proteomics technologies will fill the gaps in understanding the global profile of genes and proteins that assist microbial modification into AREs.

Antibiotic-resistant extremophiles and environment

Food and Drug Administration (FDA) approved and clinically proven safest dose of different classes of antibiotics that has ability to inhibit microbial growth in the society are being considered as regular antibiotics. However, over the past few decades' extensive use of antibiotics has enforced microorganisms to adapt and survive in the presence of approved dosages, thereby evolving into the superbug. Adapted microorganisms, which are able to thrive at higher dosage of different classes of antibiotics [i.e., aminoglycosides, ansamycins, carbacephem, cephalosporins (various generations), glycopeptides, lincosamides, lipopeptide, macrolides, β-lactams, sulfonamides, and quinolones], otherwise lethal to normal microorganisms, are being referred as 'AREs'.

Unmetabolized drugs from the extensive use of antibiotics in human and animal therapeutics are excreted in urine and fecal matter and end up in recipient waters (Diwan et al. 2010). In addition, the antibiotics find their way into the environment from pharmaceutical plants as a result of dumping of wastes and unused antibiotics (Kummerer 2009). In developing countries, the higher levels (31 g/L) of various antibiotics, chemicals and heavy metals were detected in recipient waters (Larsson et al. 2007). Despite the efforts of the wastewater treatment, the high levels of antibiotics were detected 17 km downstream of the site, finding their ways to get into groundwater used for human consumption (Fick et al. 2009).

In addition to aquatic environments, hospitals are prime sites in which bacteria are continuously exposed to the antibiotics. Diwan et al. (2010) reported 236.6 μg/L ciprofloxacin in the wastewater from hospital. In Europe, the first generation cephalosporin usage increased by $\sim$3 times and the second generation by 20 % between 1997 and 2009. A substantial increase of the third generation was also reported in other countries such as France, Italy, and Russia (Versporten et al. 2011). In Singaporean hospitals, the prescription of monitored antibiotics was increased from 233.12 defined daily doses (DDD)/1,000 inpatient-days in 2006 to 254.38 DDD/1000 inpatient-days in 2010. In Switzerland, the total consumption of systemic antibiotics rose from 46.1 DDD/100 inpatient-days to 54.0 DDD/100 inpatient-days between 2004 and 2008 (Pluss-Suard et al. 2011). These over-usages of antibiotics resulted into remains and excretion of unmetabolized antibiotics and their presence in wastewater providing a perfect environment to antibiotic sensitive microorganisms to adapt and/or mutate in which the AREs can thrive. The overuse of antibiotics in humans and animals makes them the best reservoir where bacteria can be adapted to high dose of antibiotics under perfect living conditions. The microbial communities exposed to the high levels of antibiotics under

both in vivo and in vitro conditions circumvent to thrive under great selective pressure forcing them to evolve into AREs.

With continued modification of microbial genome, several of the organisms that have developed antibiotic resistance from nature have also been found in human cultures. Clinical strains of *A. baumannii* showed resistance to minimal inhibitory concentration (MICs) of over 64 mg/L Imipenem, 64 mg/L Sulbactam, and 512 mg/L Fosfomycin (Santimaleeworagun et al. 2011). Table 1 summarizes few AREs that have shown resistance to MIC of various antimicrobial agents. It has also been found that the presence of metallo-β-lactamase has shown to have a higher MIC in *P. aeruginosa* than the non metallo-β-lactamase producing strains in patients with pneumonia in intensive care units (Vitkauskiene et al. 2011). *P. aeruginosa* has been found to be resistant to MICs of meziocillin (75 mg/L), ceftazidime (30 mg/L), and cefepime (30 mg/L) in patients with lower respiratory tract infections acquired in intensive care unit (Ozer et al. 2012). Four isolates of *Salmonella entrica* were found to be resistant to MICs of antibiotic Ceftriaxone (256 mg/L) from human clinical specimens (Firoozeh et al. 2011). The ability of these organisms to survive and thrive above the MIC can characterize them as AREs.

In addition to the excessive use of antibiotics in human therapeutics, the wide use of antibiotics in livestock has also led to the adaptation of antibiotic-resistant bacteria into AREs. A recent study in Ghana indicated that over 98 % of the farmers used antibiotics on animals to prevent infections and many even used them on a weekly basis (Donkor et al. 2012). Recently, it was determined that the wide use of antibiotics in poultry and pigs in Australia caused several strains of *Campylobacter* sp. (isolated from fecal samples) to develop resistance to MIC of lincomycin (51–100 %), ampicillin (33.3–60.2 %), and tetracycline (5.6–40.7 %) (Obeng et al. 2012). Similarly, in Switzerland, a countrywide survey found that third-generation cephalosporin-resistant *E. coli* were found in broilers, swine, and cattle (Endimiani et al. 2012). Many strains in animals were found to have a higher resistance of drugs when compared to humans. For example, *E. coli* isolated from animals revealed a higher resistance than that of human isolates for ampicillin (Donkor et al. 2012). In addition, the overall prevalence of multiple drug resistance among the *E. coli* isolates was 91.6 % whereas in the human isolates was only 70.6 % (Donkor et al. 2012). The large families of antibiotics have various novel means of inhibiting cellular growth in microorganisms (Blanchard 2005; Sherman 2005; Clardy et al. 2006). However, many bacteria have developed novel mechanisms to combat the deadly effects of antibiotics. The antibiotic-resistant extremophiles have multiple molecular mechanistic adaptations to eliminate antibiotics from their cytoplasm as well as inactivate them, thus rendering them ineffective.

Table 1 Potential antibiotic-resistant microorganisms (AREs) in human subjects

Microorganism	Antibiotics	MIC (mg/L)	Ref.
A. baumannii AB307	Imipenem	64	Santimaleeworagun et al. (2011)
	Sulbactam	64	
A. baumannii AB167	Fosfomycin	256	Santimaleeworagun et al. (2011)
A. baumannii AB315	Fosfomycin	512	
P. aeruginosa	Ciprofloxacin	64	Vitkauskiene et al. (2011)
	Amikacin	256	
	Piperacillin	256	
	Mezlocillin	75	Ozer et al. (2012)
	Piperacillin	100	
S. enterica	Ceftriaxone	256	Firoozeh et al. (2011)
H. pylori	Metronizadole	8	Eisig et al. (2011)
	Clarithromycin	1	
S. pneumonniae	Cefaclor	>32	Kiffer and Pignatari (2011)
	Azithromycin	>32	
H. influenzae	Amoxicillin	16	
M. catarralis	Amoxicillin	32	

Antibiotic efflux pumps

Most pathogens resist the action of antibiotics through pumps, which drive microbial metabolic products including physiological substrates, non-antibiotic substrates, and antibiotics into (influx) or outside (efflux) the cell (Liang et al. 1995; Brown et al. 1999; Jariyawat et al. 1999; Ribera et al. 2002). In clinically relevant microorganisms, five different protein-family-based efflux pumps have been identified: the multidrug and toxic compound extrusion (MATE) family (Brown et al. 1999), the adenosine triphosphate (ATP) binding cassette (ABC) superfamily, such as P-type ATPase (van Veen and Konings 1998), the small multidrug resistance (SMR) family (Paulsen et al. 1996), the major facilitator superfamily (MFS) (Marger and Saier 1993), and the resistance-nodulation-cell division (RND) family (Saier et al. 1994). The three major types of efflux pumps MFS, SMR and RND are discussed below.

The natural functions of the various efflux pumps are still a topic of debate. However, the physiological role of these systems is evasion of naturally produced toxic molecules, thereby allowing the bacterium to survive in its ecological niche. Natural functions suggested for efflux pumps include removal of metabolic products, removal of

toxins, and buffering the organism against surges in pools of potentially toxic metabolites (Helling et al. 2002). It has been also suggested that the natural function of RND pumps might include the efflux of molecules required for cell-to-cell signaling such as quorum sensing (Rahmati et al. 2002). The MATE transporters are known to confer resistance to multiple cationic toxic agents, including fluoroquinolones, as H^+ and Na^+ antiporters. However, the substrate profiles for this family are generally narrower than those of the RND transporters. To this day, only about 20 MATE transporters have been characterized (Kuroda and Tsuchiya 2009).

MFS efflux pumps

MFS transporters are known to represent the largest group of secondary active transporters. These transporters are antiporters that are thought to function as monomers. However, in Gram-negative bacteria, MFS efflux systems can function as components of tripartite systems together with additional membrane fusion proteins and outer membrane channels (Pao et al. 1998). These systems enable the transporter to efficiently export the substrates across the double membranes of Gram-negative bacteria. This system has the largest family of transport proteins and it contains many important efflux pumps. Two major types of pumps that exist in this category are 14 transmembrane segments and 12 transmembrane segments.

QacA and QacB pumps are involved in actively pumping biocides and dyes such as ethidium bromide, benzalkonium chloride, cetyltrimethylammonium bromide, pentamideine isethionate, and chlorhexidine (Paulsen et al. 1996). EmrB of *E. coli* also belonging to this category allows for resistance of compounds such as carbonyl cyanide *m*-chlorophenylhydrazone and antibiotics such as nalidizic acid and thiolactomycin (Lomovskaya and Lewis 1992). Floyd et al. (2010) observed that LmrS, a MFS efflux pump of *S. aureus*, was able to actively pump a 16-fold increase in linezolid and tetraphenylphosphonium and an eightfold increase in sodium dodecyl sulfate, trimethoprim, and chloramphenicol. NorA of *S. aureus* is able to actively pump puromycin, tetraphenylphosphonium, and fluoroquinolones (Neyfakh et al. 1993). In addition to this, *S. aureus* also contains NorB and NorC. In about one-half of *S. aureus* infections more than half of the strains had overexpression of NorB and NorC, two chromosomally coded pumps (DeMarco et al. 2007).

In addition to *S. aureus*, MFS pumps have been found in *A. baumannii* and *E. coli*. A MFS pump AmvA belonging to *A. baumannii* had decreased susceptibility to antibiotics, disinfectants, dyes, and detergents, as well as greatly enhanced efflux activity (Rajamohan et al. 2010). Smith et al. (2009) identified a multidrug pump, EmrD-3, belonging to the MFS of transporters and shared homology with the Bcr/CflA subfamily in *Vibrio cholera*. In *E. coli*, this pump was able to effectively lower intracellular concentrations of ethidium bromide, linezolid, tetraphenylphosphonium chloride, rifampin, erythromycin, minocycline, trimethoprim, and chloramphenicol.

SMR efflux pumps

SMR efflux pumps were first reported in *S. aureus* plasmids, followed by chromosomes of Gram-negative bacteria. EmrE of *E. coli* is a well recognized SMR pump that function as a homodimer of a small four-transmembrane protein (Jack et al. 2001; Schuldiner 2007). The inward flux of the proton and outward flux of the substrate are coupled by the sharing of the common binding sites (Schuldiner 2007).

SMR pumps have been proven to be very effective. Expression of a cloned *abeS* gene of the SMR family from *A. baumannii* in a hypersensitive *E. coli* host KAM32 resulted in a decreased susceptibility to various classes of antimicrobial agents, detergents, and dyes. In addition, the deletion of *abeS* from *A. baumannii* caused the resistive strain to become sensitive to various antimicrobial agents (Srinivasan et al. 2009). An SMR family gene, *ssmE*, found in *Serratia marcescens* and cloned into hypersensitive *E. coli*, also produced elevated levels of resistance to antimicrobial agents. It was able to effectively efflux ethidium in an energy-dependent fashion (Minato et al. 2008). A two-component multidrug efflux pump, EbrA and EbrB, in *Bacillus subtilis* also showed an elevated energy-dependent efflux of ethidium in *E. coli*. Neither was found to be sufficient for resistance when alone, but the simultaneous expression of EbrA and EbrB in *E. coli* made it resistant to a wide array of antimicrobial agents (Masaoka et al. 2000).

RND efflux pumps

RND efflux systems function as proton/drug antiporters and are widespread among Gram-negative bacteria. The efflux systems belonging to the RND family confer resistance to aminoglycosides and decreased susceptibility to fluoroquinolones, tetracycline, chloramphenicol, erythromycin, trimethroprim, netilmicin, and meropenem. The transport proteins of this family play a predominant role in the multidrug resistance of Gram-negative bacteria. Two examples of these types of pumps are AcrB of *E. coli* and MexB of *P. aeruginosa* (Tseng et al. 1999). These pumps are associated with two other classes of proteins: the outer membrane factor family of proteins, such as TolC of *E. coli*, and membrane fusion proteins, such as ArcA of *E. coli* and MexA of *P. aeruginosa* (Dinh et al. 1994;

Paulsen et al. 1997). Expression of these pumps allows for Gram-negative bacteria to become resistant to penicillin G, oxacillin, cloxacillin, nafcillin, macrolides, novobiocin, linezolid, and fusidic acid. Inactivation of the ArcB in *E. coli* makes the bacteria completely susceptible to these agents (Nikaido 2009). The RND pumps show extremely wide substrate specificity. For example, *E. coli* AcrB pumps out not only most common antibiotics, but also dyes, detergents, and solvents. Table 2 summarizes major RND pumps and the substrates that are known to be pumped out by the organisms.

Expression of most of the RND pumps is reported to be regulated by specific regulator proteins (Li and Nikaido 2004). The MexXY, an aminoglycoside efflux pump of *P. aeruginosa*, can be expressed in the presence of its substrates (Masuda et al. 2000). However, with other pumps, the gene expression is regulated not by its substrates, but by its repressor genes and as a result, the efflux pump overexpression occurs because of mutations in the repressor gene. For example, MexAB-overproducing strains of bacteria had mutations in the *mexR* gene, a repressor for the *mexAB* gene (Cao et al. 2004). It has also been observed that in *P. aeruginosa*, the *mexZ* repressor gene upstream of *mexXY*-oprA in PA7 is mutated, leading to overexpression of *mexXY*-oprA. In addition, the effect of modifying enzymes was enhanced by the presence of this efflux pump to develop a high-level aminoglycoside resistance (Morita et al. 2012).

The evolution of RND efflux pumps has been a big threat in the development of AREs. RND pumps have made major contributions to the intrinsic and elevated resistance of clinically relevant pathogens (Poole 2005b). For example, fluoroquinoline resistance is mostly attributed to mutational alteration of the target topoisomerases; however, many strains have been found where the increased resistance has been caused by increased efflux (Mazzariol et al. 2000). In another example, significant portions of aminoglycoside-resistant clinical isolates of Gram-negative bacteria are known to owe their resistance to increased active efflux (El'Garch et al. 2007). The trend to increasingly add disinfectants to household products like soaps has caused selection for the overproduction of these RND pumps (Aiello et al. 2007). In addition, the RND pumps have been known to efflux spermidine (Neyfakh 1997), secrete secondary metabolites, protect bacteria against bile salts and detergents that are abundant in environment (Zgurskaya and Nikaido 1999), as well as toxic metals such as Co^{2+}, Ni^{2+}, Cd^{2+}, and Zn^{2+} (Goldberg et al. 1999).

Adaptation of antibiotic resistance extremophiles (AREs)

AREs and adaptive plasmids

Acquiring of a plasmid by a bacterium, which allows it to adapt to a certain environmental condition (i.e., high antibiotic environment), is known as an adaptive plasmid. The R plasmids were first discovered in Japan, and contained resistance genes for chloramphenicol, tetracycline, aminoglycosides, and sulfonamides (Clark and Pazdernik 2012). R plasmids have been widely known to contain genes for antibiotic resistance in microorganisms (Clark and Pazdernik 2012). Most of the resistant genes are components of transposons, which can insert genes in the middle of any piece of DNA. Transposon Tn21 is a large, complex, multiple composite transposon (Liebert et al. 1999) that contains genes for mercury, sulfonamide, and aminoglycoside resistance. Many resistant genes in R plasmids contain a unique 59-base 3′-sequence tag that led to the discovery of the integron (Hall and Stokes 1993).

Table 2 Substrate specificity of some RND multidrug efflux pumps

Organism	RND Pump	Substrate	Ref.
E. coli	AcrB	Acriflavine, bile salts, chloramphenicol, crystal violet, ethidium bromide, fatty acids, fluoroquinolines, macrolides, novobiocin, organic solvents, penicillins, sodium dodecylsulfate, tetracyclines, trimethoprim, Triton X-100	Nikaido et al. (1998)
	AcrD	Aminoglycosides, deoxycholate, fusidic acid, novobiocin	
P. aeruginosa	MexB	Acriflavine, aminoglycosides, chloramphenicol, cephalosporins, crystal violet, ethidium bromide, macrolides, novobiocin, organic solvents, penicillins, sodium dodecylsulfate, sulfonamides, tetracyclines, trimethoprim, triclosan	Tseng et al. (1999)
	MexD	Chloramphenicol, cephalosporins, fluoroquinolines, macrolides novobiocin, tetracyclines, triclosan	
	MexF	Chloramphenicol, fluoroquinolines, tetracyclines	
	MexY	Aminoglycosides, fluoroquinolines, macrolides, tetracyclines	

This contains a gene for the enzyme integrase, which allows the cell to insert the resistance gene at a predetermined site downstream from a strong promoter.

Once the gene is integrated, it becomes tagged, allowing it to be easily integrated into another integrin, which might contain another set of resistance genes. For example, a Tn21 already containing a sulfonamide resistance gene *sul1* and a truncated version of a multidrug efflux gene *qacE* has integrated an aminoglycoside resistance gene *aadA1* at the specific integration site *attI* (Liebert et al. 1999). Many integron structures have been found to be associated with a downstream structure called an ISCR element. This contains a presumed transposase gene that functions in an unusual open-ended transposition event and recruits various resistance genes and delivers them close to the integron structure (Toleman et al. 2006). In a study that examined the relationship between the IncP1-α plasmid RP1 and plasmids R1033 and R934, it was determined that the Tn1 and ampicillin resistance genes contained in the plasmids were acquired by the IncP1-α backbone on three separate occasions (Pinyon and Hall 2011).

Chowdhury et al. (2011) reported that a plasmid-mediated quinolone resistance gene, *qnrA1*, along with *aac(6′)-Ib-cr* and *bla* (CTX-M-3) genes, was on a mobile plasmid and able to spread to other bacterial species that were under the selective pressure of fluoroquinolones and β-lactam antimicrobials. In *Aeromonas hydrophila* infections, antibiotic treatment with flumequine, tetracycline, and trimethoprim strongly induced the expression of genes mediating conjugative transfer of the R-plasmid pRAS1 carrying the resistive genes *TcR*, *TmR*, and *SuR* (Cantas et al. 2012). IncF plasmids, which carry the *blaCTX-M-14* gene, responsible for ceftazidime and defotaxime resistance, were able to readily spread in *E. coli* from various clinical isolates, causing horizontal transfer of resistance genes (Kim et al. 2011).

Gene duplication and amplification

Gene amplification has been found in all forms of life. It is a known contributor to human and microbial evolution by generating genomic variance that is required for genetic adaptation. In the environment, microorganisms, through horizontal gene transfer, acquire necessary genes in the form of plasmids. These genes have been known to go through duplication and amplification in bacteria to adapt in the presence of various toxic drugs, including antibiotics.

The plasmid-borne amplifications involved in antibiotic resistance were first identified in *Proterus mirabilis* (Perlman and Stickgold 1977). Normark et al. (1977) observed chromosomal gene amplification in *E. coli* as microbial adaptation against antibiotics. In this strain, AmpC β-lactamase was amplified up to 30 times. Apart from the direct increase in antibiotic resistance that results from increased expression of a modifying or degrading enzyme or efflux pumps, there are also examples in which duplications have mediated more unpredicted mechanisms to increase resistance. Duplication of part of the ribosome-binding site of the macrolide resistance gene *ermA* has been found in both *S. aureus* and *E. faecalis* (Sandegren and Andersson 2009). These duplications lead to constitutive expression of *ermA* by bypassing the translational attenuation system that regulates *ermA* expression (Srinivasan et al. 2009). In another example, with macrolide treatment of *S. pneumoniae* infection, the organism developed resistance to the drug due to duplication events in the *rplV* gene, which encodes the ribosomal protein L22.

Persister cells

The antibiotic susceptibility of bacterial cells is influenced by the colony's physiological states. A consequence of this is the incidence of persister cells, discovered when high concentrations of antibiotics were unable to exterminate all the bacterial population. A persister population was always left behind (Lewis 2005). The presence of persisters is now thought to be an example of the strategy whereby bacteria naturally generate mixtures of phenotypically different populations, so that in the event of environmental change, one of them can survive (Dhar and McKinney 2007). It has been determined that the bacterial persisters are able to tolerate antibiotics by not producing hydroxyl radicals (Kim et al. 2011).

In cystic fibrosis patients, *P. aeruginosa*-mediated chronic infection of the airway showed mutations in the *mutS* gene responsible for a hypermutator phenotype (Kenna et al. 2007). In addition, various other mutations in the *mexZ* repressor caused the organism to express the *MexXY-OprM* gene and develop resistance against ofloxacin, carbenicillin, and tobramycin. With chronic antibiotic treatment for this infection, these organisms were able to develop large populations of persister cells and quickly became resistant to a wide range of antibiotics (Mulcahy et al. 2010). In *Staphylococcus epidermidis*, it is the adherent growth mode, rather than the ability to build a multilayer biofilm structure, that contributes to its high resistance to antibiotics. The presence of persister cells in coagulase-negative *Staphylococci* biofilms plays an important role in antibiotic resistance (Qu et al. 2010). In *P. aeruginosa*, it has been determined that quorum-sensing-related signaling has been able to increase the persister cells. Treatment with phenazinepyocyanin and acyl-homoserine lactone 3-OC12-HSL significantly increased the persister numbers in *P. aeruginosa* (Moker et al. 2010). The gain of mutations via hypermutator phenotype is now being considered as the mechanism by which the persister

population becomes resistant to antibiotics and therefore, the development of AREs.

Inactivating enzymes

Inactivating enzymes modify the molecular structure of antibacterial drugs to stop them from acting against targeted microorganisms. β-lactamase (the β-lactam family) is a wide group of antibiotic-resistant enzymes comprising several functional classes such as cephalosporinase, penicillinase, and metalloenzyme. Genes encoding the β-lactamases have been reported in a variety of Gram-negative species, mostly in *Enterobacteriace*, *P. aeruginosa*, and *A. baumannii* (Paterson and Bonomo 2005; Jacobson et al. 2012; Poirel et al. 2012). The β-lactamases are able to inactivate the antibiotics by catalyzing the hydrolytic opening of the β-lactam ring (Fig. 1), which leads to resistance in Gram-negative bacteria as the enzymes are concentrated in periplasmic space. Chromosomal or plasmid R-factor-controlled production of β-lactamase in microbes is the most common mechanism of acquired resistance to β-lactam antibiotics (Garcia-Fernandez et al. 2008). This mechanism can be acquired by obtaining new genes through transformation, conjugation, or bacteriophage transduction.

In *A. baumannii*, ceftazimide resistance was found to be mediated by a PER-7 β-lactamase encoded in an ISCR1 element located on a plasmid (Opazo et al. 2012). In other organisms, such as *Bacillus licheniformis* and *S. aureus*, a membrane-bound penicillin receptor (BlaR/MecR) detects the presence of β-lactam and launches a signaling cascade that leads to the synthesis of a β-lactamase (Amoroso et al. 2012). Members of *Enterobacteriaceae* have been known to encode plasmid-based β-lactamases as extended-spectrum β-lactamases (ESBLs). Dortet et al. (2012) showed that ESBLs were located on self-conjugative plasmids and were near other resistant genes belonging to the carbapenemase group of genes, such as *NDM-1*, *OXA-48*, and *OXA-181* in *Enterobacteriaceae*.

One of the main resistance mechanisms to aminoglycoside drugs, as with β-lactams, is the production of aminoglycoside-modifying enzymes (AMEs) (Poole 2005a). The enzymes aminoglycoside phosphoryltransferase, acetyltransferase, and adenyltransferase phosphorylate, acetylate and adenylate, respectively, aminoglycoside molecules and decreases their binding affinity to the ribosomal subunits (Poole 2005a). The AMEs found in *P. aeruginosa* have been encoded by the genes *aac-I*, *aac-II*, *ant-I*, and *aph-VI* (Vaziri et al. 2011). In addition, the genes *aphA6*, *aac-I*, *aacC1*, *aacC2*, *aacA4*, *aadB*, and *aadA1* have been found in *A. baumannii*. The occurrence of identical resistance genes in distinct strains indicated that horizontal gene transfer mechanism plays a major role in the dissemination of aminoglycoside resistance in *A. baumannii* (Asadollahi et al. 2011). Another AME, *AAC(3)-II* found in *E. coli*, showed a significant resistance to gentamicin and amikacin in China (Xiao and Hu 2012).

Altered target

For an antibiotic to be effective, it must be able to bind to its receptors and cause metabolic changes in bacterial cells. The site targeted by an antibacterial agent may be in a form that is not sensitive to the action of the agent. Several studies have reported that penicillin-binding protein (PBP) alterations are key to β-lactam resistance in *P. aeruginosa* (Farra et al. 2008). Substitutions of one or more amino acids in the PBP structures could lead to a reduced affinity for β-lactams (Fig. 2). As a result, the PBPs remain functional and ensure peptidoglycan synthesis, and the cell wall is not altered. In *Burkholderia pseudomallei*, it was observed that prolonged ceftazidime therapy led to resistance due to the deletion of a gene encoding a penicillin-binding protein 3 (Chantratita et al. 2011). An insertion of an aspartate residue at position 345a in PBP-2 that lowered the rate of penicillin acylation sixfold was reported in *Neisseria gonorrhoeae*. The Asp345a mutation acts by altering the interaction between the adjacent residue, Asp346, in the B2a-B2d hairpin loop and Ser363, the middle residue of the SXN active site motif (Tomberg et al. 2012). Banerjee et al. (2008) showed that *S. aureus* is resistant to β-lactam antibiotics as it expresses penicillin-binding protein 2a, which is a low-affinity penicillin-binding protein.

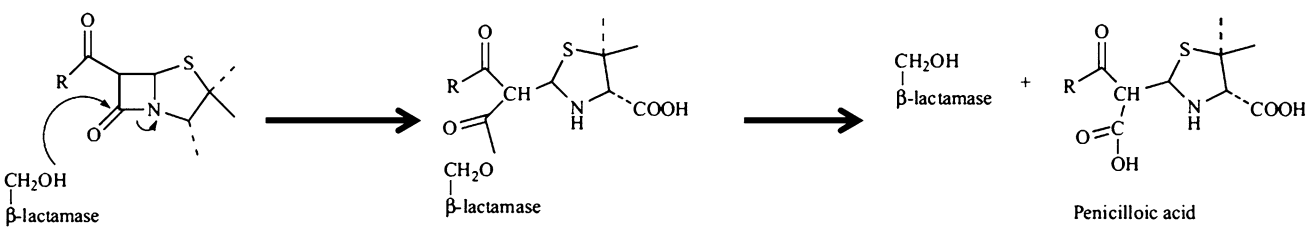

Fig. 1 β-Lactamase-mediated inactivation of penicillin. β-Lactamase deactivates β-lactam antibiotics catalyzing the hydrolytic opening of the β-lactam ring making the antibiotic ineffective

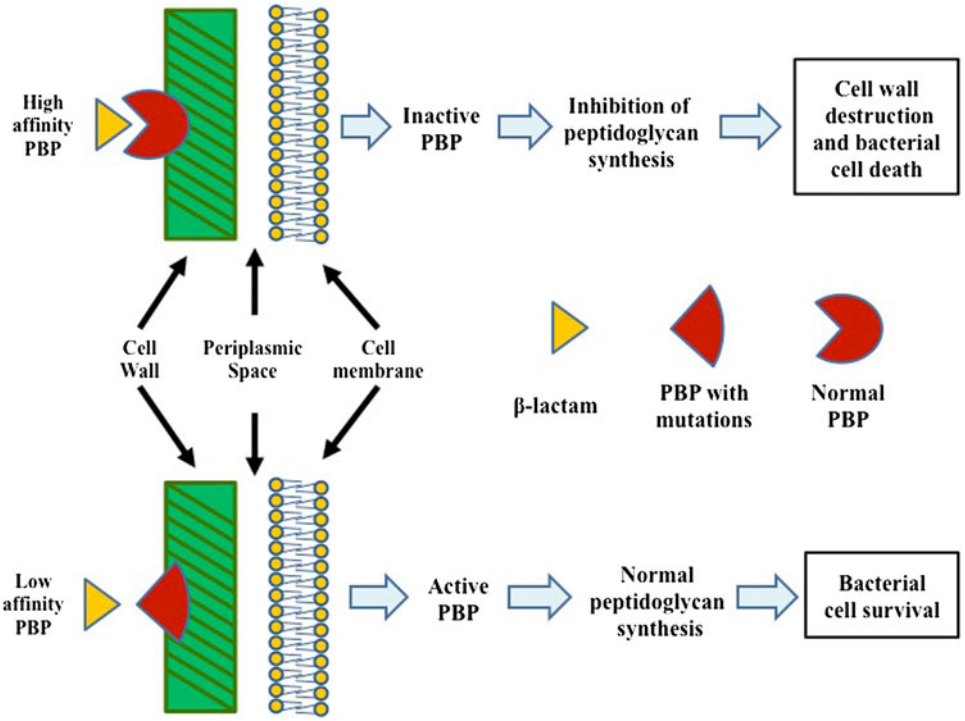

Fig. 2 Low affinity of penicillin-binding protein (PBP) to β-lactam causes bacterial survival. Mutations in the genes coding for PBP may reduce the affinity for β-lactam thereby protecting the bacterial cell wall from being destroyed and increase their survival in high levels of β-lactams

Membrane permeability

Gram-positive and Gram-negative bacteria differ in the structure of their cell walls. Gram-positive bacteria possess a permeable cell wall that usually does not restrict the penetration of antibiotics. It is hypothesized that small and hydrophilic antibiotics cross the membrane via porins, whereas hydrophobic antibiotics diffuse through the membrane directly. A decrease in the number of porins leads to a decrease in the permeability of hydrophilic drugs. Low outer-membrane permeability to hydrophilic compounds contributes substantially to intrinsic resistance to antimicrobials such as β-lactams (Livermore 2001). For example, even highly active anti-pseudomonal β-lactams, such as imipenem, can have their activity affected by the loss of an outer membrane porin (Gotoh and Nishino 1990). Since most porin channels are nonspecific, the entry of other small hydrophilic agents, such as tetracyclines, aminoglycosides, and chloramphenicol, might also be affected by porin loss (Gotoh and Nishino 1990, Sobel et al. 2003). Several molecular genetic events such as gene deletion or reduced genomic expression of the gene encoding the porin channel could lead to loss of porins. Repression of the genes encoding the porins due to mutated proteins produced by the bacterium can also be a factor in increasing the antibiotic resistance potential of that organism.

P. aeruginosa is a major pathogen that infects cystic fibrosis and immune-compromised patients. The antibiotic resistance of *P. aeruginosa* is due to the outer membrane protein H, which is an eight-stranded β-barrel protein with four extracellular loops of equal size (Edrington et al. 2011). Champlin et al. (2005) determined that outer cell envelope impermeability played a big role in resistance to high triclosan concentrations in *P. aeruginosa*.

Integration of "-omics" and antibiotic-resistance

The genome (genomics DNA) of an organism is its entire set of hereditary information that is converted to mRNA (transcriptome) for protein translation. The proteome of an organism is the entire set of proteins that are expressed in the organism under uniform or specific environmental conditions. Due to the recent considerable increase in antibiotic-resistant bacteria, understanding the mechanisms of resistance at the molecular level is extremely important to control the growth of multidrug-resistant strains and to develop new therapeutic strategies. This also involves the study of structure and functional interactions between proteins. Potential genes and proteins involved in antibiotic resistance mechanisms can be identified and studied via advanced genomics and proteomics techniques (Nagaraj and Singh 2010; Singh et al. 2011) (Fig. 3). Recent advances in next-generation sequencing, genomics, and proteomics have opened the door for new drug designs that can revolutionize the treatment of pathogens that have become resistant to the standard of treatment antibiotics (Nagaraj and Singh 2010; Fothergill et al. 2012).

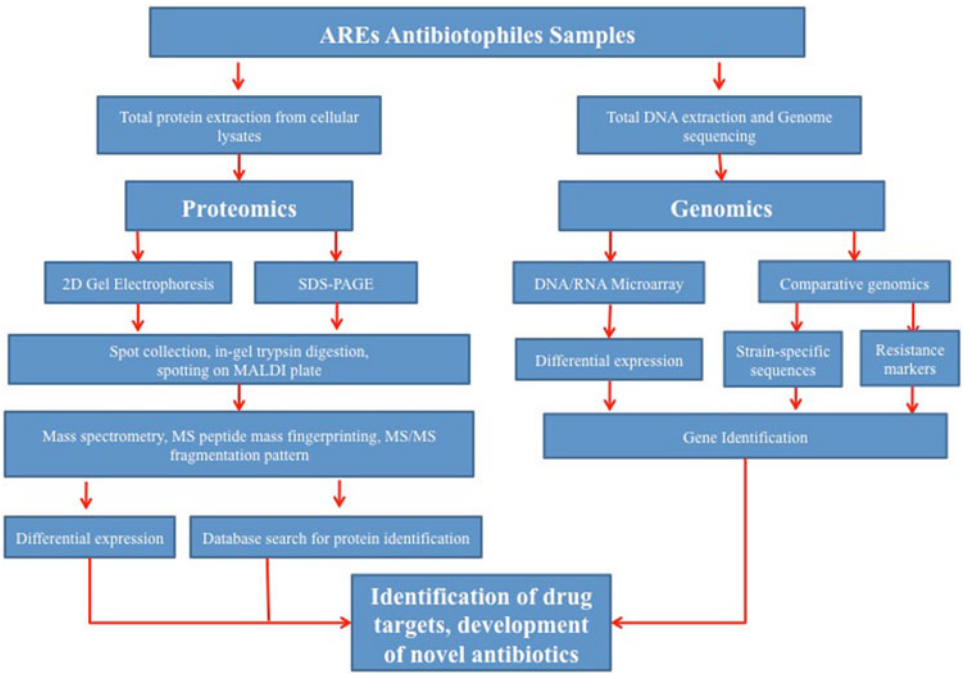

Fig. 3 Typical workflow for '-omics' based genomics and proteomics approach in identifying genes and proteins involved in antibiotic resistance in AREs. Variety of techniques including 2DE, SDS-PAGE, DNA/RNA microarray, and comparative genomics can be used to identify the genes and proteins involved in molecular mechanisms in the development of antibiotic resistance in AREs

Genomics

The complete genome sequences of many bacterial pathogens are now available and can be used for genome organization, including comparisons through microarrays (Ishii et al. 2007). Sun et al. (2012) identified critical virulence factors involved in antibiotic resistance mechanisms via high-throughput methods involving a growth-based screen on a library of 55,000 small molecules. The microarray analysis confirmed genes involved in antibiotic resistance in the presence of various different types of antibiotics (Sun et al. 2012).

Comparative genome analysis of the microorganism *Streptococcus suis* showed horizontal gene transfer as an evolutionary force in determining multidrug resistance with different gene modulations (Hu et al. 2011). In another study of multidrug-resistant pathogenic *E. coli*, the bla (CMY-2) plasmid lineage appeared to have been derived from an ancestral IncA/C plasmid type harboring *floR-tetAR-strAB* and Tn21-like accessory modules (Fernandez-Alarcon et al. 2011). A comparative genomic analysis of *Klebsiella pneumoniae* strain 1162281 with another strain of *K. pneumoniae* revealed a core set of 3,631 conserved orthologous proteins, which suggested a large component of the genetic and phenotypic diversity of the isolate was largely in part due to horizontal gene transfer (Kumar et al. 2011). In curated lists of over 400 antibiotic resistance genes, *K. pneumoniae* 1162281 showed the presence of additional efflux pumps, extended-spectrum β-lactamases, and multiple mechanisms of fluoroquinolone resistance, unlike susceptible strains (Kumar et al. 2011). The genome analysis of *Salmonella enteric* serovar *typhimurium* T000240 strain revealed that its multidrug resistance was due to the acquisition of a genomic island via IS1 derivatives on its chromosome (Izumiya et al. 2011).

Several individual sets of genes were studied by polymerase chain reaction (PCR) to reveal the severity of antibiotic resistance in bacteria. *S. pneumoniae* isolates were screened for the presence of antibiotic resistance genes and real-time PCR assays were used to identify *mef(E)*, *mef(A)*, *erm(B)*, and *tet(M)* genes (Bowers et al. 2012). Araujo et al. (2011) tested multidrug-resistant *Enterococcal* isolates for macrolide (*ermA*, *ermB*, and *ermC*), tetracycline (*tetM*, *tetK*, and *tetL*), vancomycin (*vanA*, *vanB*, *vanC-1*, and *vanC-2/3*), and *vatD*, *vatE*, *aac(6′)-aph(2″)*, *aph(3′)*, and *ant(6′)* resistance genes. It was determined that some isolates possessed a *vanA* or acquired type genotype, while others had an intrinsic mechanism of resistance, *vanC1*. The *vanA*-containing isolates showed resistance to tetracycline and erythromycin and possessed the *tetM*, *tetL*, and *ermB* genes (Radhouani et al. 2010a). Metagenomic analyses of ancient DNA from 30,000-year-old Beringian permafrost sediments revealed a complete vancomycin-resistant element *vanA*, confirming that antibiotic resistance is a natural phenomenon that predates the modern selective pressure of today's large antibiotic use (D'Costa et al. 2011). Multiplex PCR was used by Porrero et al. (2012) to confirm that isolates were indeed methicillin-resistant *S. aureus* (MRSA), and then multilocus sequence typing was conducted to characterize the isolates and further determine the genes involved in antibiotic resistance.

In addition to comparative genomics, transcriptomic studies have also provided insight into the genes involved in antibiotic resistance. A transcriptomic screening revealed a possible increase in the metabolism and transport of carbohydrates in linezolid-resistant *S. pneumoniae* mutants. A global analysis of gene expression highlighted that metabolism alterations were associated with linezolid resistance in *S. pneumoniae* (Feng et al. 2011). DNA microarrays were used to compare the gene expression profile of *S. pneumoniae* M22, a multidrug-resistant mutant, with the strain M4. The strain *S. pneumoniae* M22 expressed 22 genes at higher levels than M4, including genes responsible for branched-chain amino acid biosynthesis and two genes, *patA* and *patB*, with sequences matching ABC transporter proteins (Marrer et al. 2006). Table 3 lists various other genes differentially expressed and found in AREs.

Proteomics

Proteomics technology characterizes the global proteins in an organism at a given time under certain conditions. Because the mechanism of antibiotic resistance is mediated from DNA to RNA to proteins, proteomics can be used to study and characterize the novel pumps and enzymes used to combat antibiotics. Two-dimensional gel electrophoresis (2-DE) has proven to be an excellent tool in studying the proteins involved in the molecular mechanisms of antibiotic resistance. A comparative analysis of two vancomycin-resistant *Enterococci* (*E. faecium* SG41 and *E. durans* SG3) on 2-DE showed 60 modulated protein spots corresponding to 97–45 kDa molecular weight proteins (Radhouani et al. 2010b). The proteomic study also revealed that antibiotic-resistant cells produced peptidoglycan precursors that terminated in the depsipeptide D-alanine-2-D-hydroxy acid rather than the dipeptide D-alanine-D-alanine, which prevents binding of antibiotics such as vancomycin (Radhouani et al. 2010b). Twelve differentially expressed proteins related to bacterial virulence, antibiotic resistance, and DNA protection were identified in a piperacillin/tazobactam-resistant (PTZ) strain of *E. coli* using 2-DE followed by MALDI-TOF/TOF-MS (dos Santos et al. 2010). Several proteins that were observed to increase in abundance in the PTZ strain were OmpA, glyceraldehyde-3-phosphate dehydrogenase A, and superoxide dismutase Fe-SOD (dos Santos et al. 2010). These proteins may play a role in dismantling the structure of the antibiotics and making them nonfunctional. MRSA has long been recognized as an important antibiotic-resistant pathogen in humans; a 2-DE proteomic analysis of *S. aureus* ST398 revealed 227 proteins unique to *S. aureus*, many of which were involved in virulence and antibiotic resistance (Monteiro et al. 2012).

The global proteome was screened in two penicillin mutants of *S. pneumonia* and revealed a number of differentially expressed proteins, including PstS, a subunit of the phosphate ABC transporter (Soualhine et al. 2005). Inactivation of PstS led to an increased susceptibility to penicillin. In addition, increased levels of PstS mRNA were found in 12 different isolates responsible for increased penicillin resistance (Soualhine et al. 2005). In a proteomics study of *S. pneumoniae*, Feng et al. (2011) reported that modulated glycolytic proteins, enzymes, and transporters involved in the metabolism of sugars were overexpressed in antibiotic-resistant strain R6M2-LZD. The components of a phosphotransferase (PTS) system (spr0562, spr0563, and spr0564 proteins) were also reported to be overexpressed (Feng et al. 2011).

In addition to 2-DE, mass spectrometry (MS/MS) has also proven effective to study the underlying mechanisms of antibiotic resistance. The proteomic profile of rifampicin-resistant *Brucella abortus* was examined and compared to that of a non-resistant strain using MS-drive comparative proteomics; the resistant strain contained a mutation in the *rpoB* gene. In the same study, 12 836 MS/MS spectra identified 6,753 peptides corresponding to 456 proteins, of which 39 were differentially regulated in the resistant strain and were involved in various metabolic pathways (Sandalakis et al. 2012). From this study, it can be hypothesized that various proteins, such as pyruvate decarboxylase, urocanatehydratase, bifunctional protein FolD, and an aminotransferase class-III: maltose-binding protein could potentially regulate antibiotic resistance (Sandalakis et al. 2012). Using an MS/MS technique, Piras et al. (2012) were able to identify the proteins that were differentially regulated in a multidrug-resistant *E. coli* as those involved in the metabolic pathways, including malate dehydrogenase, fructose-bisphosphatealdolase, triosephosphateisomerase, phosphoglucomutase, and others. Various membrane and transport proteins, such as thiosulfate-binding protein, zinc transport protein B, outer membrane protease OmpP, and Ox2 may also play a role in antibiotic resistance (Piras et al. 2012).

After protein spots are resolved using 2-DE, MALDI-MS is a useful tool for identifying peptides to analyze plasmid-encoded antibiotic resistance genes and proteins. This technology has been previously used to investigate β-lactamase activities in various Gram-negative bacteria (Hooff et al. 2012). In addition to 2-DE, LC–MS has also been used for comparative proteomics analysis of clinical isolates of daptomycin-resistant *S. aureus*, which revealed that differentially regulated proteins were involved in the cell wall associated with targets and biofilm formation (Fischer et al. 2011). Table 4 summarizes differentially expressed proteins involved in the major metabolic pathways that have been recently found in antibiotic-resistant

Table 3 Differentially regulated proteins in antibiotic-resistant bacteria as identified using genomic techniques

Organism	Gene	Regulation	Description	Ref.
Streptococcus sp. MGAS166	*copZAY*	Up	Ion export	Sun et al. (2012)
	speB		Strepahesin-virulence	
	spy1135-1137		Nucleotide metabolism	
	ntplECABD	Down	V type ATP synthase	
	atpEBFHGDC		ATP synthase	
	sagABCDEFGHI		Streptolysin S-virulence	
	accDAC, *fabZBFG*, *DKPH*		Lipid metabolism	
	ptsBCD, *glgP*, *malMEGDCX*, *lacABCDEF*		Carbohydrate metabolism	
	oppABCD, *dppBCDE*, *arcABC*/ *Spy1541-1549*		Amino acid metabolism	
Streptococcus suis R61	*mef(E)*	Up	Erythromycin resistance	Hu et al. (2011)
	SSUR61_1068		Amikacin and dibekacin resistance	
	SSUR61_2113		Streptomycin A resistance	
	SSUR61_1983		*β*-Lactamase_B	
Klebsiella pneumoniae 1162281	*SHV-75*	Up	*β*-Lactamase type BLA	Kumar et al. (2011)
	blaP1		*β*-Lactamase type BLA	
	cmlA1		Chloramphenicol resistance protein	
	norA		Multidrug efflux protein	
	acrA, *acrB*		Acridine/acriflavin resistance protein	
	yceE (mdtG)		Drug efflux system protein MdtG	
	macB (ybjZ)		Macrolide transporter ATP-binding protein	
Klebsiella pneumoniae JH1	*SHV-60*	Up	*β*-Lactamase type BLA	
	norA		Multidrug efflux protein	
	acrA, *acrB*		Acridine/acriflavin resistance protein	
	yceE (mdtG)		Drug efflux system protein MdtG	
	macB (ybjZ)		Macrolide transporter ATP-binding protein	
Acinetobacter baumannii UMB001	*adeFGH operon*	Up	Efflux pump	Sahl et al. (2011)
	adeA and *adeB*		adeABC efflux pump complex	
	adeFGH operon		Efflux pump	
	adeA and *adeB*	Down	adeABC efflux pump complex	
	adeC		Outer membrane protein AdeC	
	adeFGH operon	Up	Efflux pump	
	adeA and *adeB*		AdeABC efflux pump complex	
	adeC	Down	Outer membrane protein AdeC	

microorganisms via proteomics platforms. These proteins may play a role in the metabolism of antibiotics and in making them inactive, enabling the microorganisms to survive.

Future directions and challenges

Currently, there is an ongoing need to define the molecular mechanisms behind the antibiotic resistance of AREs. These may lead to the discovery of new channel proteins or responsive genes that the AREs induce to evolve toward extreme antibiotic resistance, and further our understanding of AREs. Additional studies will reveal the unique means by which AREs efflux or inactivate antibiotic drugs, or how they alter the binding sites of antibiotics, rendering them inactive. Further studies need to be conducted to determine the efficiency of the various antibiotic resistance mechanisms used by the AREs. This will help the pharmacological industry to develop precise drugs that target the proteins and genes that contribute most to antibiotic resistance.

Table 4 Differentially expressed proteins in antibiotic-resistant microorganisms identified using proteomic techniques

Organism	Enzyme	Regulation	Biological process	Ref.
S. pneumoniae 1974M2	Lactate oxidase, fructokinase, putative alcohol dehydrogenase, dihydrolipoamide *S*-acetyltransferase, glucokinase 6-phosphofructokinase	Up	Energy metabolism	Feng et al. (2011)
	Fructose bisphosphatealdolase, 3-ketoacyl-acyl carrier protein reductase, acetyl-CoA carboxylase alpha subunit, hydroxymyristoyl-dehydrogenase	Down		
	Ribosomal protein S18	Up	Protein translation	
	Ribosomal protein S6			
	Ribosomal protein S2	Down		
B. abortus	Dihydrodipicolinate synthetase	Up	Amino acid biosynthesis	Sandalakis et al. (2012)
	Glutamine synthetase			
	2-Dehdro-3-deoxyphosphooctonate aldolase		Cell envelope process	
	Catalase		Detoxification	
	Glyceraldehyde 3-phosphate dehydrogenase		Energy metabolism	
	Beta-ketoacyl synthase			
	Adenine phosphoribosyltransferase		Nucleotide and nucleoside metabolism	
	Periplasmic binding protein		Transport and binding	
E. coli (MDR)	Malate dehydrogenase	Up	Energy metabolism	Piras et al. (2012)
	Fructose bisphosphatealdolase			
	Isocitrate dehydrogenase ATP synthase gamma chain, fumaratereductase iron-sulfur subunit	Down		
	Curved DNA-binding protein	Up	Molecular chaperon	
	Outer membrane protease ompP		Ion transport	
	Thiosulfate-binding protein			
	Phosphoserine aminotransferase		Amino acid biosynthesis	
E. durans SG3	Chaperone protein dnaK	Up	Stress response	Radhouani et al. (2010b)
	Tryptophan repressor-binding protein		Tryptophan operon regulation	
E. faecalis SG41	Chaperonin groL		Protein folding	
	D-Alanine-D-alanine ligase		Cell wall biogenesis/ peptidoglycan biosynthesis	
E. coli (Ec-PTZ)	Outer membrane protein A	Up	Cell invasion, serum resistance, conjugation	dos Santos et al. (2010)
	Alkyl hydroperoxidereductase subunit F		Stress response	
	Chaperone protein clpB			
	Fumaratereductase iron-sulfur subunit		Energy metabolism	
	Transaldolase B	Down	Pentose-phosphate shunt	
	Glyceraldehyde 3-phosphate dehydrogenase A		Energy metabolism	
	Alkyl hydroperoxidereductase subunit C		Stress response	

The variety of genomic and proteomic methods discussed above can be useful in determining the genes, proteins, and molecular mechanisms involved in antibiotic resistance (Fig. 3). DNA microarrays and transcriptomics have been useful in determining the differential gene expression of antibiotic-resistant strains. 2-DE followed by MS/MS and MALDI-TOF/TOF-MS can be useful to identify and compare the protein expressions that are involved in the molecular mechanisms of antibiotic resistance. However, these technologies can only reveal the

primary and secondary protein structures; additional methods are still needed to reveal the tertiary or even quaternary structures of proteins involved in antibiotic resistance mechanisms. Microbial genomics and proteomics are driving the new era of therapeutic discovery, but this process of researching new drugs to fight antibiotic resistance has been very slow to progress. Further challenges that remain include studying the metabolomics and interactomics of AREs to fully understand the metabolic profiles of these organisms.

Conclusion

AREs are becoming prevalent and raising serious health issues and challenges to the pharmaceutical industry. New ways to study the survival strategies of AREs are needed. The combined effects of the different mechanisms of antibiotic resistance discussed in this article have led to the development of AREs, and their study is inevitable. Further, the availability of genome sequences of many AREs and the integration of "omics" technologies into the field have revolutionized the study of microbial strategies for thriving under otherwise deadly conditions. These survival strategies could lead us to categorize antibiotic-resistant microorganisms under novel group of extremophiles: AREs. Existing gene sequences from other extremophiles and the discovery of new targets through "omics" integration will allow us to grow our understanding of the resistance mechanisms and create clinical treatments to effectively fight the growing threat of antibiotic-resistant pathogens.

References

Aiello AE, Larson EL, Levy SB (2007) Consumer antibacterial soaps: effective or just risky? Clin Infect Dis 45(Suppl 2):S137–S147

Amoroso A, Boudet J, Berzigotti S, Duval V, Teller N, Mengin-Lecreulx D, Luxen A, Simorre JP, Joris B (2012) A peptidoglycan fragment triggers beta-lactam resistance in *Bacillus licheniformis*. PLoS Pathog 8:e1002571

Araujo C, Torres C, Goncalves A, Carneiro C, Lopez M, Radhouani H, Pardal M, Igrejas G, Poeta P (2011) Genetic detection and multilocus sequence typing of vanA-containing *Enterococcus* strains from mullets fish (*Liza ramada*). Microb Drug Resist 17:357–361

Asadollahi K, Taherikalani M, Maleki A, Alizadeh E, Valadbaigi H, Soroush S, Maleki H, Asadollahi P, Emaneini M (2011) Diversity of aminoglycoside modifying enzyme genes among multidrug resistant *Acinetobacter baumannii* genotypes isolated from nosocomial infections in Tehran hospitals and their association with class 1 integrons. Acta Microbiol Immunol Hung 58:359–370

Baldoni D, Haschke M, Rajacic Z, Zimmerli W, Trampuz A (2009) Linezolid alone or combined with rifampin against methicillin-resistant *Staphylococcus aureus* in experimental foreign-body infection. Antimicrob Agents Chemother 53:1142–1148

Banerjee R, Gretes M, Basuino L, Strynadka N, Chambers HF (2008) In vitro selection and characterization of ceftobiprole-resistant methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 52:2089–2096

Blanchard JS (2005) Old approach yields new antibiotic. Nat Med 11:1045–1046

Bowers JR, Driebe EM, Nibecker JL, Wojack BR, Sarovich DS, Wong AH, Brzoska PM, Hubert N, Knadler A, Watson LM, Wagner DM, Furtado MR, Saubolle M, Engelthaler DM, Keim PS (2012) Dominance of multidrug resistant CC271 clones in macrolide-resistant *Streptococcus pneumoniae* in Arizona. BMC Microbiol 12:12

Breidenstein EB, de la Fuente-Nunez C, Hancock RE (2011) *Pseudomonas aeruginosa*: all roads lead to resistance. Trends Microbiol 19:419–426

Brown MH, Paulsen IT, Skurray RA (1999) The multidrug efflux protein NorM is a prototype of a new family of transporters. Mol Microbiol 31:394–395

Cantas L, Midtlyng PJ, Sorum H (2012) Impact of antibiotic treatments on the expression of the R plasmid tra genes and on the host innate immune activity during pRAS1 bearing *Aeromonas hydrophila* infection in zebrafish (*Danio rerio*). BMC Microbiol 12:37

Canton R, Morosini MI (2011) Emergence and spread of antibiotic resistance following exposure to antibiotics. FEMS Microbiol Rev 35:977–991

Cao L, Srikumar R, Poole K (2004) MexAB-OprM hyperexpression in NalC-type multidrug-resistant *Pseudomonas aeruginosa*: identification and characterization of the nalC gene encoding a repressor of PA3720-PA3719. Mol Microbiol 53:1423–1436

Carattoli A (2009) Resistance plasmid families in *Enterobacteriaceae*. Antimicrob Agents Chemother 53:2227–2238

Champlin FR, Ellison ML, Bullard JW, Conrad RS (2005) Effect of outer membrane permeabilisation on intrinsic resistance to low triclosan levels in *Pseudomonas aeruginosa*. Int J Antimicrob Agents 26:159–164

Chantratita N, Rholl DA, Sim B, Wuthiekanun V, Limmathurotsakul D, Amornchai P, Thanwisai A, Chua HH, Ooi WF, Holden MT, Day NP, Tan P, Schweizer HP, Peacock SJ (2011) Antimicrobial resistance to ceftazidime involving loss of penicillin-binding protein 3 in *Burkholderia pseudomallei*. Proc Natl Acad Sci USA 108:17165–17170

Chowdhury G, Pazhani GP, Nair GB, Ghosh A, Ramamurthy T (2011) Transferable plasmid-mediated quinolone resistance in association with extended-spectrum β-lactamases and fluoroquinolone-acetylating aminoglycoside-6′-*N*-acetyltransferase in clinical isolates of *Vibrio fluvialis*. Int J Antimicrob Agents 38:169–173

Clardy J, Fischbach MA, Walsh CT (2006) New antibiotics from bacterial natural products. Nat Biotechnol 24:1541–1550

Clark D, Pazdernik N (2012) Molecular biology. Elsevier, Waltham, MA

Copeland E, Choy N, Gabani P, Singh OV (2012) Biosynthesis of extremolytes: radiation resistance and biotechnological implications. In: Singh OV (ed) Extremophiles: sustainable resources and biotechnological implications. Wiley, New York

D'Costa VM, King CE, Kalan L, Morar M, Sung WW, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R, Golding GB, Poinar HN, Wright GD (2011) Antibiotic resistance is ancient. Nature 477:457–461

DeMarco CE, Cushing LA, Frempong-Manso E, Seo SM, Jaravaza TA, Kaatz GW (2007) Efflux-related resistance to norfloxacin, dyes, and biocides in bloodstream isolates of *Staphylococcus aureus*. Antimicrob Agents Chemother 51:3235–3239

Dhar N, McKinney JD (2007) Microbial phenotypic heterogeneity and antibiotic tolerance. Curr Opin Microbiol 10:30–38

Dinh T, Paulsen IT, Saier MH Jr (1994) A family of extracytoplasmic proteins that allow transport of large molecules across the outer membranes of gram-negative bacteria. J Bacteriol 176:3825–3831

Diwan V, Tamhankar AJ, Khandal RK, Sen S, Aggarwal M, Marothi Y, Iyer RV, Sundblad-Tonderski K, Stalsby-Lundborg C (2010) Antibiotics and antibiotic-resistant bacteria in waters associated with a hospital in Ujjain, India. BMC Public Health 10:414

Donkor ES, Newman MJ, Yeboah-Manu D (2012) Epidemiological aspects of non-human antibiotic usage and resistance: implications for the control of antibiotic resistance in Ghana. Trop Med Int Health. doi:10.1111/j.1365-3156.2012.02955.x

Dortet L, Poirel L, Al Yaqoubi F, Nordmann P (2012) NDM-1, OXA-48 and OXA-181 carbapenemase-producing *Enterobacteriaceae* in Sultanate of Oman. Clin Microbiol Infect 18:E144–E148

dos Santos KV, Diniz CG, Veloso Lde C, de Andrade HM, Giusta Mda S, Pires Sda F, Santos AV, Apolonio AC, de Carvalho MA, Farias Lde M (2010) Proteomic analysis of *Escherichia coli* with experimentally induced resistance to piperacillin/tazobactam. Res Microbiol 161:268–275

Edrington TC, Kintz E, Goldberg JB, Tamm LK (2011) Structural basis for the interaction of lipopolysaccharide with outer membrane protein H (OprH) from *Pseudomonas aeruginosa*. J Biol Chem 286:39211–39223

Eisig JN, Silva FM, Barbuti RC, Navarro-Rodriguez T, Moraes-Filho JP, Pedrazzoli J Jr (2011) *Helicobacter pylori* antibiotic resistance in Brazil: clarithromycin is still a good option. Arq Gastroenterol 48:261–264

El'Garch F, Jeannot K, Hocquet D, Llanes-Barakat C, Plesiat P (2007) Cumulative effects of several nonenzymatic mechanisms on the resistance of *Pseudomonas aeruginosa* to aminoglycosides. Antimicrob Agents Chemother 51:1016–1021

Endimiani A, Rossano A, Kunz D, Overesch G, Perreten V (2012) First countrywide survey of third-generation cephalosporin-resistant *Escherichia coli* from broilers, swine, and cattle in Switzerland. Diagn Microbiol Infect Dis 73:31–38

Engstrom A, Morcillo N, Imperiale B, Hoffner SE, Jureen P (2012) Detection of first- and second-line drug resistance in *Mycobacterium tuberculosis* clinical isolates by pyrosequencing. J Clin Microbiol 50:2026–2033

Erill I, Campoy S, Mazon G, Barbe J (2006) Dispersal and regulation of an adaptive mutagenesis cassette in the bacteria domain. Nucleic Acids Res 34:66–77

Farra A, Islam S, Stralfors A, Sorberg M, Wretlind B (2008) Role of outer membrane protein OprD and penicillin-binding proteins in resistance of *Pseudomonas aeruginosa* to imipenem and meropenem. Int J Antimicrob Agents 31:427–433

Feng J, Billal DS, Lupien A, Racine G, Winstall E, Legare D, Leprohon P, Ouellette M (2011) Proteomic and transcriptomic analysis of linezolid resistance in *Streptococcus pneumoniae*. J Proteome Res 10:4439–4452

Fernandez-Alarcon C, Singer RS, Johnson TJ (2011) Comparative genomics of multidrug resistance-encoding IncA/C plasmids from commensal and pathogenic *Escherichia coli* from multiple animal sources. PLoS ONE 6:e23415

Fick J, Soderstrom H, Lindberg RH, Phan C, Tysklind M, Larsson DG (2009) Contamination of surface, ground, and drinking water from pharmaceutical production. Environ Toxicol Chem 28:2522–2527

Firoozeh F, Shahcheraghi F, Zahraei Salehi T, Karimi V, Aslani M (2011) Antimicrobial resistance profile and presence of class I integrongs among *Salmonella enterica* serovars isolated from human clinical specimens in Tehran, Iran. Iran J Microbiol 3:112–117

Fischer A, Yang SJ, Bayer AS, Vaezzadeh AR, Herzig S, Stenz L, Girard M, Sakoulas G, Scherl A, Yeaman MR, Proctor RA, Schrenzel J, Francois P (2011) Daptomycin resistance mechanisms in clinically derived *Staphylococcus aureus* strains assessed by a combined transcriptomics and proteomics approach. J Antimicrob Chemother 66:1696–1711

Floyd JL, Smith KP, Kumar SH, Floyd JT, Varela MF (2010) LmrS is a multidrug efflux pump of the major facilitator superfamily from *Staphylococcus aureus*. Antimicrob Agents Chemother 54:5406–5412

Fothergill JL, Winstanley C, James CE (2012) Novel therapeutic strategies to counter *Pseudomonas aeruginosa* infections. Expert Rev Anti Infect Ther 10:219–235

Garcia-Fernandez A, Chiaretto G, Bertini A, Villa L, Fortini D, Ricci A, Carattoli A (2008) Multilocus sequence typing of IncI1 plasmids carrying extended-spectrum β-lactamases in *Escherichia coli* and *Salmonella* of human and animal origin. J Antimicrob Chemother 61:1229–1233

Garrison MW, Anderson DE, Campbell DM, Carroll KC, Malone CL, Anderson JD, Hollis RJ, Pfaller MA (1996) *Stenotrophomonas maltophilia*: emergence of multidrug-resistant strains during therapy and in an in vitro pharmacodynamic chamber model. Antimicrob Agents Chemother 40:2859–2864

Gill MM, Usman J, Kaleem F, Hassan A, Khalid A, Anjum R, Fahim Q (2011) Frequency and antibiogram of multi-drug resistant *Pseudomonas aeruginosa*. J Coll Physicians Surg Pak 21:531–534

Goldberg M, Pribyl T, Juhnke S, Nies DH (1999) Energetics and topology of CzcA, a cation/proton antiporter of the resistance-nodulation-cell division protein family. J Biol Chem 274:26065–26070

Gotoh N, Nishino T (1990) Decreases of the susceptibility to low molecular weight beta-lactam antibiotics in imipenem-resistant *Pseudomonas aeruginosa* mutants: role of outer membrane protein D2 in their diffusion. J Antimicrob Chemother 25:191–198

Hall RM, Stokes HW (1993) Integrons: novel DNA elements which capture genes by site-specific recombination. Genetica 90:115–132

Helling RB, Janes BK, Kimball H, Tran T, Bundesmann M, Check P, Phelan D, Miller C (2002) Toxic waste disposal in *Escherichia coli*. J Bacteriol 184:3699–3703

Higgins CF (2007) Multiple molecular mechanisms for multidrug resistance transporters. Nature 446:749–757

Hooff GP, van Kampen JJ, Meesters RJ, van Belkum A, Goessens WH, Luider TM (2012) Characterization of β-lactamase enzyme activity in bacterial lysates using MALDI-mass spectrometry. J Proteome Res 11:79–84

Hu P, Yang M, Zhang A, Wu J, Chen B, Hua Y, Yu J, Chen H, Xiao J, Jin M (2011) Comparative genomics study of multi-drug-resistance mechanisms in the antibiotic-resistant *Streptococcus suis* R61 strain. PLoS ONE 6:e24988

Ishii N, Nakahigashi K, Baba T, Robert M, Soga T, Kanai A, Hirasawa T, Naba M, Hirai K, Hoque A, Ho PY, Kakazu Y, Sugawara K, Igarashi S, Harada S, Masuda T, Sugiyama N, Togashi T, Hasegawa M, Takai Y, Yugi K, Arakawa K, Iwata N, Toya Y, Nakayama Y, Nishioka T, Shimizu K, Mori H, Tomita M (2007) Multiple high-throughput analyses monitor the response of *E. coli* to perturbations. Science 316:593–597

Izumiya H, Sekizuka T, Nakaya H, Taguchi M, Oguchi A, Ichikawa N, Nishiko R, Yamazaki S, Fujita N, Watanabe H, Ohnishi M, Kuroda M (2011) Whole-genome analysis of *Salmonella enterica* serovar *typhimurium* T000240 reveals the acquisition of a genomic island involved in multidrug resistance via IS1 derivatives on the chromosome. Antimicrob Agents Chemother 55:623–630

Jack DL, Yang NM, Saier MH Jr (2001) The drug/metabolite transporter superfamily. Eur J Biochem 268:3620–3639

Jacobson RK, Minenza N, Nicol M, Bamford C (2012) VIM-2 metallo-beta-lactamase-producing *Pseudomonas aeruginosa* causing an outbreak in South Africa. J Antimicrob Chemother 67:1797–1798

Jariyawat S, Sekine T, Takeda M, Apiwattanakul N, Kanai Y, Sophasan S, Endou H (1999) The interaction and transport of beta-lactam antibiotics with the cloned rat renal organic anion transporter 1. J Pharmacol Exp Ther 290:672–677

Kenna DT, Doherty CJ, Foweraker J, Macaskill L, Barcus VA, Govan JR (2007) Hypermutability in environmental *Pseudomonas aeruginosa* and in populations causing pulmonary infection in individuals with cystic fibrosis. Microbiology 153:1852–1859

Kiffer CR, Pignatari AC (2011) Pharmacodynamic evaluation of commonly prescribed oral antibiotics against respiratory bacterial pathogens. BMC Infect Dis 11:286

Kim J, Bae IK, Jeong SH, Chang CL, Lee CH, Lee K (2011) Characterization of IncF plasmids carrying the blaCTX-M-14 gene in clinical isolates of *Escherichia coli* from Korea. J Antimicrob Chemother 66:1263–1268

Kumar R, Patel DD, Bansal DD, Mishra S, Mohammed A, Arora R, Sharma A, Sharma RK (2010) Extremophiles: sustainable resource of natural compound—extremolytes. In: Singh OV, Harvey SP (eds) Sustainable biotechnology: sources of renewable energy. Spring Press, UK, pp 279–294

Kumar V, Sun P, Vamathevan J, Li Y, Ingraham K, Palmer L, Huang J, Brown JR (2011) Comparative genomics of *Klebsiella pneumoniae* strains with different antibiotic resistance profiles. Antimicrob Agents Chemother 55:4267–4276

Kummerer K (2009) Antibiotics in the aquatic environment—a review—part I. Chemosphere 75:417–434

Kuroda T, Tsuchiya T (2009) Multidrug efflux transporters in the MATE family. Biochim Biophys Acta 1794:763–768

Larsson DG, de Pedro C, Paxeus N (2007) Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J Hazard Mater 148:751–755

Lewis K (2005) Persister cells and the riddle of biofilm survival. Biochemistry (Mosc) 70:267–274

Li XZ, Nikaido H (2004) Efflux-mediated drug resistance in bacteria. Drugs 64:159–204

Liang R, Fei YJ, Prasad PD, Ramamoorthy S, Han H, Yang-Feng TL, Hediger MA, Ganapathy V, Leibach FH (1995) Human intestinal H+/peptide cotransporter. Cloning, functional expression, and chromosomal localization. J Biol Chem 270:6456–6463

Liebert CA, Hall RM, Summers AO (1999) Transposon Tn21, flagship of the floating genome. Microbiol Mol Biol Rev 63:507–522

Livermore DM (2001) Of *Pseudomonas*, porins, pumps and carbapenems. J Antimicrob Chemother 47:247–250

Lomovskaya O, Lewis K (1992) Emr, an *Escherichia coli* locus for multidrug resistance. Proc Natl Acad Sci USA 89:8938–8942

Marger MD, Saier MH Jr (1993) A major superfamily of transmembrane facilitators that catalyse uniport, symport and antiport. Trends Biochem Sci 18:13–20

Marrer E, Satoh AT, Johnson MM, Piddock LJ, Page MG (2006) Global transcriptome analysis of the responses of a fluoroquinolone-resistant *Streptococcus pneumoniae* mutant and its parent to ciprofloxacin. Antimicrob Agents Chemother 50:269–278

Masaoka Y, Ueno Y, Morita Y, Kuroda T, Mizushima T, Tsuchiya T (2000) A two-component multidrug efflux pump, EbrAB, in *Bacillus subtilis*. J Bacteriol 182:2307–2310

Masuda N, Sakagawa E, Ohya S, Gotoh N, Tsujimoto H, Nishino T (2000) Contribution of the MexX–MexY–oprM efflux system to intrinsic resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 44:2242–2246

Mazzariol A, Tokue Y, Kanegawa TM, Cornaglia G, Nikaido H (2000) High-level fluoroquinolone-resistant clinical isolates of *Escherichia coli* overproduce multidrug efflux protein AcrA. Antimicrob Agents Chemother 44:3441–3443

Mesbah NM, Wiegel J (2008) Life at extreme limits: the anaerobic halophilic alkalithermophiles. Ann NY Acad Sci 1125:44–57

Minato Y, Shahcheraghi F, Ogawa W, Kuroda T, Tsuchiya T (2008) Functional gene cloning and characterization of the SsmE multidrug efflux pump from *Serratia marcescens*. Biol Pharm Bull 31:516–519

Moker N, Dean CR, Tao J (2010) *Pseudomonas aeruginosa* increases formation of multidrug-tolerant persister cells in response to quorum-sensing signaling molecules. J Bacteriol 192:1946–1955

Monteiro R, Vitorino R, Domingues P, Radhouani H, Carvalho C, Poeta P, Torres C, Igrejas G (2012) Proteome of a methicillin-resistant *Staphylococcus aureus* clinical strain of sequence type ST398. J Proteomics 75:2892–28915

Morita Y, Tomida J, Kawamura Y (2012) Primary mechanisms mediating aminoglycoside resistance in the multidrug-resistant *Pseudomonas aeruginosa* clinical isolate PA7. Microbiology 158:1071–1083

Mulcahy LR, Burns JL, Lory S, Lewis K (2010) Emergence of *Pseudomonas aeruginosa* strains producing high levels of persister cells in patients with cystic fibrosis. J Bacteriol 192: 6191–6199

Nagaraj NS, Singh OV (2010) Using genomics to develop novel antibacterial therapeutics. Crit Rev Microbiol 36:340–348

Neyfakh AA (1997) Natural functions of bacterial multidrug transporters. Trends Microbiol 5:309–313

Neyfakh AA, Borsch CM, Kaatz GW (1993) Fluoroquinolone resistance protein NorA of *Staphylococcus aureus* is a multidrug efflux transporter. Antimicrob Agents Chemother 37:128–129

Nikaido H (2009) Multidrug resistance in bacteria. Annu Rev Biochem 78:119–146

Nikaido H, Basina M, Nguyen V, Rosenberg EY (1998) Multidrug efflux pump AcrAB of *Salmonella typhimurium* excretes only those beta-lactam antibiotics containing lipophilic side chains. J Bacteriol 180:4686–4692

Normark S, Edlund T, Grundstrom T, Bergstrom S, Wolf-Watz H (1977) *Escherichia coli* K-12 mutants hyperproducing chromosomal beta-lactamase by gene repetitions. J Bacteriol 132:912–922

Obeng AS, Rickard H, Sexton M, Pang Y, Peng H, Barton M (2012) Antimicrobial susceptibilities and resistant genes in *Campylobacter* strains isolated from poultry and pigs in Australia. J Appl Microbiol. doi:10.1111/j.1365-2672.2012.05354.x

Okesola AO (2011) Community-acquired methicillin-resistant *Staphylococcus aureus*—a review of literature. Afr J Med Med Sci 40:97–107

Opazo A, Sonnevend A, Lopes B, Hamouda A, Ghazawi A, Pal T, Amyes SG (2012) Plasmid-encoded PER-7 beta-lactamase responsible for ceftazidime resistance in *Acinetobacter baumannii* isolated in the United Arab Emirates. J Antimicrob Chemother 67:1619–1622

Ozer B, Duran N, Onlen Y, Savas L (2012) Efflux pump genes and antimicrobial resistance of *Pseudomonas aeruginosa* strains isolated from lower respiratory tract infections acquired in an intensive care unit. J Antibiot (Tokyo) 65:9–13

Pao SS, Paulsen IT, Saier MH Jr (1998) Major facilitator superfamily. Microbiol Mol Biol Rev 62:1–34

Paterson DL, Bonomo RA (2005) Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 18:657–686

Paulsen IT, Skurray RA, Tam R, Saier MH Jr, Turner RJ, Weiner JH, Goldberg EB, Grinius LL (1996) The SMR family: a novel family of multidrug efflux proteins involved with the efflux of lipophilic drugs. Mol Microbiol 19:1167–1175

Paulsen IT, Park JH, Choi PS, Saier MH Jr (1997) A family of gram-negative bacterial outer membrane factors that function in the export of proteins, carbohydrates, drugs and heavy metals from gram-negative bacteria. FEMS Microbiol Lett 156:1–8

Perlman D, Stickgold R (1977) Selective amplification of genes on the R plasmid, NR1, in *Proteus mirabilis*: an example of the induction of selective gene amplification. Proc Natl Acad Sci USA 74:2518–2522

Pinyon JL, Hall RM (2011) Evolution of IncP-1alpha plasmids by acquisition of antibiotic and mercuric ion resistance transposons. Microb Drug Resist 17:339–343

Piras C, Soggiu A, Bonizzi L, Gaviraghi A, Deriu F, De Martino L, Iovane G, Amoresano A, Roncada P (2012) Comparative proteomics to evaluate multi drug resistance in *Escherichia coli*. Mol BioSyst 8:1060–1067

Pluss-Suard C, Pannatier A, Kronenberg A, Muhlemann K, Zanetti G (2011) Hospital antibiotic consumption in Switzerland: comparison of a multicultural country with Europe. J Hosp Infect 79:166–171

Poirel L, Bonnin RA, Nordmann P (2012) Genetic support and diversity of acquired extended-spectrum beta-lactamases in Gram-negative rods. Infect Genet Evol 12:883–893

Poole K (2005a) Aminoglycoside resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 49:479–487

Poole K (2005b) Efflux-mediated antimicrobial resistance. J Antimicrob Chemother 56:20–51

Porrero MC, Wassenaar TM, Gomez-Barrero S, Garcia M, Barcena C, Alvarez J, Saez-Llorente JL, Fernandez-Garayzabal JF, Moreno MA, Dominguez L (2012) Detection of methicillin-resistant *Staphylococcus aureus* in Iberian pigs. Lett Appl Microbiol 54:280–285

Poudel A, Nakajima C, Fukushima Y, Suzuki H, Pandey BD, Maharjan B, Suzuki Y (2012) Molecular characterization of multidrug-resistant *Mycobacterium tuberculosis* isolated in Nepal. Antimicrob Agents Chemother 56:2831–2836

Qu Y, Daley AJ, Istivan TS, Rouch DA, Deighton MA (2010) Densely adherent growth mode, rather than extracellular polymer substance matrix build-up ability, contributes to high resistance of *Staphylococcus epidermidis* biofilms to antibiotics. J Antimicrob Chemother 65:1405–1411

Radhouani H, Pinto L, Coelho C, Sargo R, Araujo C, Lopez M, Torres C, Igrejas G, Poeta P (2010a) MLST and a genetic study of antibiotic resistance and virulence factors in vanA-containing *Enterococcus* from buzzards (*Buteo buteo*). Lett Appl Microbiol 50:537–541

Radhouani H, Poeta P, Pinto L, Miranda J, Coelho C, Carvalho C, Rodrigues J, Lopez M, Torres C, Vitorino R, Domingues P, Igrejas G (2010b) Proteomic characterization of vanA-containing *Enterococcus* recovered from Seagulls at the Berlengas Natural Reserve, W Portugal. Proteome Sci 8:48

Rahmati S, Yang S, Davidson AL, Zechiedrich EL (2002) Control of the AcrAB multidrug efflux pump by quorum-sensing regulator SdiA. Mol Microbiol 43:677–685

Rajamohan G, Srinivasan VB, Gebreyes WA (2010) Molecular and functional characterization of a novel efflux pump, AmvA, mediating antimicrobial and disinfectant resistance in *Acinetobacter baumannii*. J Antimicrob Chemother 65:1919–1925

Ribera A, Ruiz J, Jiminez de Anta MT, Vila J (2002) Effect of an efflux pump inhibitor on the MIC of nalidixic acid for *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* clinical isolates. J Antimicrob Chemother 49:697–698

Rice LB (2012) Mechanisms of resistance and clinical relevance of resistance to β-lactams, glycopeptides, and fluoroquinolones. Mayo Clin Proc 87:198–208

Rossolini GM, Thaller MC (2010) Coping with antibiotic resistance: contributions from genomics. Genome Med 2:15

Sahl JW, Johnson JK, Harris AD, Phillippy AM, Hsiao WW, Thom KA, Rasko DA (2011) Genomic comparison of multi-drug resistant invasive and colonizing *Acinetobacter baumannii* isolated from diverse human body sites reveals genomic plasticity. BMC Genomics 12:291

Saier MH Jr, Tam R, Reizer A, Reizer J (1994) Two novel families of bacterial membrane proteins concerned with nodulation, cell division and transport. Mol Microbiol 11:841–847

Sandalakis V, Psaroulaki A, De Bock PJ, Christidou A, Gevaert K, Tsiotis G, Tselentis Y (2012) Investigation of rifampicin resistance mechanisms in *Brucella abortus* using MS-driven comparative proteomics. J Proteome Res 11:2374–2385

Sandegren L, Andersson DI (2009) Bacterial gene amplification: implications for the evolution of antibiotic resistance. Nat Rev Microbiol 7:578–588

Santimaleeworagun W, Wongpoowarak P, Chayakul P, Pattharachayakul S, Tansakul P, Garey KW (2011) In vitro activity of colistin or sulbactam in combination with fosfomycin or imipenem against clinical isolates of carbapenem-resistant *Acinetobacter baumannii* producing OXA-23 carbapenemases. Southeast Asian J Trop Med Public Health 42:890–900

Schuldiner S (2007) When biochemistry meets structural biology: the cautionary tale of EmrE. Trends Biochem Sci 32:252–258

Sherman DH (2005) The Lego-ization of polyketide biosynthesis. Nat Biotechnol 23:1083–1084

Singh OV, Gabani P (2011) Extremophiles: radiation resistance microbial reserves and therapeutic implications. J Appl Microbiol 110:851–861

Singh OV, Nagaraj NS, Gabani P (2011) Systems biology: integrating '-omics' oriented approaches to determine foodborne microbial toxins. In: Sahu SC, Casciano DA (eds) Handbook of systems toxicology: from omcis technology to nanotechnology. Wiley, West Sussex

Smith KP, Kumar S, Varela MF (2009) Identification, cloning, and functional characterization of EmrD-3, a putative multidrug efflux pump of the major facilitator superfamily from *Vibrio cholerae* O395. Arch Microbiol 191:903–911

Sobel ML, McKay GA, Poole K (2003) Contribution of the MexXY multidrug transporter to aminoglycoside resistance in *Pseudomonas aeruginosa* clinical isolates. Antimicrob Agents Chemother 47:3202–3207

Soualhine H, Brochu V, Menard F, Papadopoulou B, Weiss K, Bergeron MG, Legare D, Drummelsmith J, Ouellette M (2005) A proteomic analysis of penicillin resistance in *Streptococcus pneumoniae* reveals a novel role for PstS, a subunit of the phosphate ABC transporter. Mol Microbiol 58:1430–1440

Srinivasan VB, Rajamohan G, Gebreyes WA (2009) Role of AbeS, a novel efflux pump of the SMR family of transporters, in resistance to antimicrobial agents in *Acinetobacter baumannii*. Antimicrob Agents Chemother 53:5312–5316

Sun H, Xu Y, Sitkiewicz I, Ma Y, Wang X, Yestrepsky BD, Huang Y, Lapadatescu MC, Larsen MJ, Larsen SD, Musser JM, Ginsburg D (2012) Inhibitor of streptokinase gene expression improves survival after group A *Streptococcus* infection in mice. Proc Natl Acad Sci USA 109:3469–3474

Taccone FS, Cotton F, Roisin S, Vincent JL, Jacobs F (2012) Optimal meropenem concentrations to treat multidrug-resistant *Pseudomonas aeruginosa* septic shock. Antimicrob Agents Chemother 56:2129–2131

Toleman MA, Bennett PM, Walsh TR (2006) ISCR elements: novel gene-capturing systems of the 21st century? Microbiol Mol Biol Rev 70:296–316

Tomberg J, Temple B, Fedarovich A, Davies C, Nicholas RA (2012) A highly conserved interaction involving the middle residue of the SXN active-site motif is crucial for function of class B

penicillin-binding proteins: mutational and computational analysis of PBP 2 from *N. gonorrhoeae*. Biochemistry 51:2775–2784

Tseng TT, Gratwick KS, Kollman J, Park D, Nies DH, Goffeau A, Saier MH Jr (1999) The RND permease superfamily: an ancient, ubiquitous and diverse family that includes human disease and development proteins. J Mol Microbiol Biotechnol 1:107–125

van Veen HW, Konings WN (1998) The ABC family of multidrug transporters in microorganisms. Biochim Biophys Acta 1365: 31–36

Vaziri F, Peerayeh SN, Nejad QB, Farhadian A (2011) The prevalence of aminoglycoside-modifying enzyme genes (aac (6′)-I, aac (6′)-II, ant (2″)-I, aph (3′)-VI) in *Pseudomonas aeruginosa*. Clinics (Sao Paulo) 66:1519–1522

Versporten A, Coenen S, Adriaenssens N, Muller A, Minalu G, Faes C, Vankerckhoven V, Aerts M, Hens N, Molenberghs G, Goossens H (2011) European Surveillance of Antimicrobial Consumption (ESAC): outpatient cephalosporin use in Europe (1997–2009). J Antimicrob Chemother 66(Suppl 6):vi25–vi35

Vitkauskiene A, Skrodeniene E, Dambrauskiene A, Baksyte G, Macas A, Sakalauskas R (2011) Characteristics of carbapenem-resistant *Pseudomonas aeruginosa* strains in patients with ventilator-associated pneumonia in intensive care units. Medicina (Kaunas) 47:652–656

Woodford N, Livermore DM (2009) Infections caused by Gram-positive bacteria: a review of the global challenge. J Infect 59(Suppl 1):S4–S16

Xiao Y, Hu Y (2012) The major aminoglycoside-modifying enzyme AAC(3)-II found in *Escherichia coli* determines a significant disparity in its resistance to gentamicin and amikacin in China. Microb Drug Resist 18:42–46

Zgurskaya HI, Nikaido H (1999) Bypassing the periplasm: reconstitution of the AcrAB multidrug efflux pump of *Escherichia coli*. Proc Natl Acad Sci USA 96:7190–7195