

Patents on antivirulence therapies

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Abstract

Antivirulence therapy inhibits bacterial virulence factors, thus preventing the development of infection without affecting bacterial growth. The development of new antibiotics is complicated by the increasing incidence of antibiotic resistance in pathogenic bacteria. Antivirulence therapy is a promising alternative to traditional antibiotic therapy for the treatment of infectious disease, either alone or in combination with antibiotic treatment. In this review, we consider patents concerning inhibition of several bacterial virulence factors: adhesion/colonization, secretion systems, cellular signalling systems and antimicrobial resistance mechanisms. Finally, we emphasize the importance of analyzing new targets and/or molecules in this field and of considering possible resistance mechanisms.

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Core tip: Antimicrobial resistance in nosocomial pathogens has increased dramatically in recent years. The development of new molecules, therapies and/or new combinations for the eradication of these pathogens is therefore imperative. A new line of research in this area is called "Antivirulence Therapy".

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INTRODUCTION

Microbial virulence is the ability of a microbe to cause disease. Antivirulence therapies are constituted on inhibition of bacterial virulence and do not influence bacterial growth. Bacteria appreciate their environment and, once in the host they respond by starting a plan determined for the activation of virulence factors. Hence, antivirulence strategies have the ability to interfere in the recognition of the host signals that alarm the bacteria localized in the place of infection and/or that activate specific virulence factors implicated to development of the infection. If the development of virulence factors is prevented, the bacteria will be less able to colonize. Moreover, this tactic will not directly kill bacteria, so initially the evolutionary pressure for the development of resistant strains would be lower than with conventional antibiotics^[1]. Inhibition of the following systems enables interruption of the process of bacterial infection: toxin production, adhesion and colonization, bacterial secretory systems, cell-to-cell signalling pathways, and antibiotic resistance mechanisms, such as efflux pumps (multidrug resistance) (Figure 1)^[2]. In this review, we provide details of patents concerning the inhibition of each of these mechanisms, except for toxin production, which is specific to certain pathogens such as *Bacillus anthracis* (which causes anthrax) and *Clostridium spp.* (which causes gangrene)^[1].

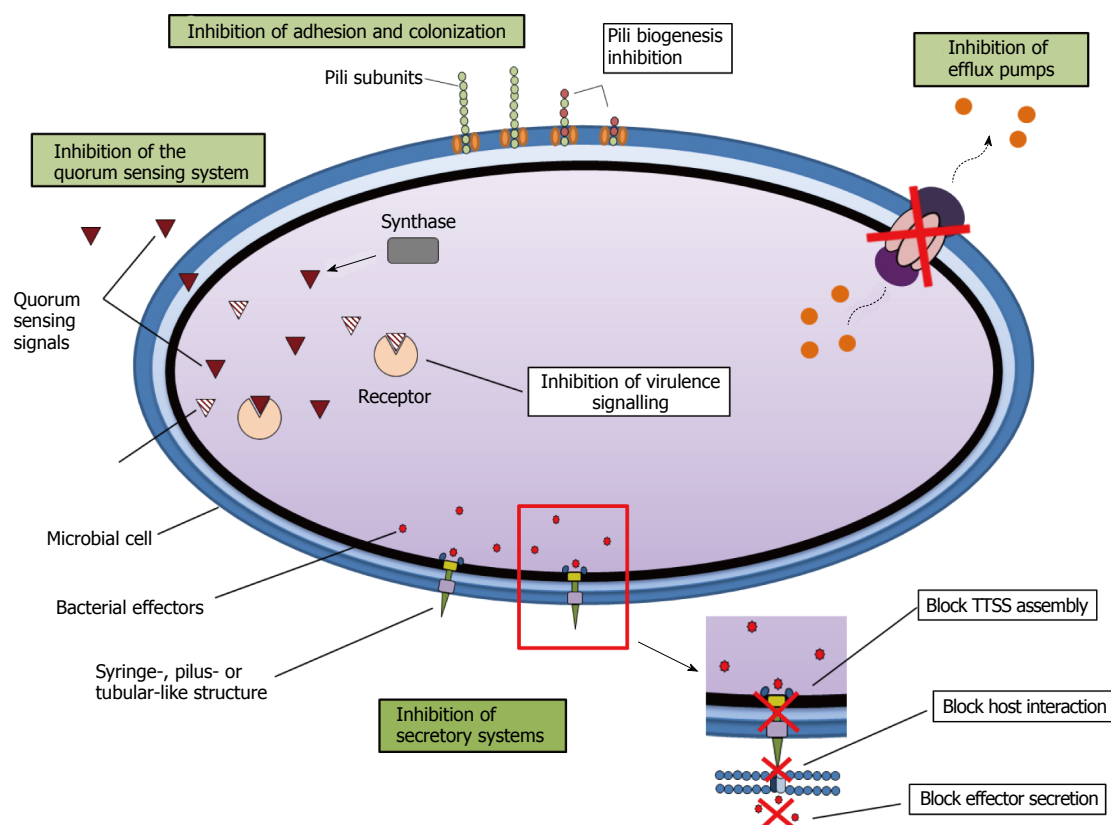


Figure 1 Anti-virulence strategies to combat bacteria-mediated disease (quorum quenchers). TTSS: Type III secretion system.

ADHESION AND COLONIZATION MECHANISMS

Microorganisms adhere to host cells in order to colonize the host and begin infection. The majority of the bacteria own a determined host interval and will only infect hosts that express specific receptors for bacterial adhesion traits on their cell surface. Besides, once inside the host, bacteria will only infect the cells (tissue) that have the adequate receptor. Attachment of bacteria to a host cell is a complicated process managed by adhesin on the bacteria and the receptor on the cell. However, adherence is frequently only the first step in the infection course, that besides implicates internalization, deeper tissue penetration and likely systemic spread. Bacteria have different kinds of elements for adhere to the host surface, including- but not only-pili, fimbriae and in some cases flagella^[3]. Adhesion can be inhibited by the following strategies: (1) prevention of adhesion complex assembly [as in the pili of uropathogenic *Escherichia coli* (*E. coli*)], for which the compounds bicyclic 2-pyridones (pilicides)^[4] and Virstatin^[5] have been developed; and (2) prevention of elongation and formation of a functional pilus.

A search carried out in patent databases^[6,7] revealed a total of 26 patent applications related to strategies that interfere with adhesion and colonization mechanisms (Table 1). These include the use of probiotics such as *Lactobacillus reuteri*, *Bifidobacterium infantis*, *Bifidobacterium lactis*, *Lactobacillus acidophilus* and *Lactobacillus casei*, for in-

hibition of *Candida* colonization, and also *Enterococcus faecium* LJS-01, which displays a strong capacity to adhere to intestinal epithelial cells and also good antimicrobial activity. The following proteins have also been described: decorin binding protein, which prevents colonization by *Borrelia*; collagen binding protein, isolated from *Staphylococcus aureus*; C3 binding polypeptide, isolated from *Streptococcus agalactiae*; novel fluorinated linker compounds; and Zn-releasing calcium phosphate. Finally, the following targets have been identified for vaccine development: capsular polysaccharide, EtpA flagellin and pyruvate-ferredoxin oxidoreductase adhesin protein.

BACTERIAL SECRETORY SYSTEMS

Many bacteria have a specialized excretory system that resembles a syringe through which bacterial toxins (effector proteins) are injected into the host cell. These systems work by imitating host proteins, thus altering the signalling pathways and enabling development of the disease^[8]. Three different secretion systems are implicated in the translocation of bacterial effectors into host cells, III, IV and VI^[9].

The type III secretion system (TTSS) comprises some proteins that form a spire-like construction through which the bacteria inject the effector proteins from the bacterial cytoplasm to the cytoplasm of eukaryotic host cells. These secreted effector proteins often modify signal transduction in the host cells to improve microbial survival, invasion or attachment^[10,11]. The type IV secretory

Table 1 Patents concerning the inhibition of bacterial adhesion and colonization

Patent title	Description	Application date	Inventors	Publication number
Capsular polysaccharide adhesion antigen preparation, purification and use	General method for preparing pure capsular exopolysaccharide adhesins strains of adhesin coagulase-negative staphylococci to produce vaccines	1994	Pier Gerald B	US5980910 (A)
Method for inhibiting microbial adhesion on surfaces	A method for inhibiting microbial adhesion on surfaces in contact with an aqueous system is disclosed and involves adding a treatment comprising an alkyl sulfosuccinate surfactant to the system	1995	Wright J Barry; Michalopoulos Daniel L	US5512186
Method and apparatus for preventing adhesion and colonization of bacteria in medical devices	Activation of compounding photochemicals for preventing and eliminating adherence and colonization of bacteria	1996	Prescott Marvin A	WO9806340 (A1)
Decorin binding protein compositions and methods of use	DNA segments encoding these proteins and anti-(decorin binding protein) antibodies for use in the prevention of <i>Borrelia</i> colonization in an animal	1996	Guo Betty P; Hoeoek Magnus; Hanson Mark	WO9727301 (A1)
Collagen binding protein compositions and methods of use	Disclosed are the <i>cna</i> gene and <i>cna</i> -derived nucleic acid segments from <i>Staphylococcus aur.</i> Also disclosed are Col Binding Protein (CBP) for use in the treatment of pathological infections, and in particular, for use in the prevention of bacterial adhesion to Col	1997	Hoeek Magnus; Patti Joseph M; House-Pompeo Karen; Sthanam Narayana; Symersky Jindrich	US6288214 (B1)
Surfactants for reducing bacterial adhesion onto surfaces	Inhibiting microbial colonization (ethylene oxide units) of a surface in contact with an aqueous system	1997	Donlan Rodney M; Elliot David L; Kapp Nancy J; Wiatr Christopher L; Rey Paula	US6039965 (A)
Composition of treatment of Candidiasis	Inhibition of adhesion of <i>Candida</i> colonization by using probiotics (<i>Lactobacillus reuteri</i> , <i>Bifidobacterium infantis</i> , <i>Bifidobacterium lactis</i> , <i>Lactobacillus acidophilus</i> not viable and non-viable <i>Lactobacillus casei</i>)	1998	Dohnlek Margaret H; Wagner Robert Doug; Balish Edward; Hilty Milo D	WO9917788 (A1)
Antimicrobial adhesion surface	The invention provides an implantable medical device with a hydrophilic coating to limit <i>in vivo</i> colonization of bacteria and fungi	1999	Zhong Samuel P	US6468649 (B1)
Anti-bacterial compounds directed against pilus biogenesis, adhesion and activity; co-crystals of pilus subunits and methods of use thereof	The invention relates to novel compounds that mimic a chaperone G1 beta-strand or an amino terminal motif of a pilus subunit	2000	Hultgren Scott J; Sauer Frederic G; Waksman Gabriel; Fuetterer Klaus; Choudhury Devapriya; Knight Stefan D; Barnhart Michelle	US7041465 (B1)
C3 binding polypeptide of <i>Streptococcus agalactiae</i> group b- <i>Streptococcus</i>	The invention involves the identification of a human complement C3 binding polypeptide and the nucleic acid that encodes the polypeptide from <i>Streptococcus agalactiae</i>	2000	Smith Beverly L; Ferrieri Patricia	US6582950 (B1)
Compounds directed against pilus biogenesis and activity in pathogenic bacteria, methods and compositions for synthesis thereof	Novel fluorinated linker compounds and methods of synthesis are provided. Methods for using the fluorinated linker compounds in methods of solid-phase synthesis of the N-substituted amino acid compounds are also disclosed (inhibiting or preventing the formation of a pilus chaperone-subunit complex)	2000	Kihlberg Jan; Larsson Andreas; Svensson Anette; Fex Tomas; Hultgren Scott J; Pinkner Jerry	WO2001020995
DbpA compositions and methods of use	The DBP gene and decorin protein compositions of <i>Borrelia burgdorferi</i> are disclosed The DBP protein and antigenic epitopes derived from them are contemplated for use in preventing bacterial adhesion to decorin	2000	Guo Betty P; Hook Magnus	US6312907
Composition and method for controlling microbial adhesion and biofilm formation of surfaces	The invention describes how coating of surfaces with an extract, particularly a fish extract, can significantly reduce microbial adhesion, attachment, colonization and biofilm formation on surfaces	2003	Gram Lone Kirsten; Vogel Birtefonnesbech; Bagge-Ravn Dorte	WO03092382 (A1)
Packaged antimicrobial medical device and method of preparing same	An antimicrobial suture assembly (halogenated hydroxyl ethers, acyloxydiphenyl ethers, and combinations thereof) to substantially inhibit bacterial colonization	2004	Scalzo Howard; Fischer Jerome A; Rothenburger Stephen	US2004220614 (A1)
Sealing material	A sealing material is presented (fluoropolymer layer, a reinforcing layer and an adhesive) to hinder growth and colonization of bacteria	2004	Patel Malay; Napolitano Michael; Hanrahan James R; Chu Chaokang	US2005250398 (A1)

Zn-releasing calcium phosphate (Zn-CaP) compounds for antimicrobial coating on orthodontic appliances and dental implants	Compositions of Zn-releasing calcium phosphate (Zn-CaP) compounds for use as anti-bacterial coatings for orthodontic brackets and dental implants	2006	Legeros Racquel Z; Legeros John P; Park Jae Hyun; Mijares Dindo	WO2007022211 (A2)
Composition for the administration of biologically active principles in gynaecological and rectal conditions and uses thereof	The invention relates to a composition for the administration of biologically active substances in gynaecological and rectal conditions, as well as the uses of said composition	2007	Strozzi Gianpaolo; Mogna Luca	US2010092440 (A1)
Enhanced treatments to kill or debilitate pathogenic microorganisms of a mammalian body	The novel treatments involve the use of anti-adhesive polysaccharide molecules to abolish or reduce the adhesion of <i>Helicobacter pylori</i>	2008	Nifantiev Nikolay; Wieland Gerhard D	US2011245198 (A1)
Non-leaching surface-active film compositions for microbial adhesion prevention	Coating (surfactant) useful to prevent bacterial colonization on a variety of surface including surfaces of medical devices	2008	Gruening Rainer; Qu Xin; Merritt Karen; Chen Paul N; Falevich Vitaly	MX2008009326 (A)
Prevention and treatment of Gram-negative, flagellated bacterial infections	EtpA which binds to the conserved region of the flagellin protein located at the tip of the flagella in Gram-negative bacteria (development vaccine)	2008	Fleckenstein James M	US2011206694 (A1)
Method for coating medical device ¹	Method for coating a medical device to prevent bacterial adhesion, colonization and device-associated infection (isocyanate-terminated polymer)	2010	Stopek Joshua	JP2011019902 (A)
Novel <i>Enterococcus faecium</i> LJS-01 and its use as a probiotic ¹	<i>Enterococcus faecium</i> LJS-01 shows good antimicrobial activity and strong capacity to adhere to intestinal epithelial cells	2010	Lin Chuen-FU; Wu Cheng-Nan; Lu Cheng-Hsiung; Hsu Wei-Li; Chiou Ming-Tang	TW201143631 (A)
Method for detecting colonization characteristic of lactobacillus in gastrointestinal tract on basis of green fluorescent protein ¹	The invention relates to a method for detecting the colonization characteristic of lactobacillus in the gastrointestinal tract on the basis of green fluorescent protein	2011	Yanping Wang; Jingrui Wang; Jinju Wang	CN102604877 (A)
Prevention of bacterial adhesion ¹	Prevention of adhesion of microorganisms on hard surfaces by the semi-permanent modification thereof during the cleaning process. A cleaning agent that contains surface-active polymers is used to prevent the bacterial colonization of hard surfaces	2011	Veith Birgit; Weide Mirko; Corbellini Francesca; Giesen Brigitte; Stumpe Stefan; Breves Roland; Barreleiro Paula; Karten Stefan; Bockmuehl Dirkl; Meier Frank	WO2012010700 (A1)
Pyruvate-ferredoxin oxidoreductase (PFO) adhesive protein as a target for inhibiting the adherence of <i>Trichomonas vaginalis</i> and as a diagnosis and vaccinal target for trichomoniasis ¹	Novel function of PFO upon participating in the cytoadherence of the <i>Trichomonas vaginalis</i> parasite to the hosting cell. The present invention enables development of vaccines for preventing the adhesion (and therefore the colonization) of parasites to the vaginal mucosa	2011	Verastegui Rossana Arroyo	MX2011011361 (A)
Vacuum assisted percutaneous appliance ¹	This device is stabilized by fibroblast in-growth and inhibits bacterial colonization	2012	Kantrowitz Allen B; Mortin Chris; Wadsworth JR Daniel C	US2013006186 (A1)

¹Those published from 2010 onwards are highlighted.

system is utilized to transfer bacterial DNA or bacterial effector proteins to eukaryotic cells. This system also forms a duct between the bacterial and eukaryotic cell cytoplasm. It is a pilus-like structure rather than a spire construction^[9]. Type VI secretion systems form tubular construction; however, exactly how these systems assemble and give effector proteins into the eukaryotic host cells stays in great measure unknown^[12].

Although a lot of various types of TTSS are known, there are a restricted number of manners of inhibiting them: (1) prevention of assembly of the TTSS; (2) inhibition of interplay with the eukaryotic host cells; and (3) inhibition of secretion of the effector proteins.

Three components that are capable of inhibiting bacterial secretion systems have been reported^[13]: (1) inhibitors of the type III secretion systems such as acylated hydrazones

of salicylaldehydes in *Chlamydia* and *Shigella* infections; (2) 2-amino-5-arylidene thiazolidinone in *Salmonella*, *Pseudomonas* and *Yersinia* infections; and (3) dirylacrylonitrile, which inhibits sortase A and has shown *in vitro* activity against *S. aureus*.

A search of the patent database (up to April 2014) revealed 22 patents involving inhibition of the proteins related to secretion systems (Table 2). All of these are based on methods that describe how to identify inhibitors and target proteins of bacterial secretion systems. Two proteins groups are associated with these secretion systems: Inc and HpaB group proteins.

CELL-TO-CELL SIGNALLING: QUORUM SENSING

Cell-cell communication, or quorum sensing (QS), is a

Table 2 Patents concerning the inhibition of bacterial secretion systems

Patent title	Description	Application date	Inventors	Publication number
Method for screening for inhibitors and activators of type III secretion machinery in <i>Gram-negative</i> bacteria	This invention relates to mutant strains of Gram-negative bacteria that constitutively secrete proteins <i>via</i> the type III secretion machinery and to methods of identifying molecules that are able to activate or inhibit secretion in wild-type strains of Gram-negative bacteria	2001	Demers Brigitte; Sansonetti Philippe; Parsot Claude	US6696249 (B1)
Method of detecting substance inhibiting type III secretion mechanism of bacterium and the function of secretory protein thereof	A method whereby a substance specifically inhibiting the type III secretion mechanism and the function of a type III secretory protein secreted therefrom can be detected in large amounts within a short period of time without depending on any animal infection experiments	2001	Omura Satoshi; Abe Akio	KR1020020086208
Secreted <i>Chlamydia</i> polypeptides and method for identifying such polypeptides by their secretion by a type III secretion pathway of a <i>Gram-negative</i> bacteria	The present invention uses a heterologous secretion system, namely a type III system, to investigate whether some <i>Chlamydia</i> proteins, especially Inc proteins and other proteins exhibiting a similar hydropathy profile, might be secreted and demonstrates that these hybrid proteins are secreted by the type III secretion system of <i>Shigella flexneri</i>	2003	Subtil Agathe; Parsot Claude; Dautry-Varsat Alice	US2004131624 (A1)
Bacterial system for protein transport in eukaryotic cells	Development of a system for the targeted transport of proteins into eukaryotic cells by using a type III secretion system and bacteria strains that are mutated in hpaB or homogenous genes. The inventive bacterial system is used to transport bacterial proteins into eukaryotic cells, in order to influence or modify cellular processes such as gene expression, growth, development and defence/resistance mechanisms	2005	Bonas Ulla; Buettner Daniela	WO2005085417 (A2)
Methods of identifying modulators of bacterial type III protein secretion system	Provides methods for identifying inhibitors or activators of bacterial type III protein secretion system by using a recombinant beta-lactamase that can be secreted by a type III protein secretion system. The assay could be easily adapted to a high throughput mode to allow daily screening of several tens of thousands compounds	2005	Goldschmidt Raul; Loeloff Michael	WO2005113791 (A2)
Pharmaceutical composition for the treatment of bacterial infections and sepsis	The invention involves a pharmaceutical composition comprising at least one glycogen synthase kinase 3 β ; inhibitor, at least one Rho-kinase inhibitor, and an optional adequate pharmaceutical carrier for producing a drug for the preventive or therapeutic treatment of bacterial infectious diseases by synergistically increasing synthesis and secretion of type II. A secretory phospholipase A2 into the bloodstream so as to boost the body's inherent resistance to infections	2005	Menschikowski Mario; Hagelgans Albert; Siebert Gabriele	WO2005120475
Pyridone compounds as inhibitors of bacterial type III protein secretion systems	Provides compounds that inhibit type III protein secretion useful for the treatment and prevention of bacterial infections, particularly those caused by <i>Gram-negative</i> bacteria, and methods for their use	2005	Li Xiaobing	US2005256137 (A1)
Methods for stimulating an immune response using bacterial antigen delivery system	Provides methods for stimulating and/or increasing an immune response against tumor antigens through the use of the type III secretion system of bacteria. The invention also relates to the preparation of antigen presenting cells from peripheral blood mononuclear cells by using bacteria with a type III secretion system	2006	Old Lloyd J; Ritter Gerd; Nishikawa Hiroyoshi; Gnjjatic Sacha; Galan Jorge E	US2009324651 (A1)
Screening system for inhibitors and activators of type III secretion machinery in <i>Gram-negative</i> bacteria	Provides a screening system (comprising inhibitors and activators of type III secretion machinery) that directly transfers pathogenic proteins of <i>Gram-negative</i> bacteria into a host cell to identify substances capable of activating or inhibiting the secretion of type III protein secretion system	2006	Hwang In Gyu; Moon Jae Sun; Kim Sung Uk	KR20080051240 (A)
Application of bovine lactoferrin for preparing a medicinal agent for inhibition of bacteria growth	The invention refers to a new application of bovine lactoferrin for preparing a medicinal agent for inhibiting bacteria growth. The bovine lactoferrin inhibits the growth of bacterial pathogens expressing the type III secretory system	2007	Makmakhon Robert Dzh; Kliari Tomas; Ochoa Tereza	RU2007140789 (A)
Bacterial secretion system and uses	-	2007	Gey Van Pittius Nicolaas Claudius; Warren Robin Mark; Van Helden Paul David	ZA200706520 (A)
Biopolymer and protein production using type III secretion systems of <i>Gram-negative</i> bacteria	Provides proteins, polynucleotide, expression cassette, vector and bacterium compositions for obtaining proteins of interest by expression of same in Gram-negative bacteria with a type III secretion system. Also provides uses for the proteins obtained in the manufacture of isolated proteins and pharmaceutical compositions	2007	Voigt Christopher Ashby; Widmaier Daniel Matthew	WO2008019183 (A2)

Use of the <i>Pseudomonas syringae</i> effector protein HopU1 related to its ability to ADP-ribosylate eukaryotic RNA binding proteins	The invention provides novel methods for modulation of the innate immune response of a plant to infection caused by <i>Pseudomonas syringae</i> , which injects effector proteins into host cells <i>via</i> a type III protein secretion system. Also provides methods for enhancing or suppressing the innate immune response of the plant	2007	Alfano James R; Fu Zheng Qing; Elthon Thomas E	WO2008042026 (A2)
Method and means for preventing and inhibiting type III secretion in infections caused by Gram-negative bacteria	Discloses a means of decreasing bacterial virulence in a mammal or in a plant by inhibition of the type III secretion system at concentrations that do not prevent or substantially reduce bacterial growth. Also disclosed are a therapeutic method and a pharmaceutical composition	2008	Elofsson Mikael	US2010099674 (A1)
Carboplatin compound inhibiting secretion system of phytopathogenic Gram-negative bacteria and biocontrol agent of plant diseases with this compound	Provides an agent for preventing plant diseases, containing carboplatin compounds, to selectively suppress secretion system related to plant pathogenicity	2009	-	KR20110048335 (A)
Inhibition of quorum sensing-mediated processes in bacteria	Provides methods for identifying molecules that can be used to positively and negatively manipulate quorum-sensing-mediated communication to control bacterial behavior. Methods of inhibiting quorum sensing-mediated activity in Gram-negative bacteria are provided wherein the activity is pathogenicity, bioluminescence, siderophore production, type III secretion, or metalloprotease production	2009	Bassler Bonnie; Swem Lee	US2011123586 (A1)
Type III secretion inhibitors, analogs and uses thereof	The invention relates to compounds and compositions useful for inhibiting type III secretion systems in pathogenic bacteria, such as <i>Yersinia pestis</i> , and uses of such inhibitors in the treatment and prevention of disease	2009	Goguen Jon; Pan Ning; Lee Kyungae	US2011034463 (A1)
5-substituted-2-imino-thiazolidinone compounds and their use as inhibitors of bacterial infection ¹	Provides a method for inhibiting Gram-negative bacterial pathogenesis, a method of screening for compounds that inhibit type III secretion in <i>Gram-negative</i> bacteria, and compounds that inhibit type III secretion in <i>Gram-negative</i> bacteria	2010	Felise Heather B; Miller Samuel I; Kline Toni	US2011039849 (A1)
Methods for Identifying Inhibitors of the type III Secretion System ¹	Provides a method for determining whether a test compound can inhibit the function of the type III secretion system. The method identifies drug candidates that are highly specific anti-bacterial agents for treating diseases caused by Gram-negative bacteria with a T3SS	2010	Marlovits Thomas C; Radics Julia; Schmied Wolfgang	US2013130283 (A1)
Attenuated <i>Salmonella</i> inducible secretory expression oral vaccine presentation system and application thereof ¹	The invention comprises an attenuated salmonella inducible secretory expression oral vaccine presentation system containing an antigen expression carrier. The system is controlled by a promoter induced by induction of a bacteria excretion signal, and it uses the attenuated salmonella as the host of the antigen expression carrier	2011	Zichun Hua; Guo Chen	CN102335421 (A)
Bacterial mediated delivery of nuclear protein into pluripotent and differentiated cells ¹	A modified <i>Pseudomonas aeruginosa</i> type III secretion system has been developed that efficiently delivers selected proteins into a host cell	2011	Jin Shouguang; Bichsel Candace	WO2012012605 (A2)
Inhibitors of bacterial type III secretion system ¹	Discloses organic compounds showing the ability to inhibit effector toxin secretion or translocation mediated by bacterial type III secretion systems. These inhibitor compounds are useful for combating infections by <i>Gram-negative</i> bacteria with such type III secretion systems	2012	Moir Donald T.; Aiello Daniel; Peet Norton P; Williams John D; Torhan Matthew	US2014142134 (A1)

¹Those published from 2010 onwards are highlighted.

widespread phenomenon in bacteria that is used to co-ordinate gene expression between local populations^[13]. Bacterial populations can use QS communication to coordinate the execution of important biological functions, many of which are involved in pathogen virulence, *e.g.*, biofilm formation, extracellular polysaccharide production, host colonization, motility, bioluminescence, transfer of plasmids by conjugation, and biosynthesis of antibiotics and siderophores.

All QS systems utilize small, secreted signalling molecules known as autoinducers (AIs): (1) AI-1 molecules are N-acyl-homoserine lactones (AHLs); (2) AI-2 molecules are heterocyclic furanosyl-borates; (3) AI-3 signals are catecholamines and finally; and (4) AI-4 signals are cyclic peptides. Some other QS signals go beyond these classes,

e.g., *Pseudomonas* quinolone signal and diffusible signal factor. New molecules will undoubtedly be discovered as the study of QS expands to species of bacteria yet to be investigated.

Targeting bacterial virulence (quorum quenchers, Figure 1) is an alternative focusing to antimicrobial therapy that offers a hopeful opportunity to inhibit pathogenesis and its consequences without producing immediately the death the target bacterium. Bacterial virulence factors have been shown to be potential targets for drug design and therapeutic intervention for Gram-negative pathogens^[1]. Numerous quorum sensing inhibitors have been reported in the literature^[1,2].

In 2012, Romero *et al.*^[14] published an article about patents concerning quorum quenching (QQ) (*i.e.*, the

mechanisms that cause the inactivation of QS communication systems)^[15].

A search of patent databases (up to August 2011) revealed a total of 45 applications related to strategies for interfering with QS systems as a method of fighting microbial infections. Following the bias in the literature, the vast majority of the patented technologies based on the inhibition of QS mechanisms target AHL signals, whereas only 5 out of 45 patent cases are based on the inhibition of AI-2 signals and only 4 are based on the inhibition of peptide-based QS signals from Gram-positive bacteria. In this review, a search of more recent reports (up to April 2014) was conducted, revealing 32 patents concerning the inhibition of QS systems (Table 3). QQ occurs in *Lysobacter enzymogenes*, *Shewanella piezotolerans*, *Bacillus pumilus*, *Tenacibaculum discolor* (cect 7426) and novel alpha-proteobacteria. The molecules involved in QQ distinguish inhibitors of the AI-2 signals (triazol derivatives, furan compounds and phosphorylated, branched dihydroxy-pentane-dione) and inhibitors of AHLs [(oxododecanoyl)-L-homoserine lactone and bicyclic furanones with low toxicity]. Furanones, which are naturally occurring compounds, appear to be the most widely studied QQ compounds. These compounds are toxic to *Artemia* and rotifers, which will limit their use in humans^[15]. However, the use of C-30, a synthetic furanone, at non toxic concentrations, significantly reduced the pathogenicity of *Vibrio anguillarum* in rainbow trout^[16]. Other patented compounds involved in QQ include honaucin A, 2-methylthiopyrrolidines, lovastatin and hydroxytyrosol. Finally, one enzyme (OLB-26) is known to be involved in QQ.

MECHANISMS OF RESISTANCE: MULTIDRUG RESISTANCE PUMPS

Multidrug resistance (MDR) efflux pumps have multiple functions in natural microbial ecosystems. In clinical environments, MDR pumps are implicated in the following: (1) resistance to antimicrobial compounds localized on mucosal surfaces (colonization factor)^[17,18]; (2) efflux virulence factors^[19]; (3) QS-regulated expression of virulence traits^[20]; and (4) antibiotic resistance, which is a code element in patients under treatment^[21]. All of these roles are important for the survival, colonization and pathogenic outcome of virulent bacteria in clinical environments. In nonclinical environments, MDR pumps may be associated to resistance to heavy metals^[22] and organic solvents^[23] (plant colonization factor). Bacterial efflux pumps are classified into five families according to their composition, number of transmembrane spanning regions, energy sources and substrates: the resistance-nodulation-division (RND) family, the major facilitator superfamily, the adenosine triphosphate-binding cassette superfamily, the small multidrug resistance family, and the multidrug and toxic compound extrusion family^[17,18].

Several MDR pump inhibitors were published^[24]. We consider two examples of RND inhibitors pumps: (1)

1-(1-naphthylmethyl)-piperazine and phenyl-arginine- β -naphthylamide. These act as inhibitors of RND efflux pumps and virulence traits in *Vibrio cholerae*, such as the cholera toxin and the toxin-coregulated pilus^[25], and were suggested as a suitable tool for the treatment of cholera infections; and (2) Of 12 trifluoromethyl ketone compounds tested, 6 proved to be effective inhibitors of the quorum-sensing response by *Chromobacterium violaceum* 026, as well as inhibitors of the RND efflux pumps of CV026 and *E. coli*. This result is of clinical applicability and may be used for the prevention of QS responses of infecting bacteria^[26].

We found 22 patents related to the inhibition of the MDR efflux in patent databases up to April 2014 (Table 4). Most of these are screening methods for microbial efflux pump inhibitors. Moreover, pump inhibitors are described as potentiators of the action of antiseptics, disinfectants and antimicrobial agents such as tigecycline. Finally, polyamine molecules are inhibitors of bacterial efflux pumps that could be used in combination with other drugs such as antibiotics, as well as pharmaceutical compositions thereof.

FUTURE PROSPECTS: RESISTANCE TO ANTIVIRULENCE COMPOUNDS

Although antivirulence therapies are novel in the field of treatment of infectious diseases, several studies involving clinical strains have demonstrated the development of mechanisms of resistance, especially to Quorum Quenching compounds^[27]. *Vibrio cholerae* strains that are resistant to virstatin have also been described; the mechanisms whereby these strains colonize their hosts are independent of the elaboration of the toxin co-regulated pilus^[28].

The first evidence that cells develop resistance to QQ compounds has been reported by Maeda *et al.*^[29] (published ahead of print in 2011). These authors worked with a concentration of brominated furanone C-30 [the gold standard for QQ compounds, and which is a synthetic brominated furanone 4-bromo-5-(bromomethylene)-2(5H)-furanone] that did not influence growth in rich medium (*i.e.*, it did not inhibit growth) and utilized both transposon mutagenesis and spontaneous mutants to detect resistant bacteria.

The mechanism of this resistance was overexpression of the MexAB-OprM multidrug resistance operon due to mutations in the gene repressors *mexR* and *nalC*, resulting in efflux of the compound C-30. This quorum quenching compound showed a reduced capacity to decrease some QS-controlled virulence traits and phenotypes in the *mexR* mutant, and the pathogenicity of the *mexR* mutant against the nematode *Caenorhabditis elegans* was not decrease by the inclusion of C-30. Importantly, these authors also worked with cells from cystic fibrosis patients (Liverpool epidemic strain 12142) with *mexR* and *nalC* mutations^[30] to demonstrate that, even in the absence of the QS inhibitor, cells develop resistance to quorum quenching compounds in the pathogenic state when

Table 3 Patents concerning the inhibition of quorum sensing systems

Patent title	Description	Application date	Inventors	Publication number
Anti-inflammatory and quorum sensing inhibition compounds and methods of making and using them	This invention generally relates to novel compositions based on a structure designated as "Honaucin A", including Honaucin A variants and analogs, and pharmaceutical compositions, liposomes and nanoparticles comprising them, and methods of making and using them	2011	Gerwick William H; Gerwick Lena; Choi Huykjae; Villa Francisco A; Smith Jennifer; Rowley David C	WO2011153502 (A2)
Composition for oral use	A method for suppressing dental caries by regulating biofilm formation by the bacteria that cause dental caries instead of controlling these bacteria	2011	Tsugane Takanori; Saeki Yoji	EP2620160
Conjugates of acyl homoserine lactone and catalase a from <i>Pseudomonas aeruginosa</i>	The present invention relates to the acyl homoserine lactone N-3-(oxododecanoyl)-L-homoserine lactone or butyryl L-homoserine lactone and <i>Pseudomonas aeruginosa</i> KatA protein, or an antigenic portion conjugate thereof, used to treat <i>Pseudomonas aeruginosa</i> infections by limiting biofilm formation and inhibiting a range of quorum-sensing dependent virulence factors	2011	Kyd Jennelle M; Cooley Margaret	WO/2012/083382
Enzyme bag containing quorum quenching enzyme immobilized silica for inhibiting biofilm formation and membrane bioreactor system for water treatment system using the bag	This invention relates to an enzyme bag containing silica-immobilized enzyme for inhibiting biofilm formation. A membrane bioreactor system for water treatment using the bag is provided for stable implementation of filtering operations over a long period of time by improving the performance of operational processes	2011	Lee Chung Hak; Yang Cheon Seok; Lee Jung Kee; Han Jong Yun; Lee Chung Hak; Yang Cheon Seok; Lee Jung Kee; Han Jong Yun	KR20120134724 (A)
Fluidizable carrier with biofilm formation-inhibiting microorganisms immobilized therein and membrane water treatment apparatus using the same	This invention relates to a biofilm formation-inhibiting microorganism immobilized fluidizable carrier in which a biofilm formation-inhibiting microorganism is fixed therein and a membrane water treatment apparatus including the same are provided to increase a membrane cleaning period	2011	Lee Chung Hak; Kim Sang Ryoung; Lee Jung Kee	KR20130034935 (A)
Methods of disrupting quorum sensing to affect microbial population cell density	The invention relates to the modulation of quorum sensing mechanisms in a microorganism for the purpose of exploiting the fermentation capabilities of the microorganism	2011	Marrs Barry; Swalla Brian M	US2011124522 (A1)
Quorum-sensing signal molecular preparation and application thereof in tobacco waste treatment	The invention relates to the field of environmental biotechnology, in particular to preparation of a quorum sensing signal molecule and application in processing tobacco waste	2011	Meizhen Wang; Hongzhen He; Huajun Feng; Xin Zheng; Dongsheng Shen; Zhenmei LV; Hang Min	CN102392051 (A)
Synthetic analogs of bacterial quorum sensors	The invention relates to synthetic analogs of bacterial quorum sensing molecules, and methods of use of these	2011	Iyer Rash; Ganguly Kumkum; Silks Louis A	US2012071430 (A1)
System and method for reversing the antibiotic tolerance of bacterial persister cells	The present invention relates to antibiotics and, more particularly, to a system and method for decreasing the tolerance of bacterial persister cells to antibiotics	2011	Ren Dacheng; Pan Jiachuan	EP2603576
Triazole compounds as well as preparation method and application thereof	The present invention relates to triazole derivatives, the preparation method and as the autoinducer-2 (AI-2) quorum sensing inhibitors, belonging to anti-AI-2 quorum sensing type drug technology	2011	Minyong Li; Lvpei Du; Peng Zhu	CN102219753 (A)
Use of a novel alpha-proteobacteria for quorum quenching	The invention (concerning the fields of biology, molecular biology, and aquaculture) specifically relates to a new α -proteobacteria capable of degrading/V-acyl-homoserine lactones (AHLs) for control of bacterial infectious diseases and prevention of biofilm formation	2011	Otero Casal Ana Maria; Romero Bernardez Manuel	WO2011154585 (A1)
2-methylthiopyrrolidines and their use for modulating bacterial quorum sensing	Formula (I) compounds are disclosed and their use in inhibiting quorum sensing in bacteria is reported	2012	Malladi Venkata L; Schnepfer Lisa; Sobczak Adam J; Mathee Kalai; Wnuk Stanislaw F	WO/2012/174511
Compositions for regulating or modulating quorum sensing in bacteria, methods of using the compounds, and methods of regulating or modulating quorum sensing in bacteria	The report encompasses compounds and compositions that are useful as specific AI-2 antagonists for the control of bacterial quorum sensing and methods for inhibiting or attenuating microbial virulence, biofilm formation and drug resistance	2012	Wang Binghe; Ni Nanting; Wang Junfeng; Lu Chung-Dar; Chou Han-Ting; Li Minyong; Zheng Shilong; Cheng Yunfeng; Peng Hanjing	EP2529793 (A2)
Construction and application of unmarked <i>Lysobacter enzymogenes</i> engineering strain capable of preventing plant bacteriosis	The invention (within the field of microbial genetic engineering), specifically relates to a plant bacterial disease that can prevent dissolving enzyme production strains of <i>Bacillus</i> unmarked engineering construction and application	2012	Liu Fengquan; Qian Guoliang	CN102943061 (A)

Furan compound and preparation method and application of furan compound	The invention relates to furan derivatives, the method of preparation and as the AI-2 quorum sensing inhibitors, are anti-AI-2 type of quorum sensing	2012	Minyong Li; Peng Zhu	CN102603683 (A)
Method for increasing output of microbial lovastatin based on quorum sensing mechanism	The invention relates to the pharmaceutical raw material fermentation industry, in particular to a method of increasing microbial production of lovastatin	2012	Li Haoming	CN102925509 (A)
Method for quickly identifying food-borne pathogen bacterial biofilm formation inhibitor	The invention relates to the field of food microbiology control technology, in particular to the rapid identification of an inhibitor of foodborne bacterial biofilm formation	2012	Wenyan Zhang; Hongmei Zhang; Zhihua Tao; Wenyan Zhou	CN102706821 (A)
Phosphorylated and branched dihydroxy-pentane-one analogs as quorum sensing inhibitors in bacteria	The invention provides compositions and methods for modulating quorum sensing in microbes and can be used in prophylactic methods or therapy for bacterial infections and for reduction of biofilms. The compounds are AI-2 analogs and as such have structures similar to 4,5-dihydroxy-2,3-pentanedione that can act as agonists/antagonists of quorum sensing	2012	Sintim Herman; Bentley William E; Roy Yarnika; Smith Jacqueline	US2012294900 (A1)
Preparation method of imprinted polymer of bacterial quorum sensing signal molecule AI-1	The present invention relates to bacterial quorum sensing signal molecules AI-1 imprinted polymer preparation	2012	Xin Li; Ling Wang	CN102604010 (A)
Probiotics for biological control against <i>Vibrio</i> sp.	The invention relates to probiotics for biological control against <i>Vibrio</i> sp., and in particular, to a newly isolated bacillus strain that degrades quorum-sensing signal molecules of the pathogenic bacteria <i>Vibrio</i> sp., and inhibits biofilm formation	2012	Yang Si Yong; Woo Seo Hyung; Kang In Hye; Im Hyun Jung	WO2012105805
Quorum sensing inhibitor against a pathogenic microorganism, and an antibacterial composition using the same	A quorum sensing inhibitor and an antibacterial composition using the same are provided to suppress quorum sensing between bacteria, and to prevent or treat infection or diseases	2012	Undescribed	KR101243696
<i>Shewanella piezotolerans</i> 34# and application thereof to algae inhibition	The invention relates to the field of biotechnology, in particular to a marine bacterium <i>Shewanella</i> and the inhibition of algal growth	2012	Zhou Jin; Yin Peng	CN103173383 (A)
Simple method for testing disease resistance of pathogenic bacteria quorum-quenching gene prokaryotic expression product	The invention relates to a prokaryotic expression product of disease resistance testing methods, in particular to test pathogen populations prokaryotic expression product quenching effect of a simple disease, and belongs to the field of gene function identification techniques	2012	Ouyang Lejun; Huang Zhenchi; Zeng Fuhua; Li Limei; Li Heng	CN102972220 (A)
Use of quorum sensing inhibitors and biofilm dispersing agents for controlling biofilm-associated implantable medical device related infections	The invention generally relates to implantable medical devices and, more specifically, to the use of quorum sensing inhibitors and/or biofilm dispersing agents to control biofilm-associated infections related to the use of implantable medical devices	2012	Samade Richard; Dinesh Prashant; Nabutovsky Yelena; Bornzin Gene A; Poore John W; Karicherla Annapurna; Dalal Nirav	US2014005605 (A1)
<i>Bacillus pumillus</i> microbial preparation with quorum sensing system inhibiting effect	The invention relates to <i>Bacillus pumillus</i> with a quorum sensing system inhibiting effect. The invention has the advantages that <i>Chromobacterium violaceum</i> is used for screening out a bacterial strain F3-1. It can be used to produce a microbial preparation capable of preventing and treating aquatic bacterial diseases	2013	Song Zengfu; Fan Bin; Chen Biao	CN103525723 (A)
Bicyclic furanones with low toxicity for microbial control	The invention relates to a class of bicyclic brominated furanone structures with reduced toxicity and high activity for inhibiting biofilm formation and quorum sensing by microbes	2013	Luk Yan-Yeung; Yang Sijie	US2013197077 (A1)
Method for detecting quorum sensing quenching bacterial strain	The invention relates to a method for detecting a quorum sensing quenching bacterial strain. The method comprises the addition of a bacterial strain to be detected in a PIPES (1,4-piperazinediethanesulfonic acid) buffer solution of pH 6	2013	Zhang Xiaohua; Tang Kaihao; Shi Xiaochong; Zhang Yunhui	CN103215342 (A)
Quorum-quenching enzyme OLB-26, and coding gene and application thereof	The invention relates to a quorum sensing quenching enzyme OLB-26 and its coding gene and application	2013	Zhou Zhigang; Zhang Meichao; Yang Yalin; Xu Li; He Suxu; Li Qing; Yu Qiang	CN103275949 (A)
Targeted enzymatic degradation of quorum-sensing peptides	The present invention generally relates to the fields of microbiology and wound care. More particularly, it concerns methods and compositions for inhibiting biofilms in wounds and on medical devices	2013	Alarcon Rodolfo; McNulty Amy K	US20130253382
Use of ellagitannins as inhibitors of bacterial quorum sensing	Materials and methods for the inhibition of bacterial QS are described. Methods of treating bacterial infections by administration of one or more ellagitannins in amounts effective for inhibiting bacterial QS are also provided	2013	Athee Kalai; Adonizio Allison L; Ausubel Frederick; Clardy Jon; Bennett Bradley; Downum Kelsey	US2013317094 (A1)
Use of hydroxytyrosol and derivatives thereof as quorum quenchers	The quorum quenching activity of formula (I) or (II) compounds, such as hydroxytyrosol (HT), hydroxytyrosol acetate (HTA), 3,4-dihydroxyphenylacetic acid (DOPAC) and derivatives thereof are described	2013	Au Ón Calles David; Allende Prieto Ana; Fábregas Casal Jaime; Gómez-Acebo Gullón Eduardo	WO2014060581 (A1)
Use of the cect 7426 strain for generating quorum quenching of the autoinducer-2 signal (ai-2)	The invention relates to the use of a bacterial strain of the species <i>Tenacibaculum discolor</i> in the control of infectious diseases and for inhibiting biofilm formation caused by bacteria, through the inhibition of quorum sensing signals type AI -2. The invention applies to the field of molecular biology	2013	Otero Casal, Ana María; Romero Bernárdez Manuel	WO2014057151 (A1)

Table 4 Patents concerning the inhibition of multidrug resistance systems

Patent title	Description	Application date	Inventors	Publication number
Method for screening for non-tetracycline efflux pump inhibitors	Provides screening methods for inhibitors of microbial efflux pumps and pharmaceutical compositions containing such efflux pump inhibitors as well as methods for treating microbial infections by use of these compositions	1995	Trias; Joaquim Chamberland; Suzanne Hecker; Scott J Lee; Ving J	US5989832 A
Efflux pump inhibitors	Provides screening methods for inhibitors of microbial efflux pumps and pharmaceutical compositions containing such efflux pump inhibitors. Also provides methods for treating microbial infections using those compositions	1996	Trias Joaquim; Hecker Scott J; Chamberland Suzanne; Lee Ving J	CA2217865
Methods and compositions for reducing bacterial tolerance of disinfectants and organic solvents	Methods and compositions useful for manipulating bacterial resistance to non-antibiotic antibacterial compositions, disinfectants and organic solvents, and for rendering bacterial cells susceptible to non-antibiotic antibacterial compositions	1997	Levy Stuart B	WO9917607 (A2)
Methods and compositions for reducing bacterial tolerance to antibacterials, disinfectants and organic solvents	Methods and compositions useful for manipulating bacterial resistance to non-antibiotic antibacterial compositions, disinfectants and organic solvents, and methods for rendering bacterial cells susceptible to non-antibiotic antibacterial compositions	1997	Levy Stuart B	US6068972 (A)
Inhibitors of cellular efflux pumps of microbes	Describes compounds that are inhibitors of efflux pump in bacteria. Also describes methods of preparing and using such compounds and the pharmaceutical compositions that include such compounds	2001	De Souza Noel John; Patel Mahesh Vithalbhai; Gupte Shrikant V; Upadhyay Dilip J; Shukla Milind Chintaman; Chaturvedi Nishith C; Bhawar Satish B; Nair Sheela Chandrasekharan; Jafri Mohammed A; Khorakiwala Habil Fakhruddin	US2002177559 (A1)
Drug discovery and increased potency of antiseptics and disinfectants based on high extracellular pH, the disablement of cellular efflux pumps, and the unexpected synergism therebetween	Methods for increasing the therapeutic potency of amphipathic compounds, <i>e.g.</i> , antiseptics and disinfectants, and the exploitation of these discoveries in the screening of small molecules, and libraries thereof, for biological activity in prokaryotes and eukaryotes	2002	Lewis Kim; Hsieh Peichung	US2003118541 (A1)
Methods to study and mechanisms of biofilm-related antibiotic resistance	Discloses various genes that encode proteins shown to play a role in microbial resistance of an organism in a biofilm and homologs thereof. Describes methods of identifying a compound that modulates microbial resistance of an organism in a biofilm and also genes encoding proteins that play a role in biofilm resistance	2002	O'Toole George A; Mah Thien-Fah	US2003166030 (A1)
Minicell-based gene therapy	Provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery	2002	Sabbadini Roger A; Berkley Neil; Surber Mark W	US2003199088 (A1)
Potentiators of antibacterial activity	The invention relates to compounds that potentiate the activity of antibacterials, compositions useful in treating bacterial infection, and related methods. Also relates to a method of inhibiting bacterial efflux of an antibiotic, thereby increasing its efficacy	2004	Grossman Trudy H	US20040887719 20040709
Substituted polyamines as inhibitors of bacterial efflux pumps	Methods of treating bacterial infections, including those caused by multidrug resistant bacteria, by using polyamine efflux pump inhibiting compounds, optionally in combination with other drugs such as antibiotics. The pharmaceutical compositions of the polyamines are also reported	2004	Nelson Mark L; Alekshun Michael N	US2004204378 (A1)
Essential and important genes of <i>Pseudomonas aeruginosa</i> and the use thereof to design or identify antibacterial agents	Database of candidate essential genes in <i>Pseudomonas aeruginosa</i> , and other important genes that, when mutated, produce a growth attenuated phenotype. The invention includes methods for confirming the need for or importance of candidate genes, methods for using those genes to screen for new antibacterial drugs, the antibacterial agents identified using the disclosed methods, and also methods of using the same for treating and preventing <i>Pseudomonas</i> infection	2005	Bruce Kim F; Warrenner Paul; Hou Kevin	US2007196829 (A1)
Method for increasing the susceptibility of peptide deformylase inhibitors by using efflux pump inhibitors	Provides methods and compositions for increasing the susceptibility of PDF inhibitors against Gram-negative organisms by using efflux pump inhibitors	2005	Dean Charles Richard; Ryder Neil Stewart	CA2569681

Products and methods for <i>in vivo</i> secretion of monatin	Provides products and methods for the <i>in vivo</i> production of monatin sweetener. The products include microorganisms that are genetically modified to secrete or to improve secretion of monatin, to produce monatin, and to both secrete/ improve secretion and produce monatin	2006	Laplaza Jose; Anderson James C; Desouza Mervyn L; Hicks Paula M; Kollmann Sherry R	WO2006113897
Rhamnose-inducible expression systems and methods	Describes rhamnose-inducible expression constructs which may include at least one rhamnose-inducible regulatory element expressing a regulatory protein and one promoter that is inducible by the regulatory protein. An open reading frame expressing a protein of interest may be placed under control of the promoter. Also describes optimized Shine-Dalgarno sequences for use with the promoter	2006	Surber Mark W	US2007122881 (A1)
Enhancement of tigecycline potency using efflux pump inhibitors	Discloses Efflux Pump Inhibitor (EPI) compounds that can be co-administered with antimicrobial agents for the treatment of infections caused by drug resistant pathogens and methods of treatment and pharmaceutical compositions for co-administering tigecycline with an EPI	2007	Glinka Tomasz; Bostian Keith; Lomovskaya Olga; Surber Mark; Sun Dongxu	US20070832626 20070801
Method or agent for inhibiting the function of efflux pump of <i>Pseudomonas aeruginosa</i>	Discloses a method comprised of modifying any amino acid residue selected from 100 th to 109 th and 311 th to 320 th amino acid residues in an amino acid sequence for mature OprM protein, as well as an agent for inhibiting the function of an efflux pump of <i>Pseudomonas aeruginosa</i> with good efficiency. Further disclosed is a method for screening the agent	2007	Yoshihara Eisaku; Inoko Hidatoshi	CA2641988
Method or agent for inhibiting the function of efflux pump of <i>Pseudomonas aeruginosa</i>	Provides a method for inhibiting the function of the drug efflux pump of <i>Pseudomonas aeruginosa</i> , comprising modification of any amino acid residue selected from 100 th to 109 th or 311 th to 320 th amino acid residues in the amino acid sequence of mature OprM protein. Also reports an agent with such inhibitory effect, as well as a screening method	2007	Yoshihara Eisaku; Inoko Hidatoshi	WO2007/091395
Microbial production of aromatic acids	Method for the microbial production of aromatic acids from a fermentable carbon substrate using a host cell capable of producing said aromatic acid, and comprising an efflux pump for said aromatic acid. A preferred host cell comprises a member of the proton-dependent resistance/nodulation/cell division (RND) family of efflux pumps	2007	Wery Jan	US2007259409 (A1)
Near-infrared electromagnetic modification of cellular steady-state membrane potentials	Discloses systems and methods for applying near-infrared optical energies and dosimetries to alter the bioenergetic steady-state trans-membrane and mitochondrial potentials (DeltaPsi-steady) of all irradiated cells through an optical depolarization effect. This membrane depolarization provides the ability to potentiate antimicrobial, antifungal and/or antineoplastic drugs against only targeted undesirable cells	2007	Bornstein Eric	US2008139992
<i>In vivo</i> gene sensors	Describes methods and compositions for the detection of target genes that permit the selective expression of an effector gene in those cells expressing the target gene, thus selectively targeting these cells for treatment or elimination. The methods and compositions described may also permit the selective expression of an agent such as a therapeutic gene product, in a targeted population of cells	2009	Collins James J; Lu Timothy Kuan-Ta	WO2009/137136
Methods of reducing microbial resistance to drugs	Provides methods of treating infection, screening for inhibitors of AcrAB-like efflux pumps, and enhancing antimicrobial activity of drugs. Pharmaceutical composition comprising an inhibitor of an AcrAB-like efflux pump and an antimicrobial agent are also provided	2009	Oethinger Margaret; Levy Stuart B	US2009298873
Minicells displaying antibodies or derivatives thereof and comprising biologically active compounds ¹	Minicells are used to deliver biologically active compounds, including polypeptides, nucleic acids, small molecules, drug molecules, and chemotherapeutic agents. In some cases, the minicell displays ligands or binding moieties that target the minicell to a desired host cell	2011	Sabbadini Roger A; Berkley Neil; Surber Mark W; Klepper Robert	US20070725196 20070316

¹Those published from 2010 onwards are highlighted.

coexisted with the pressures of antibiotic treatment; so, antimicrobial treatment can induce to resistance to QQ compounds.

Intensified efforts are needed to establish whether resistance may develop to other QQ compounds, as is the case of lactonase or acylase enzymes in connection with AHL autoinducers.

CONCLUSION

Antimicrobial resistance in nosocomial pathogens is in-

creasing worldwide. Mortality rates of patients infected with drug-resistant pathogens have increased by approximately 50% in recent years. Hospitals have become breeding grounds for extremely resistant pathogens, exacerbating the risk associated with hospitalization. These pathogens are extremely resistant to last line antimicrobials. If the current trend continues, the beginning of a “post-antibiotic era” is predicted. Development of novel antibiotics has almost totally ceased, at least against Gram-negative bacteria, and the prospects for a reversal of this trend are bleak. It is therefore imperative to de-

velop new molecules, therapies and/or new combinations of these for the eradication of resistant pathogens. In this review, we discuss some examples of patented molecules that act by inhibiting different bacterial virulence mechanisms (adhesion/colonization and quorum sensing mechanisms, and secretory and efflux pump systems) and which open the way to studying potential new treatments for infections caused by multiresistant bacteria.

Novel targets and molecules must be discovered for antivirulence therapies, taking into account the possible development of resistance mechanisms. Further study of combinations of these compounds with other antimicrobials for the treatment of infectious diseases is also important.

REFERENCES

- 1 **Rasko DA**, Sperandio V. Anti-virulence strategies to combat bacteria-mediated disease. *Nat Rev Drug Discov* 2010; **9**: 117-128 [PMID: 20081869 DOI: 10.1038/nrd3013]
- 2 **Beceiro A**, Tomás M, Bou G. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? *Clin Microbiol Rev* 2013; **26**: 185-230 [PMID: 23554414]
- 3 **Proft T**, Baker EN. Pili in Gram-negative and Gram-positive bacteria - structure, assembly and their role in disease. *Cell Mol Life Sci* 2009; **66**: 613-635 [PMID: 18953686 DOI: 10.1007/s00018-008-8477-4]
- 4 **Svensson A**, Larsson A, Emténäs H, Hedenström M, Fex T, Hultgren SJ, Pinkner JS, Almqvist F, Kihlberg J. Design and evaluation of piliocides: potential novel antibacterial agents directed against uropathogenic *Escherichia coli*. *Chembiochem* 2001; **2**: 915-918 [PMID: 11948880]
- 5 **Shakhnovich EA**, Hung DT, Pierson E, Lee K, Mekalanos JJ. Virstatin inhibits dimerization of the transcriptional activator ToxT. *Proc Natl Acad Sci USA* 2007; **104**: 2372-2377 [PMID: 17283330]
- 6 **European Patent Office**. Espacenet: Patent search. [accessed 2014 April]. Available from: URL: <http://worldwide.espacenet.com>
- 7 **United States Patent and Trademark Office**. Patent Full-Text and Image Database. [accessed 2014 April]. Available from: URL: <http://patft.uspto.gov/netathtml/PTO/search-adv.htm>
- 8 **Galán JE**, Cossart P. Host-pathogen interactions: a diversity of themes, a variety of molecular machines. *Curr Opin Microbiol* 2005; **8**: 1-3 [PMID: 15694849]
- 9 **Filloux A**, Hachani A, Bleves S. The bacterial type VI secretion machine: yet another player for protein transport across membranes. *Microbiology* 2008; **154**: 1570-1583 [PMID: 18524912 DOI: 10.1099/mic.0.2008/016840-0]
- 10 **Keyser P**, Elofsson M, Rosell S, Wolf-Watz H. Virulence blockers as alternatives to antibiotics: type III secretion inhibitors against Gram-negative bacteria. *J Intern Med* 2008; **264**: 17-29 [PMID: 18393958 DOI: 10.1111/j.1365-2796.2008.01941.x]
- 11 **Stavrinides J**, McCann HC, Guttman DS. Host-pathogen interplay and the evolution of bacterial effectors. *Cell Microbiol* 2008; **10**: 285-292 [PMID: 18034865]
- 12 **Filloux A**. The type VI secretion system: a tubular story. *EMBO J* 2009; **28**: 309-310 [PMID: 19225443 DOI: 10.1038/emboj.2008.301]
- 13 **Fuqua WC**, Winans SC, Greenberg EP. Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. *J Bacteriol* 1994; **176**: 269-275 [PMID: 8288518]
- 14 **Romero M**, Acuña L, Otero A. Patents on quorum quenching: interfering with bacterial communication as a strategy to fight infections. *Recent Pat Biotechnol* 2012; **6**: 2-12 [PMID: 22420877]
- 15 **Dong YH**, Wang LY, Zhang LH. Quorum-quenching microbial infections: mechanisms and implications. *Philos Trans R Soc Lond B Biol Sci* 2007; **362**: 1201-1211 [PMID: 17360274]
- 16 **Rasch M**, Buch C, Austin B, Slierendrecht WJ, Ekmann KS, Larsen JL, Johansen C, Riedel K, Eberl L, Givskov M, Gram L. An inhibitor of bacterial quorum sensing reduces mortalities caused by Vibriosis in rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Syst Appl Microbiol* 2004; **27**: 350-359 [PMID: 15214641]
- 17 **Jerse AE**, Sharma ND, Simms AN, Crow ET, Snyder LA, Shafer WM. A gonococcal efflux pump system enhances bacterial survival in a female mouse model of genital tract infection. *Infect Immun* 2003; **71**: 5576-5582 [PMID: 14500476]
- 18 **Martínez JL**, Sánchez MB, Martínez-Solano L, Hernández A, Garmendia L, Fajardo A, Alvarez-Ortega C. Functional role of bacterial multidrug efflux pumps in microbial natural ecosystems. *FEMS Microbiol Rev* 2009; **33**: 430-449 [PMID: 19207745 DOI: 10.1111/j.1574-6976.2008.00157.x]
- 19 **Hirakata Y**, Srikumar R, Poole K, Gotoh N, Suematsu T, Kohno S, Kamihira S, Hancock RE, Speert DP. Multidrug efflux systems play an important role in the invasiveness of *Pseudomonas aeruginosa*. *J Exp Med* 2002; **196**: 109-118 [PMID: 12093875]
- 20 **Pearson JP**, Feldman M, Iglewski BH, Prince A. *Pseudomonas aeruginosa* cell-to-cell signaling is required for virulence in a model of acute pulmonary infection. *Infect Immun* 2000; **68**: 4331-4334 [PMID: 10858254]
- 21 **Martínez JL**, Baquero F. Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. *Clin Microbiol Rev* 2002; **15**: 647-679 [PMID: 12364374]
- 22 **Silver S**, Phung LT. Bacterial heavy metal resistance: new surprises. *Annu Rev Microbiol* 1996; **50**: 753-789 [PMID: 8905098]
- 23 **Ramos JL**, Duque E, Gallegos MT, Godoy P, Ramos-Gonzalez MI, Rojas A, Teran W, Segura A. Mechanisms of solvent tolerance in gram-negative bacteria. *Annu Rev Microbiol* 2002; **56**: 743-768 [PMID: 12142492]
- 24 **Kourtesi C**, Ball AR, Huang YY, Jachak SM, Vera DM, Khondkar P, Gibbons S, Hamblin MR, Tegos GP. Microbial efflux systems and inhibitors: approaches to drug discovery and the challenge of clinical implementation. *Open Microbiol J* 2013; **7**: 34-52 [PMID: 23569468 DOI: 10.2174/1874285801307010034]
- 25 **Bina XR**, Philippart JA, Bina JE. Effect of the efflux inhibitors 1-(1-naphthylmethyl)-piperazine and phenyl-arginine-beta-naphthylamide on antimicrobial susceptibility and virulence factor production in *Vibrio cholerae*. *J Antimicrob Chemother* 2009; **63**: 103-108 [PMID: 19010827 DOI: 10.1093/jac/dkn466]
- 26 **Varga ZG**, Armada A, Cerca P, Amaral L, Mior Ahmad Subki MA, Savka MA, Szegedi E, Kawase M, Motohashi N, Molnár J. Inhibition of quorum sensing and efflux pump system by trifluoromethyl ketone proton pump inhibitors. *In Vivo* 2012; **26**: 277-285 [PMID: 22351670]
- 27 **García-Contreras R**, Maeda T, Wood TK. Resistance to quorum-quenching compounds. *Appl Environ Microbiol* 2013; **79**: 6840-6846 [PMID: 24014536 DOI: 10.1128/AEM.02378-13]
- 28 **Shakhnovich EA**, Sturtevant D, Mekalanos JJ. Molecular mechanisms of virstatin resistance by non-O1/non-O139 strains of *Vibrio cholerae*. *Mol Microbiol* 2007; **66**: 1331-1341 [PMID: 17986190]
- 29 **Maeda T**, García-Contreras R, Pu M, Sheng L, Garcia LR, Tomás M, Wood TK. Quorum quenching quandary: resistance to antivirulence compounds. *ISME J* 2012; **6**: 493-501 [PMID: 21918575 DOI: 10.1038/ismej.2011.122]
- 30 **Tomás M**, Doumith M, Warner M, Turton JF, Beceiro A, Bou G, Livermore DM, Woodford N. Efflux pumps, OprD porin,

AmpC beta-lactamase, and multiresistance in *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. *Antimicrob*

Agents Chemother 2010; **54**: 2219-2224 [PMID: 20194693 DOI: 10.1128/AAC.00816-09]

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