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EDITORIAL

What is the purpose of launching the *World Journal of Clinical Infectious Diseases*?

Shyam Sundar

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Figure 1 Editor-in-Chief of the *World Journal of Clinical Infectious Diseases*. Shyam Sundar, MD, FRCP (London), FAMS, FNASc, FASc, FNA, Professor, Infectious Diseases Research Laboratory, Department of Medicine, Banaras Hindu University, Varanasi 221005, India.

Abstract

Launching of the *World Journal of Clinical Infectious Diseases* (*WJCID*) could have been possible due to efforts of the publisher, members of the editorial board, all the authors and definitely our readers. I congratulate everyone for making it possible. Pathogenic organisms of various origin cause infectious diseases often resulting in symptomatic illness. *WJCID* is an open access peer reviewed journal that will be published bimonthly. *WJCID* will primarily emphasize on topics relevant to infections affecting human and animal health yet articles from other diseases and relevant issues will also be encouraged. *WJCID* welcomes articles from either basic or applied research in different disciplines like Epidemiology of communicable and non-communicable infections, Immunology and Genetics. *WJCID* covers topics like Host-Parasites interactions, Vector biology, development of advanced tools for diagnosis, genetic susceptibility to diseases, and disease prevention and vector control. *WJCID* will work as an important resource of basic and applied research in the field of infections. It is widely recommended that clinical implementations of basic and applied research be encouraged for the benefit to each stream. So again I welcome everyone and assure that *WJCID* will be a great platform where you can feel free to share your valuable results, discuss new hypothesis and research problems and update yourself with the most recent advancements made in the field of infections.

Key words: Infectious disease; Pathogen; Peer-reviewed; Open access; Journal

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INTRODUCTION

I am Shyam Sundar, a Professor at Banaras Hindu University, India (Figure 1), together with Lihua Xiao, DVM, PhD, Senior Scientist, Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, United States, we will be co-Editor-in-Chief for the *World Journal of Clinical Infectious Diseases* (*World J Clin Infect Dis*, *WJCID*,

online ISSN 2220-3176, DOI: 10.5495). It is a matter of great joy to introduce the *WJCID* as a new member of infectious disease series for discussing health related issues and new technologies. This will provide a new platform to exchange views related to clinical infectious diseases, to focus on obstacles and to overcome them to improve global health situation. I would like to congratulate all the contributors to support beginning of *WJCID*!

I take this opportunity to formally announce that the first issue of the *WJCID* on which preparation was initiated on December 16, 2010, is officially published on December 30, 2011 and from now on will be published bimonthly. *WJCID* is a peer reviewed open access (OA) journal. The editorial Board of *WJCID* consists of 106 distinguished infectious disease experts from 35 countries. Being an OA journal *WJCID* delimits the problems of access to readers and lets them make maximum use of the data. I am sure the questions like the purpose of launching *WJCID*, its scope and the selection of content, every reader would have in their mind. In the next section I will try to address these issues.

Infectious diseases or communicable diseases comprise if clinically evident illness or symptoms of infections caused by various pathogenic agents in the host[1]. Further there could be infections that can spread directly or indirectly from one person to other or zoonotic diseases which are infectious diseases of animals which can cause disease when transmitted to humans. One way of proving that a given disease is "infectious", is to satisfy Koch's postulates (first proposed by Robert Koch), which demands that the infectious agent be identified only in patients and not in healthy controls, and that patients who contract the agent also develop the disease.

Pathogenic organisms can be of any origin like virus, bacteria, fungi, protozoan and other multicellular parasites and sometimes disease transmission can involve some vectors also which can be of both biological and non-biological origin. Establishment of infection depends on many factors like mode of transmission, Vectors, Immune status of Host, Host genetic factors, *etc.* While many infections can stay asymptomatic for most or complete course of illness other convert into symptomatic diseases and are fatal if left untreated. Disease is always an outcome of Immune pathogenesis of host pathogen interaction, which is decided largely by Immune potential of host system against causative agent[2]. Most of the infections are fatal if not taken care of and cause severe mortality worldwide[3].

My main area of research is visceral leishmaniasis. Leishmanises are a group of diseases commonly caused by obligate intracellular protozoan *Leishmania*[4]. Among many forms of disease visceral leishmaniasis is the most severe and is often fatal if left untreated. Only in Indian subcontinent about 200 million people are at risk[5]. It will definitely be very interesting to deal with a broader spectrum of infections and this will help us to develop a better understanding of infectious diseases.

CONTENTS OF PEER REVIEW

In order to guarantee the quality of articles published in the journal, *WJCID* usually invites three experts to comment on the submitted papers. The contents of peer review include: (1) whether the contents of the manuscript are of great importance and novelty; (2) whether the experiment is complete and described clearly; (3) whether the discussion and conclusion are justified; (4) whether the citations of references are necessary and reasonable; and (5) whether the presentation and use of tables and figures are correct and complete.

SCOPE AND COLUMNS

WJCID will focus on a broad spectrum of topics on infectious diseases that will cover epidemiology, immunopathogenesis, genetic factors, host susceptibility to infection, vector control, novel approaches of treatment, molecular diagnostic and vaccines. It will provide a common stage to share our visions, new approaches, most advanced techniques, and to discuss research problems that will help everyone working in the field of various infections to exchange their views and to improve public health.

The journal publishes full length articles in basic and applied research showing original reports, invited reviews, Book reviews and mini reviews. Also it is open for brief case reports and short communications, commentaries, editorials and letters to Editor. *WJCID* will focus on broad range of infections like opportunistic infections, zoonotic infections, tropical and neglected tropical diseases, emerging infections, *etc.* and following topics related to these issues: (1) Causative agents discussing various pathogens; (2) Vectors and Mode of transmission; (3) Host -Pathogen Interaction and Immune-pathogenesis of the disease; (4) Epidemiology of the infection and vector control strategies; (5) Genetic factors covering both Host and pathogen; (6) Molecular diagnostic techniques vaccines; and (7) Recent advances in cell tissue culture, lab techniques, *etc.* Various other related fields like medical microbiology, pharmacology of herbs, bioinformatics, *etc.* will be included.

The columns in the issues of *WJCID* will include: (1) Editorial: To introduce and comment on the substantial advance and its importance in the fast-developing areas; (2) Frontier: To review the most representative achievements and comment on the current research status in the important fields, and propose directions for the future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (6) Review: To systemically review

the most representative progress and unsolved problems in the major scientific disciplines, comment on the current research status, and make suggestions on the future work; (7) Original Articles: To originally report the innovative and valuable findings in infectious diseases; (8) Brief Articles: To briefly report the novel and innovative findings in infectious diseases; (9) Case Report: To report a rare or atypical case; (10) Letters to the Editor: To discuss and make reply to the contributions published in *WJCID*, or to introduce and comment on a controversial issue of general interest; (11) Book Reviews: To introduce and comment on quality monographs of infectious diseases; and (12) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities worldwide on the research in infectious diseases.

So, once again I welcome you all who want to make a contribution to science. *WJCID* will make efforts to fills the gap between basic and clinical research so that fruitful implementations can take place by the crosstalk between the two streams. *WJCID* is a platform where you can feel free to share your results, raise your issues and problems and to let other take an advantage of learning from new developments in the field of infectious diseases and biomedical science.

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EDITORIAL

Statins as antifungal agents

László Galgóczy, Ildikó Nyilasi, Tamás Papp, Csaba Vágvölgyi

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Abstract

Fungal infections are increasing and their treatment is difficult, because the most widely used antifungal drugs are relatively toxic and have serious side effects. Therefore, interest has focused on safely applicable and clinically introduced non-antifungal drugs, which have potent antifungal activity. Statins were originally used as cholesterol lowering agents in human therapy, but recent studies demonstrated their *in vitro* antifungal activity against yeasts and filamentous fungi. This indicated their potential application, alone or in combination with other drugs, in the treatment of such diseases. Their effective concentrations are higher than their maximum achievable serum levels; therefore, the application of statins for the treatment of invasive fungal infections is only possible in combination with antifungal agents. These synergistic combinations establish a basis for a new safely applicable therapy. This review focuses on the antifungal activity of statins alone and in combination with antifungal and non-antifungal drugs, and their possible application in clinical therapy.

Key words: Statins; Antifungal activity; Drug interaction

Peer reviewer: Noah Isakov, PhD, Department of Microbiology and Immunology, Ben Gurion University of the Negev, POBox 653, Beer Sheva, Israel

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INTRODUCTION

The incidence of invasive fungal infections (IFIs) is increasing because of the growing number of immunocompromised hosts and the occurrence of antibiotic resistant strains. The major risk factors for these diseases are the administration of broad-spectrum antibiotics, corticosteroids and cytotoxic agents, intravenous catheters, invasive medical procedures, human immunodeficiency virus infection, poorly controlled diabetes mellitus, hematological malignancy, solid organ or bone marrow transplantation, steroid use, metabolic acidosis, deferoxamine therapy, and severe and prolonged neutropenia[1,2]. Treatment of IFIs is difficult, because the most widely applied antifungal drugs [e.g. amphotericin B (AMB)] for treatment of such disease are relatively toxic and have serious side effects. Therefore, there is a substantial interest in clinically introduced non-antifungal drugs that have potent antifungal activity and/or can act synergistically with antifungal agents to allow a decrease in their therapeutic concentrations. Such compounds would form the basis of a less toxic therapy[3]. Statins are interesting from this respect, as they have effective antifungal potential against both yeast and filamentous fungi; furthermore, they can be combined with clinically used antifungal agents.

STATINS

History of statins

Statins were discovered as cholesterol lowering drugs in the 1970s, and are the most widely prescribed medications worldwide[4].

Statins are metabolites of microorganisms (mevastatin, MEV; lovastatin, LOV; simvastatin, SIM and pravasatin, PRA) or fully synthetic compounds (atorvastatin, ATO; cerivastatin, CER; fluvastatin, FLV; pitavastatin, PIT; and rosuvastatin, ROS). The natural statins are substituted hexahydronaphthalene lactones. The first described statin, MEV, was isolated as a secondary metabolite of a *Penicillium citrinum* strain. Subsequently, further intensive fungal screenings for similar compounds revealed that a strain of both *Aspergillus terreus* and *Monascus ruber* produce a more efficient statin, LOV[5]. SIM is a post-methylated derivative of LOV[6], and PRA was isolated from the fermentation broth of an Actinobacteria species, *Nocardia autotrophica*[7].

After successful clinical trials of the natural statins, pharmaceutical companies introduced more effective and safer fully synthetic statins. The structures of synthetic statins are dissimilar and are different from the natural statins, except for the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA)-like moiety, which is responsible for HMG-CoA reductase inhibition, which, indirectly, results in their cholesterol lowering effects[8]. FLV was the first fully synthetic statin, followed by ATO, CER, PIT, and ROS[5]. CER has been withdrawn from the market because of its serious adverse effect (fatal rhabdomyolysis)[9].

Statins were observed to have unexpected antifungal effects and their potential application in the treatment of fungal diseases has been intensively studied.

Mechanism of statins' effects

Statins are competitive inhibitors of HMG-CoA reductase, which catalyses the conversion of HMG-CoA to mevalonate, a rate-limiting step in the isoprenoid biosynthetic pathway, which is involved in the synthesis of cholesterol in humans and ergosterol in fungi[10]. Statins compete with the natural substrate for the enzyme's active site, preventing the formation of a functional enzyme structure with reversible binding[11].

Thus, the effects of statins are connected with the inhibition of the synthesis of important isoprenoids, e.g. farnesyl pyrophosphate and geranylgeranyl pyrophosphate, which are important lipid attachments for the γ subunit of heterotrimeric G-proteins[12], guanosine triphosphate-binding protein Ras, and Ras-like proteins (Rho, Rab, Rac, Ral, or Rap)[12-14]. Thus, statins act as inhibitors of some G-protein actions and Ras or Ras-like signaling, which affect several important bioprocesses[15].

Figure 1 summarizes the metabolic pathway of sterols and the impact of statins in their biosynthesis[16,17].

ANTIFUNGAL ACTIVITY OF STATINS

The *in vitro* antifungal activity of statins against yeasts and filamentous fungal isolates has been frequently reported, and all the studies propose their potential application, alone or in combination, in clinical therapy. The different fungi are not equally sensitive to statins *in vitro*, e.g. SIM exhibits the strongest antifungal activity against yeasts compared to filamentous fungi, whereas the reverse is true for FLV[18]. The natural statins (e.g. SIM and LOV) mainly effect their antifungal activity in their active metabolite forms (hydrolysis of the lactone ring at pH 10), and they proved to be less effective as pro-drugs[18,19]. Generally, the synthetic statins are more effective than the natural ones[18,19].

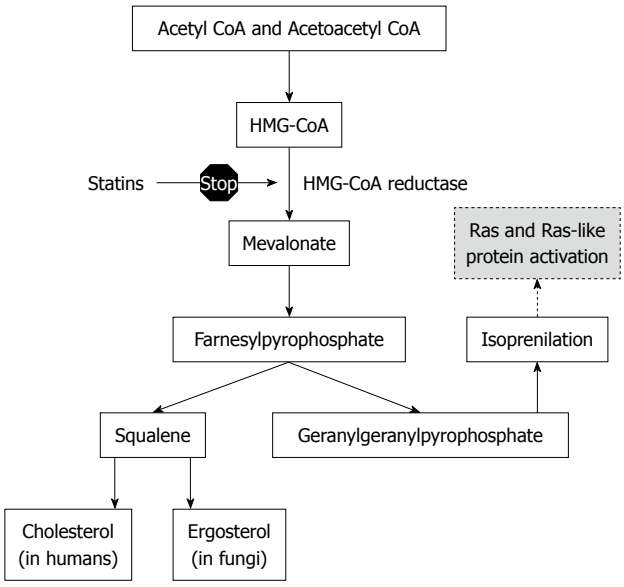

Figure 1 Metabolic pathway of sterols and the impact of statins in their bioprocess[16,17].

Antifungal activity of statins against yeasts

Statins exhibit fungicidal or fungistatic effects against yeasts in a dose dependent-manner. Data concerning the antifungal activity of various statins against yeasts are available for *Candida albicans* (*C. albicans*), *Candida glabrata* (*C. glabrata*), *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*, *Cryptococcus neoformans* (*C. neoformans*), and *Saccharomyces cerevisiae*[18,20-29]. These studies demonstrated that the various statins exhibit different antifungal effects against yeasts. SIM displayed the strongest antifungal activity, followed by FLV, ATO, ROS, and LOV. PRA proved to be completely ineffective against them. The antifungal activity of FLV is dependent on the pH of the medium[27]. Table 1 shows the available minimal inhibitory concentration (MIC) values of the investigated statins against yeast species.

The growth inhibition effect of statins on yeast cells is related to the decreasing ergosterol level, which occurs because of the inactivation of HMG-CoA reductase inactivation by statins in the isoprenoid biosynthetic pathway[16]. Ergosterol is a main constituent of the lipid layer of fungal plasma membranes, and the antifungal effect might arise from decreased membrane fluidity in the yeast cells[24]. This assumption is confirmed by the observation that supplementation with ergosterol or cholesterol reduced the antifungal effect of statins[20,25,26], and that *C. albicans* transformed from the yeast cell form to the

Table 1 Determined minimal inhibitory concentration values (μg/mL) of different statins against *Candida* species

Statin/species	ATO	FLV	LOV	PRA	ROS	SIM	Ref.
Candida albicans	128	25-128	5-64	> 128	128	8	[18,21,22,29]
Candida glabrata	32	64-> 128	128	> 128	128	16-32	[18,21,29]
Candida parapsilosis	ND	64-128	ND	ND	ND	ND	[21]
Candida tropicalis	ND	64-128	ND	ND	ND	ND	[21]
Cryptococcus neoformans	ND	16-32	ND	ND	ND	ND	[21]

ATO: Atorvastatin; FLV: Fluvastatin; LOV: Lovastatin; PRA: Pravastatin; ROS: Rosuvastatin; SIM: Simvastatin; ND: Not determined.

Table 2 Determined minimal inhibitory concentration values (μg/mL) of different statins against filamentous fungal species

Statin/Species	ATO	FLV	LOV	PRA	ROS	SIM	Ref.
Zygomycetes							
Absidia corymbifera	96[1]	> 25-3.6	> 96	> 96	33[1]	96[1]	[19,35]
Absidia glauca	ND	6.25	ND	ND	ND	ND	[35]
Cunninghamella bertholletiae	ND	ND	32-40	ND	ND	ND	[33]
Micromucor ramanniana	ND	> 25	ND	ND	ND	ND	[35]
Mortierella wolfii	> 128	ND	> 128	ND	> 128	> 128	[34]
Mucor circinelloides	ND	ND	5-40	ND	ND	ND	[33]
Mucor circinelloides f. lusitanicus	ND	> 25	ND	ND	ND	ND	[35]
Mucor hiemalis	ND	> 25	ND	ND	ND	ND	[35]
Mucor mucedo	ND	6.25	ND	ND	ND	ND	[35]
Mucor racemosus	ND	25	ND	ND	ND	ND	[35]
Mycotypha africana	8	ND	> 128	ND	8	> 128	[34]
Paecilomyces variotii	32	25	64	> 128	32	8	[29]
Rhizomucor mieheii	> 96	6.25	64-> 128	> 96	33[1]	> 96	[19,32,35]
Rhizomucor pusillus	> 96	3.125	1-3.6[1]	> 96	11[1]	33[1]	[19,32,35]
Rhizopus homothallicus	ND	ND	40-56	ND	ND	ND	[33]
Rhizopus microsporus var. oligosporus	> 96	96[1]	> 96	> 96	> 96	> 96	[19]
Rhizopus oryzae	32-96[1]	2-11[1]	32-128	> 128	> 128	64-> 96[1]	[18,19,29,33,35]
Rhizopus schipperae	ND	> 25	ND	ND	ND	ND	[35]
Rhizopus stolonifer	64		> 128		64	> 128	[34]
Saksenaea vasiformis	ND	> 25	ND	ND	ND	ND	[35]
Syncephalastrum racemosum	32-> 96	33[1]	16-> 96	> 96	32-> 96	8-> 96	[19,34,35]
Ascomycetes							
Aspergillus flavus	> 128	128	> 128	> 128	> 128	> 128	[18,29]
Aspergillus fumigatus	64-> 256	2	25	> 128	128-> 256	6.25	[18,29,37]
Aspergillus spp.	ND	ND	16-256	ND	ND	4-256	[36]
Paecilomyces variotii	32	25	64	> 128	32	8	[18]
Heterokontophyta							
Pythium insidiosum	ND	16-64	ND	ND	ND	ND	[38]

[1]MIC_{90} value. ATO: Atorvastatin; FLV: Fluvastatin; LOV: Lovastatin; PRA: Pravastatin; ROS: Rosuvastatin; SIM: Simvastatin; ND: Not determined.

pseudomycelial form upon exposure to LOV[24]. It is also proposed that antimicrobial activity based on the loss of mitochondrial DNA, and thus the respiratory function of the cell, occurs in the presence of statins[16]. Indirectly, the antifungal effect of statins might come from their negative influence on the cell signaling by the inhibition of the synthesis of lipid attachments for the γ subunit of heterotrimeric G-proteins[15], and on the cell proliferation and differentiation through inhibition of the synthesis of important isoprenoids[30]. LOV does not cause apoptotic cell death in yeasts compared to filamentous fungi[24,31].

Antifungal activity of statins against filamentous fungi

The inhibition activity of statins on the growth of filamentous fungi was revealed in the cases of several zygo-[18,19,28,31-35] and ascomycetous fungal species[18,25,28,29,36,37]. Only one article reports the antifungal activity of FLV against a Heterokontophyta fungal species, *Pythium insidiosum*[38]. In contrast to its activity against yeasts, FLV displayed the strongest antifungal activity, followed by ROS, SIM, LOV, and ATO. PRA also proved to be ineffective against them. Table 2 summarizes the determined MIC values of statins against different filamentous fungal species.

Beyond to the harmful effects on membrane fluidity and the synthesis of important isoprenoids for cell signaling and vital processes (such as cell proliferation and differentiation), and protein prenylation[16], statins induce apoptosis-like cell death in filamentous fungi[15,30,31]. The molecular mechanisms underlying the different levels of fungal resistance to statins are unknown. It is hypothesized that the resistance is connected with the different copy numbers of the HMG-CoA reductase gene (hmgR) in the case of filamentous species. This assumption is supported by the observation of Lukács *et al*[39]. In their

study, heterologous expression of the *Rhizomucor miehei hmg*R gene in *Mucor circinelloide* lowered its sensitivity to statins compared to the untransformed strain. Furthermore, supplementation of sterols to the medium reduces the antifungal activity of statins, as in the case of yeasts[25].

ANTIFUNGAL ACTIVITY OF STATINS IN DRUG COMBINATIONS

Statins are not applicable as single antifungal agents for the treatment of IFI because their MICs are much higher (about 1 order of magnitude) than their maximum achievable concentrations in human serum[11,40-45]. All the same, they should be promising agents in clinical practice because they can act additively or synergistically with antifungal agents, allowing substantial decreases in their therapeutic concentrations and their side effects[45]. Such combinations would be advantageous as the basis of a less toxic antifungal therapy[3].

Combination with antifungal agents

Statins can interact synergistically with azole antifungal agents against yeasts and can reduce their growth significantly. Fluconazole (FCZ) with LOV, and FCZ or itraconazole (ITZ) with FLV, interact synergistically on the growth of *Candida* species[21,22]; however, interaction was not demonstrated between PRA or FLV and FCZ[23]. FLV acted additively with AMB, FCZ, and ITZ against *C. albicans* and *C. neoformans*[21]. Both synergistic and additive effects were observed on the growth reduction of *C. albicans* and *C. glabrata* when primycin (PN), a non-polyene macrolide lactone antibiotic complex, was combined with FLV, LOV, or SIM[18]. Additive interactions were observed between AMB and ATO and ROS, and between nystatin (NYS) and FLV, LOV, ROS, and SIM in the case of *C. albicans* and *C. glabrata*[28]. A recent comprehensive study, where the interaction was investigated between four different azole compounds (FCZ; ITZ; ketoconazole, KTZ; and miconazole; MCZ) and six different statins (ATO, FLV, LOV, PRA, ROS, and SIM), revealed synergistic and additive interaction between these compounds against *C. albicans* and *C. glabrata*[29]. Table 3 summarizes these interactions.

Synergistic and additive interactions were revealed between statins and antifungal agents in the case of zygomycetous fungal species[18,19,28,29,33]. Significant *in vitro* synergy between statins and azole antifungal agents was demonstrated against several zygomycete fungi, though voriconazole itself was ineffective[29,33]. Remarkable antifungal effects were observed on the growth of *Rhizopus oryze* when PN was combined with statins in concentrations that could not inhibit the fungal growth alone[18]. In the case of this species, AMB and NYS also interacted additively with different statins[28]. *In vitro*, FLV and ROS acted synergistically and additively with AMB in inhibiting the growth of fungi belonging to Zygomycetes over their clinically available concentration ranges in human serum[19]. After *in vivo* tests, these concentration combinations may represent a promising basis for combined therapy in the treatment of invasive zygomycosis.

Synergistic and/or additive interaction of AMB, caspofungin, VCZ, PN with FLV on the growth reduction of *Aspergillus fumigatus* was demonstrated[28,37]. AMB acted additively with ATO and FLV against *Aspergillus flavus*[28]. Synergistic interaction was observed between PN and FLV, LOV and SIM, and an additive interaction was observed between AMB and ATO or SIM in the case of *Paecilomyces variotii*[18,28]. Additive and synergistic interactions were revealed between statins and azoles against *A. flavus*, *A. fumigatus*, and *Paecilomyces variotii*[29].

Terbinafine acted antagonistically in combination with FLV against *P. insidiosum*. Reduced antifungal activity was observed for their combination compared to when they were applied alone[38].

Combination with other drugs

Drug interactions were revealed between statins and non-antifungal drugs, which have a secondary antifungal activity. An antifungal peptide secreted by *Penicillium chrsyogenum* (*Penicillium chrsyogenum* antifungal protein; PAF) and a hexasulfonated naphtylurea, suramin (originally applied as an agent for treatment of parasitic infections) can decrease the growth of zygomyceteous fungal species in the presence of different statins[34,35]. The activities of the statin-PAF combinations on the different strains varied, and depended on the activities of the components applied separately. When a strain was resistant to one of the components, significant interactions could not be detected. On the other hand, when a strain was sensitive to both types of antifungal agents, synergistic or additive interactions were detected[34]. Interactions were not detected between FLV and suramin if the investigated strain proved to be insensitive to both compounds, but synergistic and additive interactions could be observed if the fungus was sensitive to FLV and insensitive to suramin. Antagonistic interaction was observed if the fungus was sensitive to both drugs[35].

These results are summarized in Table 4.

STATINS AS ANTIFUNGAL AGENTS IN CLINICAL THERAPY

A number of studies detail the beneficial effects of statins in transplant or non-transplant recipients with sepsis or infection[16]. One theory of the possible clinical therapy for invasive mould infection (IMI) among immunocompromised patients was created based on the observation that this disease in patients with diabetes mellitus appears to be decreasing over recent years because of the more frequent use of statins in these patients[46]. This hypothesis is well supported by the above-mentioned *in vitro* susceptibility and drug interaction studies; however, a recent retrospective case-control study suggested that, despite evidence of *in vitro* activity, statins may not decrease risk of IMI[47].

In consequence, because the *in vitro* observed MICs

Table 3 Revealed *in vitro* interactions between statins and clinically used antifungal agents against different fungi

Species	Antifungal agent	Statin	Interaction	Ref.
Yeasts				
Candida albicans	AMB	ATO, FLV	ADD	[28,21]
	FCZ, ITZ, KTZ	ATO	ADD	[29]
	FCZ, ITZ	FLV	ADD, SYN, NI	[21,23,29]
		LOV	SYN, ADD	[22,29]
	FCZ, ITZ, KTZ, MCZ	PRA	NI	[23,29]
	FCZ,KTZ	ROS	ADD	[29]
	FCZ, ITZ, KTZ	SIM	ADD	[29]
	ITZ, KTZ, MCZ	FLV, LOV	ADD	[29]
	ITZ,MCZ	ROS	SYN, ADD	[29]
	MCZ	ATO,SIM	SYN, ADD	[29]
	PN, NYS	FLV, LOV, SIM	ADD	[18,28]
Candida glabrata	AMB	ATO, ROS	ADD	[18]
	FCZ	ATO	SYN, ADD	[29]
	FCZ, ITZ	FLV	ADD, NI	[21,29]
	FCZ	LOV	SYN, ADD	[29]
	FCZ, ITZ	PRA	NI	[29]
	FCZ, ITZ, KTZ, MCZ	ROS, SIM	ADD	[29]
	ITZ, KTZ, MCZ	ATO, FLV, ROS	ADD	[29]
	KTZ, MCZ	PRA	ADD	[29]
	PN	ATO, FLV	ADD	[18]
		LOV, SIM	ADD, SYN	[18]
	NYS	LOV, ROS	ADD	[28]
Candida parapsilosis	FCZ, ITZ	FLV	ADD, SYN	[21]
Candida tropicalis	FCZ	FLV	SYN	[21]
	ITZ	FLV	ADD, SYN	[21]
Cryptococcus neoformans	FCZ, ITZ	FLV	ADD, SYN	[21]
	AMB	FLV	ADD	[21]
Filamentous fungi-Zygomycetes				
Absidia corymbifera	AMB	ROS	ADD, SYN	[19]
Cunninghamella bertholletiae	VCZ	LOV	SYN	[33]
Mucor circinelloides	VCZ	LOV	SYN	[33]
Rhizomucor mieheii	AMB	FLV, ROS	ADD, SYN	[19]
Rhizopus homothallicus	VCZ	LOV	SYN	[33]
Rhizopus microsporus var. oligosporus	AMB	FLV, ROS	ADD, SYN	[19]
Rhizopus oryzae	AMB	FLV, ROS	ADD, SYN	[19,28]
		ATO, SIM	ADD	[28]
	FCZ	FLV	NI	[29]
	FCZ, ITZ, MCZ	LOV	ADD	[29]
	ITZ, KTZ	ATO, FLV, ROS	SYN, ADD	[29]
	ITZ, KTZ, MCZ	PRA	NI	[29]
	ITZ, KTZ	SIM	NI	[29]
	KTZ	LOV	NI	[29]
	MCZ	ATO	NI	[29]
		FLV, ROS, SIM	ADD	[29]
	NYS	ATO, FLV, LOV	ADD	[28]
	PN	ATO	ADD	[18]
		LOV, ROS	SYN	[18]
	VCZ	LOV	SYN	[33]
Syncephalastrum racemosum	AMB	FLV, ROS	ADD, SYN	[28]
Filamentous fungi-Ascomycetes				
Aspergillus flavus	AMB, PN	FLV	ADD, SYN	[18,28]
	ITZ	ATO	SYN	[29]
	ITZ, KTZ, MCZ	FLV	SYN, ADD	[29]
	ITZ	LOV	ADD	[29]
	ITZ, KTZ	PRA	NI	[29]
	ITZ	ROS	ADD	[29]
	ITZ, MCZ	SIM	ADD	[29]
	KTZ	ATO, ROS	SYN, ADD	[29]
		SIM	NI	[29]
	MCZ	ATO, LOV, ROS	NI	[29]
		PRA	ADD	[29]
Aspergillus fumigatus	AMB	ATO, FLV	ADD, SYN	[28,37]
	CFG, VCZ	FLV	SYN	[37]
	FCZ, ITZ	ATO	SYN	[29]
	FCZ, ITZ, MCZ	FLV	ADD	[29]
	FCZ	LOV	SYN	[29]

	FCZ	SIM	SYN, ADD	[29]
	ITZ, KTZ, MCZ	LOV, SIM	ADD	[29]
		PRA	NI	[29]
	ITZ	ROS	SYN, ADD	[29]
	KTZ, MCZ	ATO	SYN, ADD	[29]
	KTZ	FLV	SYN, ADD	[29]
	KTZ, MCZ	ROS	ADD	[29]
Paecilomyces variotii	AMB	ATO,SIM	ADD	[28]
	PN	FLV, LOV, SIM	SYN	[18]
Filamentous fungi-Heterokontophyta				
Pythium insidiosum	TBF	FLV	ANT	[37]

ADD: Additive interaction; AMB: Amphotericin B; ANT: Antagonism; ATO: Atorvastatin; CFG: Capofungin; FCZ: Fluconazole; FLV: Fluvastatin; ITZ: Itraconazole; KTZ: Ketoconazole; LOV: Lovastatin; MCZ: Miconazole; NI: No interaction; NYS: Nystatin; PN: Primycin; PRA: Pravastatin; ROS: Rosuvastatin; SIM: Simvastatin; SYN: Synergistic interaction; TBF: Terbinafine; VCZ: Voriconazole.

Table 4 Revealed *in vitro* interactions between statins and non-antifungal drugs against zygomycetous fungi

Species	Non-antifungal drug	Statin	Interaction	Ref.
Zygomycetes				
Absidia corymbifera	SUR	FLV	SYN	[35]
Absidia glauca	SUR	FLV	ANT	[35]
Micromucor ramanniana	SUR	FLV	SYN	[35]
Mucor circinelloides f. lusitanicus	SUR	FLV	ADD, SYN	[35]
Mucor racemosus	SUR	FLV	ANT	[35]
Mycotypha africana	PAF	ATO, SIM	ADD	[34]
		LOV, ROS	ADD, SYN	[34]
Rhizomucor mieheii	SUR	FLV	ANT	[35]
Rhizomucor pusillus	SUR	FLV	ANT	[35]
Rhizopus oryzae	SUR	FLV	ADD, SYN	[35]
Rhizopus schipperae	SUR	FLV	ADD, SYN	[35]
Saksanaea vasiformis	SUR	FLV	ADD, SYN	[35]
Syncephalastrum racemosum	PAF	ATO	ADD, SYN	[34]
		ROS, SIM	SYN	[34]
	SUR	FLV	ADD, SYN	[35]

ADD: Additive interaction; ANT: Antagonism; ATO: Atorvastatin; FLV: Fluvastatin; LOV: Lovastatin; PAF: *Penicillium chrysogenum* antifungal protein; ROS: Rosuvastatin; SIM: Simvastatin; SUR: Suramin; SYN: Synergistic interaction.

of statins are higher than their concentrations achievable in human serum, their potential application to prevent or treat IMIs is only possible in combination with antifungal agents[45]. In clinical practice, the administration of statins together with antifungals, which are metabolized by different cytochrome P450 (CYP450) isoenzymes in the liver, suggests that the drug interactions with the CYP system and the serious adverse effects (e.g. myopathy) are avoidable[45].

FUTURE PROSPECTIVES

The number of antifungal agents available for treatment of IFIs is limited, and their use has been restricted because of their toxicity or unfavorable pharmacokinetic profiles[3]. Hence, research interest has focused on safe, non-antifungal drugs that are used in clinical practice and have antifungal activity.

The observed *in vitro* antifungal activities of statins and their combinations with clinically antifungal agents would create new therapies for the treatment of IFI, without serious side effects. However, there are some factors in their combined application that require increased attention in immunocompromised hosts. As a consequence of the pleiotropic beneficial effects of statins beyond their lipid lowering attributes, there is a decreased risk of chronic renal failure and an improved endothelial dysfunction)[16]. Importantly, the administration of statins together with antifungals that are predominantly metabolized by the same CYP450 isoenzymes in the liver is contraindicated, because such drug interactions with the CYP system may cause serious adverse effects[45].

Further studies, for example, *in vivo* animal model experiments, are needed to evaluate the practical efficiency and possible triggered side effects of statin-antifungal drug combinations.

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OBSERVATION

Antibiotic-resistant bugs in the 21st century: A public health challenge

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Abstract

Antimicrobial resistance, which has been reported against almost every antibiotic discovered, is one of the most urgent public health problems, threatening to undermine the effectiveness of infectious disease treatment worldwide. Since penicillin ushered in the antibiotic era in the mid 20th century, the scientific world had engaged in a war between the development of antibacterial agents and bacterial resistance. During the first decade of the 21st century, grave concern has been expressed over the evolution of multi-drug resistant staphylococci, enterococci, and mycobacteria, which pose serious clinical and public health challenge to humans. The present picture is frighteningly similar to the pre-antibiotic era, with reports of nosocomial spread and intercontinental dissemination of multi-drug resistant bacteria. For infected patients, there is no magic bullet. The microbial pathogens appear to be gaining the upper hand, coupled with a recent dramatic reduction in antibiotic research by pharmaceutical companies because of the high cost of drug research. Several compounds that have recently been developed or resurrected to treat gram-positive infections are still unable to meet the armamentarium of resistance mechanisms of these pathogens. The situation is worse for gram-negative organisms, where no new drug is currently being developed against them. A multi-disciplinary approach to combat resistance is required, which must be applied, sustained, and continuously refined. The key components for maintaining effective antimicrobial chemotherapy will include better use of existing agents, coupled with continuous investment in new and innovative technologies, which must include diagnostics and vaccines in addition to new antimicrobial agents.

Key words: Antimicrobial; Resistance; Bugs; Public health

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INTRODUCTION

The discovery and introduction of penicillin as a chemotherapeutic agent in the 1940s was greeted with great enthusiasm. A 33-year-old woman dying of a streptococcal blood stream infection in a New Haven Connecticut hospital in March 1942 was cured after careful injection of repeated doses of the "miracle" drug; she went on to live to the age of 90[1]. The enthusiasm was short lived; in 1944, *Staphylococcus aureus* (*S. aureus*) resistant to penicillin through the production of β-lactamase enzyme emerged. Methicillin, a β-lactamase resistant penicillin, was introduced into the market in 1959, but in 1961, resistance to methicillin also emerged[2]. These strains of *S. aureus* called Methicillin Resistant *Staphylococcus aureus* (MRSA) became disseminated worldwide, first as hospital-associated[3], and later as community-associated, pathogens[4].

Today, resistance by microbes has been reported against almost every antibiotic discovered, and is one of the most urgent public health problems, threatening to undermine the effectiveness of infectious disease treatment worldwide[5,6]. The present picture is frighteningly similar to the pre-antibiotic era; nosocomial epidemic spread and intercontinental dissemination of multi-drug resistant bacteria are being reported, and for patients infected with these multi-drug resistant bacteria, there is no magic bullet. The challenges of containing and controlling the threat from antibiotic-resistant pathogens are daunting. This editorial reviews certain bacteria that have evolved and adapted to a point where they pose serious clinical and public health challenge to humans.

METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

The evolution of MRSA exemplifies the genetic adaptation of an organism into a first-class multi-drug resistant bacterial pathogen. After the introduction of penicillin in 1940s and later, methicillin in 1959, *S. aureus* quickly developed resistance to these β-lactam compounds, and by 2003, more than 50% of *S. aureus* isolates recovered in United States hospitals were MRSA[7]. Increased isolation of hospital associated MRSA (HA-MRSA) was also reported by several countries in Europe, Asia, Australia, and Africa, including Nigeria[8-12]. In the early 80s, MRSA gradually emerged as a community-associated pathogen in people without identifiable risk factors. This became so pronounced at the turn of the 21st century that, at present, community-associated MRSA (CA-MRSA) is the leading cause of skin and soft tissue infections seen at US emergency rooms[13]. Such MRSA frequently causes severe infections resembling spider bites, as well as severe necrotizing fasciitis and pneumonia. The infection often produces toxins, such as the panton-valentine leukocidin and cytolytic peptides. MRSA has also acquired genes that may increase its ability to survive. A single clone, USA 300 (ST8-MRSA-IV), is responsible for most CA-MRSA infections in the United States[14]. ST80-MRSA-IV is the predominant clone in Europe, Algeria, and Tunisia, while ST88-MRSA-IV is the predominant clone reported in Nigeria[15,16]. Although such MRSA are usually susceptible to oral antibiotics, such as clindamycin, fluoroquinolones, trimethoprim-sulfamethoxazole, tetracyclines, and rifampicin, some multi-drug resistant strains are emerging[15].

VANCOMYCIN RESISTANT *STAPHYLOCOCCUS AUREUS*

For approximately 30 years, drugs used to treat MRSA infections were the glycopeptides, vancomycin, and teicoplanin[17]. MRSA then began to develop resistance to glycopeptides, evolving through a largely unknown mechanism as reduced susceptibility to vancomycin, which was associated with thickening of the pathogen's cell wall and sequestration of glycopeptides at the periphery of the cell. Such isolates were designated vancomycin (or glycopeptide) intermediately resistant *S. aureus* (VISA or GISA) and were first reported in 1997[18]. VISA is difficult for clinical laboratories to detect, but its presence is associated with therapeutic failures of glycopeptides. The Clinical and Laboratory Standards Institute, therefore, changed the susceptibility breakpoints for vancomycin testing in *S. aureus* in 2009[19], and has proposed screening tests for VISA; however, debate is ongoing regarding the usefulness of vancomycin in the treatment of serious MRSA infections. Strains of MRSA with true low-level and high-level resistance to vancomycin (Vancomycin Resistant *S. aureus* or VRSA) emerged in 2002[20]. Such resistance was due to acquisition of the *vanA* gene operon, originally described in vancomycin resistant enterococci (VRE). Eleven such isolates have been reported worldwide; nine in the US (mainly in the Michigan area), one in India, and one in Iran[21]. Certain biological constraints are believed to be responsible for the current restricted dissemination of these VRSA strains, although the potential for widespread dissemination cannot be completely ruled out. We recently isolated six VRSA isolates from chronic wound infections and osteomyelitis with high level resistance (vancomycin MIC > 256 μg/mL) in patients who had not been previously exposed to glycopeptides (Taiwo *et al*[22], 4th LAUTECH Research and Development Fair/Exhibition, May 2011; unpublished data). This suggested that VRSA carrying the *vanA* operon, which is inducible only by glycopeptides, may have disseminated. VRSA, like other strains of HA-MRSA, is often resistant to multiple drugs, including clindamycin, aminoglycosides, trimethoprim-sulfamethoxazole, rifampicin, and fluoroquinolones.

VRE

Although less virulent than MRSA, enterococci have presented therapeutic problems initially because of their "tolerance" to penicillin and vancomycin, which inhibit, but do not kill them. Enterococci are the third most common cause of infective endocarditis. The effects of penicillin tolerance on therapeutic outcomes were apparent by the late 1940s, when it became routine to add an aminoglycoside to penicillin to treat the disease. This therapy was effective until 1988, when VRE emerged[23,24]. Since the beginning of the 21st century, in the United States, enterococci have become a major reservoir of antibiotic-resistant genes. VRE has become a major cause of nosocomial infections, especially of the bloodstream, urinary tract, and surgical sites[25]. One major problem is the emergence of multidrug resistant enterococci that correlates with the predominance of a single genetic lineage, which has disseminated worldwide. Members of this lineage have acquired genetic determinants that appear to increase their success in the hospital environment, and some have developed resistance to practically

all available antibiotics. In Nigeria, we have isolated multi-resistant VRE strains[26] and strains exhibiting high-level resistance to gentamicin and streptomycin[27]. Another major problem is that enterococci harbor transferable genetic elements, which have an unusually broad host range. They can also be transferred to both Gram-negative and Gram-positive bacteria species by conjugation systems involving plasmids and transposons[28]. Transfer of the *vanA* operon has been specifically reported in patients co-colonized with VRE and MRSA[29]. The recent isolation of VRSA in Nigeria may implicate VRE, which have been locally present for some time, as the possible source of the *vanA* operon. No appropriate therapy for VRE endocarditis has been defined[30] and no agent has been approved by the Food and Drug Administration for this indication.

MULTI-DRUG RESISTANT GRAM-NEGATIVE BACTERIA

The antibiotic resistance situation is worse when it comes to nosocomial gram-negative infections, because no new antibiotics against these multi-drug resistant organisms are in the advanced stages of clinical development. *Pseudomonas aeruginosa* and *Acinetobacter* spp. are the best-known therapeutic challenges among the gram-negative bacteria. Multi-resistant strains of these, especially *Acinetobacter* spp., were reported to have caused enormous challenges in soldiers that returned to the US from Iraq and Afghanistan[31]. Resistance to the most potent antibiotics has recently extended to members of the family enterobacteriaceae, including hospital-associated strains of *Klebsiella* spp., *Escherichia coli* (*E. coli*) and *Enterobacter* spp. Such highly resistant clinical bacteria isolates have been reported in Nigeria[32]. Equally worrying is the fact that these multi-drug resistant gram-negative bacteria have been found in otherwise healthy patients outside of hospitals. For example, urinary tract infections caused by *E. coli* resistant to trimethoprim-sulphamethoxazole, fluoroquinolones, or both, and that produce extended-spectrum β-lactamases, which are capable of destroying the most potent cephalosporins, have been reported[33]. Recent major outbreaks of food poisoning caused by multi-drug resistant *Salmonella* were also reported.

Until recently, carbapenems, such as imipenem, were almost uniformly active against resistant gram-negative bacteria. However, some strains have developed very effective ways to deal with the carbapenems, including the production of β-lactamases, designated as carbapenemases, that demolish the carbapenems; changes in outer membrane porins that blocks the entry of the drug; and active pumping of the drug out of the cell using complex efflux pumps. The situation is further complicated by the permeability barrier and efflux mechanisms that also affect other classes of antibiotics, such as quinolones, aminoglycosides, and tigecycline. Moreover, the common presence of these β-lactamase genes of gram-negative bacteria in transferable mobile elements means that these genes could reach virtually any gram-negative bacteria and become a major, global threat to public health in the near future. Recognition of the presence of a carbapenemase in a gram-negative bacterium is of paramount importance, because strict infection-control measures are required to avert hospital epidemics and to prevent the dissemination of these genes to other gram-negative bacteria species[31].

MULTI-DRUG RESISTANT *MYCOBACTERIUM TUBERCULOSIS*

In early 2005, physicians at a rural hospital in KwaZulu-Natal, a province of South Africa, were concerned by a high rate of rapid death among patients infected with the human immunodeficiency virus (HIV) who had tuberculosis[34]. A study revealed the presence not only of multi-drug resistant *Mycobacterium tuberculosis* (MDR TB), but also of what came to be called extensively drug resistant TB (XDR TB). XDR TB is caused by a strain of *Mycobacterium tuberculosis* that is resistant to isoniazid and rifampicin (which defines MDR TB) in addition to any fluoroquinolone and at least one of the three following injectable drugs: capreomycin, kanamycin, and amikacin. A March 2006 report by the Centers for Disease Control and Prevention and the World Health Organization (WHO) also documented the presence of XDR TB in at least 17 countries. Though not representative at that time, the data showed that 10% of MDR TB isolates were in fact XDR TB. More representative data from the US, the Republic of Korea, and Latvia, showed that 4%, 15% and 19%, respectively, of MDR TB isolates were XDR TB strains[35]. Evidence suggests that XDR TB reflects a failure to implement the measures recommended in the WHO's stop TB strategy[36]. This strategy, which emphasizes expanding high-quality DOTS programs; addressing HIV-associated TB and drug resistance; strengthening health care systems and primary care services; encouraging all providers to follow good practices; empowering patients and communities to improve health; and enabling and promoting research, requires political commitment and will. However, in many developing countries, health is not a top priority.

A GLOOMY PICTURE

Recently, there has been a dramatic reduction in antibiotic research by pharmaceutical companies, because of the high cost of drug research, although several compounds have been developed or resurrected to treat gram-positive infections[31]. However, the available agents have important limitations. None has been shown to work better than vancomycin against MRSA. Quinupristin-dalfopristin and linezolid have important toxic effects, and resistance of MRSA to each has been observed, including linezolid-resistant VRE in patients who have never received the drug. Daptomycin has sometimes failed against MRSA and enterococci, and resistance to it has emerged. There

is little data on the effectiveness of tigecycline for enterococci infection, and the new cephalosporins (ceftobiprole and ceftaroline) are not clinically useful against ampicillin-resistant enterococci. Dalbavancin, televancin, and oritavancin are all limited in their effect on VRSA and VRE; and although iclaprim may have a role in MRSA infections, it is not useful clinically against enterococci infections. The situation for gram-negative infections is worse, as no antibiotic is currently being developed against these pathogens[31]. The resurrected polymyxins (e.g. colistin with or without rifampicin) are often the only available alternative antibiotics for some pan-resistant gram-negatives, particularly *Acinetobacter* spp.. Renal toxicity is still a major problem and reports of resistance are emerging. Similarly, *Mycobacterium tuberculosis* and atypical mycobacterium that are totally resistant to all available first and second line anti-tuberculosis drugs, as well as to all fluoroquinolones, have emerged. Some strains of MDR TB have undergone stable mutational changes that enhance their survival, virulence, nosocomial transmission, and worldwide dissemination[37].

CURRENT EFFORTS TO COMBAT ANTIBIOTIC RESISTANT BUGS: PUBLIC HEALTH PERSPECTIVES

The challenge of containing and controlling the threat from antibiotic resistant pathogens is daunting. However, few will argue against preserving the existing antimicrobials for the benefit of current and future generations. A multi-disciplinary approach is required, which must be applied, sustained, and continuously refined. The key components for maintaining effective antimicrobial chemotherapy include better use of existing agents, continuous investment in new and innovative technologies (including diagnostics and vaccines), and new antimicrobial agents.

Sound prescribing principles

While the surveillance of resistant pathogens and patterns of antibiotic prescribing need to be defined more accurately, particularly at local and individual levels, they remain the yardstick to inform strategy, policy, and practice, and are a measure of their effectiveness. To be effective and remain so, the use of antimicrobial agents needs to be supported by rigorous application of sound prescribing principles[38] in a health care environment, which strives to minimize the risks of infection by adherence to good hygiene and housekeeping practices. This is particularly important in many developing countries, where antibiotics are available over-the-counter to the populace and are used without medical authorization. It is equally important to have a sound prescribing principle when there is a serious concern about paucity of novel antibiotics, particularly against multi-drug resistant gram-negative pathogens[37]. This is a global challenge, and initiatives at national and international levels are needed to encourage investment and to strengthen research in this area[39,40]. Linking prudent prescribing and sound clinical practice remains a challenge, especially where prescribing is largely empirical. The importance of rapid diagnostic tests, including near patient testing, is increasingly supported and achievable. However, obstacles to widespread acceptance include not only cost, but also the need for greater flexibility in medical practice to permit their adoption.

Public health initiatives

Public education concerning the appropriate use of antibiotics and the risks from antimicrobial-resistant pathogens requires a repertoire of approaches. One such approach is the widespread use of routine data sets that link clinical outcome data to prescribing. The increasing recognition of the importance of a partnership between prescriber and the patient is significant. Media campaigns have dominated this approach and emphasize the importance of clear and positive messages. A structured educational approach within the context of day care nurseries and children undergoing full-time education, which also involves parents and primary care physicians, has been shown to be an effective strategy[41]. Currently, an ambitious schools educational program (e-Bug) across many European countries and funded by DG SANCO[42] is being conducted.

The European Union (EU) is strongly supportive of public education initiatives, which have been given a boost by the establishment of an annual EU Antibiotic Awareness Day (EUAAD), promoted by the European Centre for Disease Control[43]. The first EUAAD was 18 November 2008, for which each member state developed its own national program of activities. In the UK, a number of events took place within the devolved administrations and in many individual Trusts. The Advisory Committee on Antibiotic Resistance and Healthcare Associated Infections[44] organized an EUAAD symposium in the Science Museum, London entitled "Antibiotic Resistance - Myth Busting" with the objective of reviewing current problems, national surveillance strategies, the professional responses in human and veterinary medicine, and the importance of engaging with the public[45]. In addition, a school poster competition was launched to coincide with the conference, which was well supported[46]. The brief was to design an eye-catching poster to raise public awareness that antibiotics do not work on most coughs, colds, and sore throats. Recent surveillance data suggests that rates of antimicrobial resistance for a few selected pathogens are falling, a phenomenon that, until recently, would not have been considered possible. It is important to be constantly vigilant, particularly in maintaining good prescribing principles.

Social marketing framework for behavioral change

Although combating antibiotic resistance is a war that must be waged on one front by biological scientists and clinicians, social scientists also have a key role to play because of the behavioral aspects of the problem, e.g. not

taking entire prescribed regimen, skipping doses, taking antibiotics for viral illnesses, *etc*. To minimize the development and spread of antibiotic resistance, providers and current patients, as well as those who might be patients or the caretakers of patients in the future, must become sufficiently aware of the issue and engage in appropriate behavior. Although a few behavioral indicators have indicated progress in recent years, such as a decline in the UK and the US in oral antibiotic prescription rates among children[47,48], recent evidence suggests that there are still large hurdles to climb to address the broader issue. For example, although there have been multiple efforts to educate both providers and patients about the prudent use of antibiotics, survey data from a recent national probability sample of 919 US adults showed that misunderstanding continues to exist about the appropriate use of antibiotics. A substantial proportion of the population still engages in behaviors that potentially contribute to the antibiotic resistance problem[49]. The ultimate goal of social marketing is behavior change, which can only be achieved when detailed attention is paid to defining the behavioral focus.

Oligonucleotide therapeutics

There is a pressing need to develop and evaluate novel, alternative strategies to overcome resistance and reduce the morbidity and mortality associated with infections caused by antibiotic resistant bacteria. One strategy is the use of "antisense" or "antigene" agents to inhibit resistance mechanisms at the nucleic acid level. Antisense or antigene oligonucleotides bind mRNA to prevent translation or bind DNA to prevent gene transcription, respectively. Interrupting expression of resistance genes in this manner could restore susceptibility to key antibiotics, which would be co-administered with the antisense or antigene compound. This would extend the life span of existing antibiotics, which offer clinically proven therapies and are often cheaper, more effective, or less toxic than the alternatives.

Antisense molecules that bind complimentary mRNA sequences are a well-established means of modifying gene expression in mammalian systems[50]. Indeed, the manipulation of eukaryotic RNA pathways with small interfering RNAs has revolutionized research in mammalian cell biology, with libraries of custom-made molecules spanning entire genomes now commercially available. Antisense strategies have been used therapeutically in the treatment of human genetic disorders, such as muscular dystrophy and familial hypercholesterolemia[51] or viral diseases (http://www.avibio.com and http://www.isispharm.com), with some clinical trials ongoing. A small number of agents are already licensed for clinical use[52].

CONCLUSION

It is more difficult than ever to eradicate infections caused by antibiotic-resistant microbes and the problem is exacerbated by a dry pipeline for new antimicrobials with bactericidal activity against gram-negative bacteria and enterococci. A concerted effort on the part of academic researchers and their institutions, the pharmaceutical industry, social scientists, the general populace, and the government, is crucial if we are to prevent a global public health disaster.

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REVIEW

Regulation of *fim* genes in uropathogenic *Escherichia coli*

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Abstract

Uropathogenic *Escherichia coli* (UPEC) is the leading cause of urinary tract infections in women, causing significant morbidity and mortality in this population. Adherence to host epithelial cells is a pivotal step in the pathogenesis of UPEC. One of the most important virulence factors involved in mediating this attachment is the type 1 pilus (type 1 fimbria) encoded by a set of *fim* genes arranged in an operon. The expression of type 1 pili is controlled by a phenomenon known as phase variation, which reversibly switches between the expression of type 1 pili (Phase-ON) and loss of expression (Phase-OFF). Phase-ON cells have the promoter for the *fimA* structural gene on an invertible DNA element called *fimS*, which lines up to allow transcription, whereas transcription of the structural gene is silenced in Phase-OFF cells. The orientation of the *fimS* invertible element is controlled by two site-specific recombinases, FimB and FimE. Environmental conditions cause transcriptional and post-transcriptional changes in UPEC cells that affect the level of regulatory proteins, which in turn play vital roles in modulating this phase switching ability. The role of *fim* gene regulation in UPEC pathogenesis will be discussed.

Key words: Type 1 fimbriae; Type 1 pili; Gene regulation; Uropathogenic *Escherichia coli*; Urinary tract

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ROLE OF TYPE 1 PILI IN UROPATHOGENIC *ESCHERICHIA COLI* PATHOGENESIS

Uropathogenic *Escherichia coli* (UPEC) is the number one cause of urinary tract infections in the United States[1,2]. Approximately 6-7 million people are afflicted with a urinary tract infection each year in the United States at a cost of $2.5 billion per year. Urinary tract infections are modeled as ascending infections. In women, the UPEC bacteria move from the rectum to the vaginal surface to the urinary tract. Although UPEC can express several different varieties of pili, type 1 pili may be the most important in the human lower urinary tract. Agglutination of guinea pig erythrocytes in the absence of mannose is an important characteristic of type 1 pili[3,4]. Besides *Escherichia coli* (*E. coli*), type 1 pili are found on several other species within the *Enterobacteriaceae* family[5]. The role of type 1 piliated UPEC cells in the pathogenesis of human urinary tract infections was first demonstrated in the early 1980s and has continued in more recent studies[6-12]. Moreover, these human patient studies have been supported by several murine urinary tract infection model studies that have shown the importance of type 1 pili in UPEC pathogenesis[11,13-15]. This culminated in a study by Connell *et al*[16], who compared a *fimA* mutant strain to the wild-type parent to show the critical role of type 1 pili in UPEC colonization of the lower urinary tract.

GENETIC ORGANIZATION OF THE UPEC *fim* OPERON

Type 1 pili are produced from a contiguous DNA segment, labeled the *fim* operon, which encodes the genes necessary for their synthesis, assembly, and regulation. The *fim* cluster was mapped to the 98 min on the *E. coli* chromosome[17]. Nine genes have now been identified within the gene cluster (Figure 1).

The pilin structural gene, *fimA*, encodes a 158-159 amino acid polypeptide with an approximate molecular weight of 17 kDa[18,19]. Immediately upstream of the *fimA* gene is a 314-bp invertible DNA element called *fimS*, which contains the promoter for *fimA* with 9 bp inverted repeats (IRs) flanking this segment of DNA (5' TTGGGGGCCA), labeled IRL and IRR (Figure 1)[20,21]. The *fimA* promoter sequence undergoes site-specific recombination, positioning the invertible element in either the Phase-ON (piliated phenotype) or Phase-OFF (nonpiliated phenotype) orientation. This switching phenomenon is known as phase variation. Two genes upstream of the *fimS* invertible element, *fimB* and *fimE*, encode proteins thought to be involved in positioning the *fimS* DNA and will be discussed further below.

The *fimI* gene was the last gene within the *fim* operon to be characterized[22]. FimI's function is not known. Within the *fim* gene cluster, there are two additional genes involved in transport and assembly of type 1 pili: *fimC* and *fimD*. FimC is a periplasmic chaperone protein[23-25] that helps translocate the fimbrial proteins through the periplasm until the FimC-Fim protein complex reaches the FimD usher. FimD is an integral outer membrane protein that serves as an usher, allowing surface localization of the nascently forming type 1 pilus[26-28].

Although the FimA monomers comprise the bulk of the type 1 pilus structure, FimA does not mediate binding to the mannose containing receptor. An adhesin, encoded by the *fimH* gene, is responsible for this binding[29-33]. The two remaining genes in the *fim* operon are *fimF* and *fimG*. FimF and FimG are associated with FimH adhesin, forming a fibrillum structure that anchors the adhesin to the pilus shaft and controls the length of the type 1 pilus[29,30,34-37].

PHASE VARIATION'S ROLE IN TYPE 1 PILUS EXPRESSION

Phase variation is a reversible process, which, in the case of UPEC, leads to an oscillation between Phase-ON piliated cells and Phase-OFF nonpiliated cells. Using *fimA-lacZ* operon fusions, rates of 10^{-3} to 10^{-4}/cell/generation were originally calculated for type 1 pilus expression[38,39]. Phase variation results in agar and, particularly, broth cultures of UPEC to comprise a mixture of piliated and nonpiliated cells.

The site-specific recombination that allows phase variation to occur requires two trans-acting factors located proximally upstream of *fimS*, encoded by *fimB* and *fimE*[40]. Sequence analysis of *fimB* and *fimE* indicated that the predicted proteins were highly basic, a property of many DNA-binding proteins[41]. The predicted amino acid sequences show homology with the DNA binding domain of integrase[42] and contain a tetrad of conserved amino acids required for the recombinase activity[43-45]. Furthermore, FimB and FimE have 48% amino acid homology with each other[40]. Klemm[40] originally suggested that FimB and FimE might act independently to switch the *fimS* element unidirectionally, either Phase-ON to Phase-OFF or *vice versa*, *via* the two 9 bp invertible repeat elements, IRL and IRR. FimB can bind to the *fimS* element to either switch from Phase-ON to Phase-OFF or vice versa, with a slight bias towards the Phase-OFF over the Phase-ON orientation (Figure 2)[46-56]. By contrast, FimE binds to switch *fimS* from Phase-ON to Phase-OFF. In rare cases, FimE has been shown to initiate a Phase-OFF to Phase-ON switch[57] or when specific amino acid substitutions are made[45]. Orientation of the *fimS* element in the Phase-OFF position leads to the production of antisense transcripts from the *fimA* promoter[49,58].

FimB-mediated recombination occurs at the rate of 10^{-3} to 10^{-4} per cell per generation that was originally described; however, FimE-mediated switching occurs more often at a frequency of 0.3 per cell per generation[52,59]. Base substitutions within *fimS* demonstrated that FimB and FimE used the same DNA cleavage and religation sites within IRL and IRR, allowing more DNA base variations for FimB than FimE[60]. When *fimB* and *fimE* were provided in *trans* on plasmids, they affected pilin expression, suggesting that the ratio of FimB and FimE is important.

The promoters for both *fimB* and *fimE* have been mapped[61-63]. For the *fimB* gene, the number of promoters varies between one and three. Promoters P1 and P2, which were mapped by Schwan *et al*[63] in two UPEC strains (Figure 1), were confirmed by another group[61]. A potential third *fimB* promoter was also identified by Schwan *et al*[63], approximately 650 bp upstream of the *fimB* P2 promoter, and around 840 bp upstream of the translational start site of *fimB*. This third *fimB* promoter has not been confirmed by other groups and could be an anomaly. It could also be a third *fimB* promoter connected to sialic acid regulation of *fimB* (see below). Certainly, strain differences could explain the different numbers of *fimB* promoters. Only one promoter has been identified for the *fimE* gene[62].

OTHER CO-FACTOR PROTEINS THAT AFFECT PHASE SWITCHING

Besides the *fim* gene cluster, other genes and their gene products contribute to the expression of type 1 pili. Early work mapped a gene, *pilG*, at 27 min on the *E. coli* chromosome that affected inversion of the *fimS* region[21]. A mutation of the *pilG* gene increased the inversion of the *fimS* region by up to 100-fold as measured with a *fimA-lac* fusion[21]. The *pilG* locus was shown to be allelic to

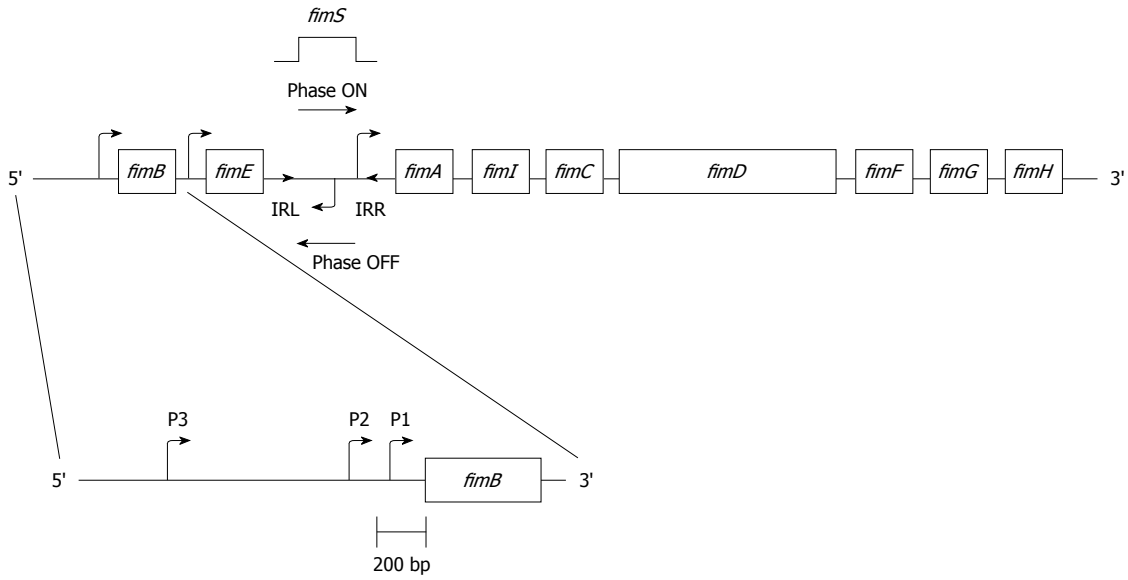

Figure 1 Schematic of the *fim* operon, including the characterized promoter sites.

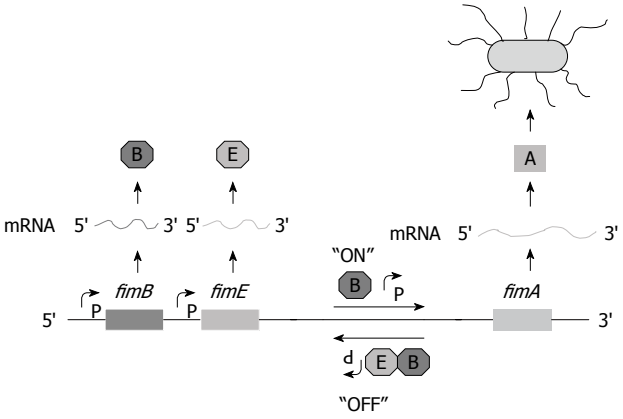

Figure 2 A schematic showing how the FimB and FimE proteins orient the *fimS* element.

bglY[64], *drdX*[65], and *osmZ*[66]. Later, it was determined that the *pilG* and *osmZ* genes were in fact alleles of the *hns* gene[66-68]. The *hns* gene encodes the H-NS global regulatory protein[69].

H-NS possibly controls the phase variation of the *fimS* region both directly and indirectly[61,62,70-74]. For a potential direct effect, H-NS binds to sequences adjacent to the *fimS* invertible element[72,75].

Indirectly, H-NS represses the transcription of both *fimB* and *fimE*[62,71,74]. H-NS binds, with a high degree of specificity, to both the P1 and P2 promoter sites for *fimB*[71,72]. The DNA-binding regulatory protein also binds to the *fimE* promoter[71]. Moreover, H-NS also represses *lrp* transcription[76], which would in turn affect the phase switching of the *fimS* element, as described below. Thus, transcriptional repression of the *fimB* and *fimE* site-specific recombinase genes would indirectly influence the position of the *fimS* element, which would indirectly affect phase variation.

Besides H-NS, integration host factor (IHF) and leucine-responsive protein (Lrp) are additional co-factors that affect type 1 pilus phase variation. Both proteins cause sharp bends in the DNA structure, introducing hairpin loops that facilitate recombination events within UPEC. IHF is a two-component protein consisting of IHF encoded by *ihfA*[77] and IHF encoded by *ihfB*[78]. Both Eisenstein *et al*[42] and Dorman *et al*[43] showed that IHF plays a role in type 1 pilus switching. Mutations in either *ihfA* or *ihfB* locked the *fimS* region in either the Phase-OFF or Phase-ON orientation[79]. In both studies, an IHF binding site (IHF Ⅱ) proximal to IRR was identified (Figure 3). In addition, an IHF binding site was also identified between IRL and the 3' end of *fimE* (IHF Ⅰ)[80]. A mutational analysis of this IHF I site demonstrated that FimB-mediated recombination was more adversely affected, suggesting a directional bias for FimB recombination[73,75,79,81,82].

The leucine-responsive regulatory protein (Lrp) is another protein that has been shown to affect the *fimS* region. Lrp is a global regulator of genes involved in metabolic functions within *E. coli*, including pili synthesis[83]. Mutations of the *lrp* gene cause a lower frequency of recombination of the *fimS* element[80,84]. Lrp binds to three distinct sites within the fimS element that are closer to the IRL site. When the high affinity sites 1 and 2 are mutated, the recombination frequency declines[79,85]. Lrp binding to the low affinity site 3 inhibits recombination[86,87]. Lrp and IHF can bend the *fimS* DNA; therefore, they would allow the proper positioning of IRL and IRR that facilitates recombination[80,87]. The levels of specific amino acids will also affect Lrp binding to the *fimS* element and subsequently phase variation[86]. Lrp binding causes an orientational bias to the *fimS* element. When neither Lrp nor IHF are present at sufficient levels, H-NS will bind and maintain the Phase-OFF orientation[88]. Although Lrp binds to multiple sites within the *fimS* element, Lrp directly regulates neither *fimB* nor *fimE*.

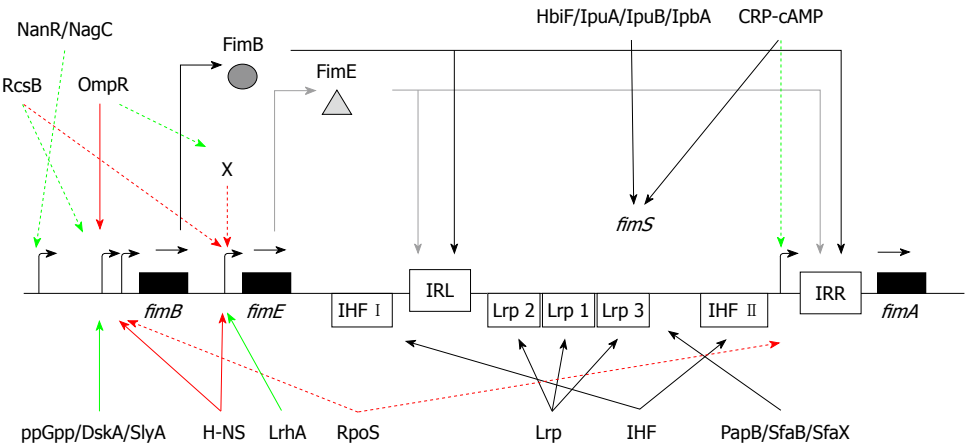

Figure 3 Schematic model of the actions of 20 auxiliary proteins on the regulation of type 1 pili. The inverted repeat left and right (IRL and IRR) are shown as open boxes. Binding sites for integration host factor (IHF I and II) and leucine-responsive protein (Lrp1, 2, and 3) are also represented as open boxes. Genes are displayed as black boxes and the promoters are shown as bent black arrows. The dark gray arrows correspond to FimB and the light gray arrows are for FimE. Black arrows signify an effect on the *fimS* element. Solid green arrows indicate confirmed binding associated with stimulatory effects, whereas dashed green arrows indicate presumed stimulatory effects. Solid red arrows indicate confirmed binding associated with repressing effects, whereas dashed red arrows indicate presumed repressing effects.

Another protein that regulates type 1 pilus expression is the LysR-type regulator, LrhA[89]. LrhA was first identified to be associated with RpoS degradation[90]. Microarray analysis of mRNA populations from an *lrhA* mutant *vs* wild-type bacteria revealed increased expression of the *fimAICDFGH* operon. Purified LrhA protein bound to the promoter regions of both *fimB* and *fimE*; however, there was higher affinity for the *fimE* promoter. The use of *fimB*- or *fimE-lacZ* translational fusions indicated there was a greater effect with the *fimE-lacZ* fusion. Thus, LrhA appears to activate *fimE*, which would repress type 1 pilus expression.

Three other proteins have unexplained effects on type 1 pilus expression in *E. coli*: OmpX, IbeA, and IbeT. Inactivation of *ompX*, encoding an outer membrane protein OmpX, caused an increased production of FimA[91]. A disruption caused by the loss of OmpX would change the cell surface, which would affect cell-surface interactions. It is likely that OmpX acts indirectly to regulate type 1 pilus expression. A deletion of the *ibeA* gene caused diminished type 1 pilus expression, as well as lower transcription of *fimB* and *fimE*, whereas an *ibeT* mutant was shown to have the *fimS* element preferentially in the Phase-OFF orientation[92]. How each of these proteins works to regulate the *fim* genes has not been determined.

The regulatory alarmone, ppGpp, has been connected to the regulation of multiple genes in *E. coli*, including the *fim* operon. ppGpp-deficient strains exhibited diminished type 1 pili expression compared to the wild-type strain[93]. Furthermore, primer-extension analysis indicated that ppGpp activated the *fimB* P2 promoter. A follow through study demonstrated that DskA, a cofactor required for ppGpp-mediated positive regulation of several amino acid biosynthesis promoters[94], also activated transcription from the *fimB* P2 promoter[95].

Besides FimB and FimE, there are four other site-specific recombinases that could affect phase switching of the *fimS* element: HbiF, IpuA, IpuB, and IpbA. The HbiF-mediated inversion of the *fimS* element occurs primarily from Phase-OFF to Phase-ON[96]. Constitutive expression of HbiF locked the *fimS* DNA in the Phase-ON position. The three other site-specific recombinases (IpuA, IpuB, and IpbA) were discovered by sequence analysis of the UPEC strain CFT073 genome because of their high homology with the *fimB* and *fimE* genes[97]. Both IpuA and IbpA bind to the *fimS* element and mediate phase switching. IpuA functions like FimB, allowing a Phase-OFF to Phase-ON switch as well as Phase-ON to Phase-OFF switching, whereas IpbA can switch *fimS* from Phase-OFF to Phase-ON. It is not clear under what environmental growth conditions these alternative site-specific recombinases affect the *fimS* element positioning.

Also linked to the *fimS* genetic switch are Rho and LeuX. Transcriptional termination of *fimE* was determined to be Rho-dependent, based on the use of a *rho* mutant or by treatment with bicyclomycin, an antibiotic that interferes with Rho[98,99]. Thus, when the phase switch is in the Phase-OFF position, there is a Rho-dependent termination of the *fimE* sense transcript, leading to a truncated, unstable mRNA that is readily degraded. Less FimE site-specific recombinase would allow FimB to bind and switch the *fimS* element to the Phase-ON position. The minor leucyl tRNA, LeuX, affects the *fimS* element switching from Phase-OFF to Phase-ON[100,101]. Placing the *leuX* gene on a multicopy plasmid caused greater expression from the *fimAICDFGH* operon[102].

All of the studies examining *fimB* regulation described above have concentrated on the P1 and P2 promoter regions. However, several other studies have shown that the intergenic region between the *yjhATS* operon and the *fimB* gene also plays a role in genetic regulation of *fimB*[103-105]. Sialic acid and N-acetylglucosamine inhibit the FimB recombinase. Two proteins, NagC (a N-acetylglucosamine-6P-responsive protein) and NanR

(a sialic acid-responsive protein), linked to sialic acid and N-acetylglucosamine catabolism[106,107], bind to two deoxyadenosine methylation sites within the intergenic region[103-105] that align with P3 *fimB* promoter described earlier[58]. In addition, NagC also binds to an operator site 212 bp closer to the *fimB* translational start site[105]. Both proteins are thought to act as antirepressors that allow *fimB* transcription to occur[103]. However, a urinary tract infection caused by type 1 piliated UPEC will elicit an inflammatory response[108], leading to increased levels of both sialic acid and N-acetylglucosamine that will, in turn, activate some cis-active regulatory protein that shuts off *fimB* transcription.

Regulatory proteins for other pilus systems can also regulate type 1 pilus expression through a cross-talk mechanism. PapB, which affects the phase variation of the pyelonephritis associated pilus (*pap*) operon[109,110], also regulates the orientation of the *fimS* element[111-113]. In contrast to FimB, PapB inhibits the Phase-OFF to Phase-ON switching. Two proteins associated with S pili, SfaB and SfaX, also have a negative effect on Phase-OFF to Phase-ON switching[111,114]. Thus, there appears to be an expression competition between the different pilus operons. These regulatory proteins that allow expression of other types of pili in other environments counter the need for type 1 pili under growth conditions where type 1 pili are not needed.

In stationary phase-grown *E. coli* cells, type 1 pilus expression is diminished compared to logarithmic grown cells. The alternative sigma factor, RpoS, which is activated during stationary phase, represses *fimB* transcription[115]. Another regulatory signal active in a logarithmic phase culture may be provided by glucose acting as a catabolite repressor by increasing internal cAMP concentrations, which allow for greater interactions with its receptor protein, CRP[116]. For type 1 pilus expression, the role of cAMP and glucose is opaque. Early studies indicated that cAMP affected pilus expression in some strains of *E. coli*[117] and in *cya* (adenyl cyclase) mutants of *Salmonella enterica* serovar Typhimurium[118]. However, in a later study, glucose had no effect on pilus expression, even when added with exogenous cAMP or when tested in adenlyate cyclase mutants[119]. Unfortunately, some of the early work was done with the CSH50 strain of *E. coli*, which has a *fimE*::IS1 mutation[52], so the role of catabolite repression remained unclear, until recently. Using a more defined system, Müller *et al*[120] have shown that CRP-cAMP directly represses the *fimA* promoter and indirectly affects phase variation by limiting the switch from Phase-OFF to Phase-ON in a logarithmic stage population.

Two other proteins that activate *fimB* transcription are RcsB and SlyA. RcsB is part of the RcsC/RcsB two-component phosphorelay regulatory system[121]. Using an *rcsB* mutant, it was shown that under neutral pH/low osmolality growth conditions, RcsB appears to activate *fimB*[122]. Growth in an acidic environment did not affect *fimB* expression in the *rcsB* strain compared to wild-type cells. Recently, the SlyA global regulator was implicated in *fimB* gene activation[123], but the growth conditions that would favor *slyA* expression were not determined.

The last accessory protein with relevance to *fim* gene regulation is OmpR. OmpR is part of the EnvZ/OmpR two-component regulatory system that regulates genes under an osmotic stress[124]. A study by Schwan *et al*[74] found that an *ompR* mutant strain had de-repressed transcription of *fimB* and *fimE* compared to wild-type cells. More recently, they found that unphosphorylated OmpR bound to the P2 promoter of *fimB* to repress *fimB* transcription[125] (Rentchler, Lovrich, and Schwan, manuscript submitted). However, through DNase I footprinting analysis, neither unphosphorylated nor phosphorylated OmpR bound directly to the *fimE* promoter, suggesting another regulatory element that is regulated by OmpR-P would directly affect *fimE* transcription.

Thus, in addition to FimB and FimE, approximately 20 different auxiliary proteins have a role to play in the regulation of one or more *fim* genes or positioning the *fimS* element. These 20 proteins are represented in a schematic model shown in Figure 3. Some of the proteins repress *fim* gene expression (e.g. H-NS, OmpR, RpoS), whereas others appear to activate *fim* gene expression (e.g. DskA, LrhA, NagC, NanR, RcsB, SlyA). How some of these proteins may affect UPEC type 1 pilus expression during the course of a human or murine urinary tract infection is described below.

ENVIRONMENTAL SIGNALS WITHIN THE URINARY TRACT AFFECTING UPEC TYPE 1 PILUS EXPRESSION

The human or murine urinary tract is a dynamic environment. In the lower urinary tract, there are ample mannose receptors for FimH-mediated attachment of type 1 piliated UPEC cells[126]. The temperature in the urinary tract is around 37℃. Although one group showed Phase-OFF to Phase-ON switching increased at lower temperatures, others have demonstrated that the *fimA* promoter element is biased in its switch from the Phase-ON to the Phase-OFF orientation in broth cultures grown at 20℃, but the switch favors FimB recombination at 37℃[59,71,127]. More recently, Kuwahara *et al*[128] demonstrated that FimB-mediated recombination could be linked to a controlled downregulation of the Phase-ON to Phase-OFF switching rate based on a temperature-dependent suppression of the interplay of the FimE recombinase.

When the UPEC cells move from the vaginal surface, which has only a slightly acidic pH/low osmolality environment, to the urethra or ascend to the bladder, there is a switch to a moderate acidic pH/moderate to high osmolality environment[129,130]. Under the slightly acidic pH/low salt growth conditions found on the vaginal surface, proteins such as SlyA or RcsB may activate *fimB* and prevent H-NS from binding, allowing type 1 pili to be created and presented on the surface of the UPEC cells for attachment. When the bacteria move from the

exterior opening of the urinary tract and ascend the urethra to the bladder, an acidic pH/moderate osmolality environment is encountered in the bladder[129,130]. A preliminary study implied that an acid tolerance system-induced protein is involved in the regulation of several *fim* genes (Schwan WR, unpublished results), which may begin to turn off the *fim* operon. Furthermore, a change in the osmolality would activate the EnvZ/OmpR two-component regulatory system, allowing OmpR to repress *fimB* transcription[74,125].

UPEC infections are ascending infections[13,131]; therefore, the presence of flagella on the UPEC cells would allow the bacteria to ascend to the kidneys. Expression of the flagella may coordinately turn off expression of the type 1 pili[132,133]. As the bacteria ascend to the kidneys, the pH would drop further and the osmolality would increase. OmpR becomes phosphorylated and activates an unknown gene whose gene product in turn potentially shuts down not only *fimB*, but also *fimE* expression. Moreover, H-NS may bind and repress both *fimB* and *fimE* at this time. This would lock the *fimS* element in the Phase-OFF position, creating nonpiliated UPEC cells. Furthermore, as the young *E. coli* population matures and moves into stationary phase, they trigger transcriptional activation of the *rpoS* gene. The acidic/high osmolality environment would cause greater translation of the *rpoS* transcripts[134], leading to more RpoS protein for repression of *fimB* transcription.

CONCLUSION

Several strains of UPEC have been shown to become nonpiliated in the murine kidney over time[13,135]. There are very few mannose receptors in human or murine kidneys[136,137] and the innate immune system is more apt to target type 1 piliated bacteria[138]; therefore, the regulatory loss of type 1 pili on UPEC cells in the human kidney would be an evolutionary advantage for these bacteria. Thus, the ability to phase vary their type 1 pilus expression offers several advantages to the UPEC. On vaginal surfaces, the outer rim of the urinary tract, and within the urethra and bladder, type 1-piliated cells benefit the bacteria because there are ample mannose receptors. When the bacteria ascend into the kidneys, the growth environment may turn off expression of an unneeded external surface structure that may target the bacteria for elimination by the host's innate defenses.

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Santa Fe, New Mexico, United States

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Cyber Security for Government Asia 2012
Kuala Lumpur, Malaysia

January 18-20, 2012
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Bangkok, Bangkok, Thailand

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International Congress on Malaria Elimination (ICME)
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Sexual Health 2012
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May 10-13, 2012
American Conference for the Treatment of HIV
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WJCID World Journal of Clinical Infectious Diseases

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Acknowledgments

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Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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