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MINIREVIEW

Is There Potential for Repurposing Statins as Novel Antimicrobials?

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Statins are members of a class of pharmaceutical widely used to reduce high levels of serum cholesterol. In addition, statins have so-called "pleiotropic effects," which include inflammation reduction, immunomodulation, and antimicrobial effects. An increasing number of studies are emerging which detail the attenuation of bacterial growth and *in vitro* and *in vivo* virulence by statin treatment. In this review, we describe the current information available concerning the effects of statins on bacterial infections and provide insight regarding the potential use of these compounds as antimicrobial therapeutic agents.

One of the major undisputed clinical breakthroughs of the 20th century was the discovery of the statin family of drugs. These compounds are renowned for their ability to lower cholesterol levels and are used to treat approximately 40 million individuals with high cholesterol levels worldwide. Since the discovery of mevastatin as a metabolic product of *Penicillium citrinum* in 1976 (1, 2), a total of nine statins have been characterized, seven of which are approved by the FDA to treat patients with high cholesterol. Structurally, statins are characterized by the presence of a conserved lactone ring (3). This structure is present as a hydrolyzed (active) form in all statins except for mevastatin, lovastatin, and simvastatin; in those statins, the lactone ring is hydrolyzed in the liver (4). Statins can be divided into two broad classes (Fig. 1). Type 1 statins are lipophilic and possess a butyryl side chain—they are said to structurally resemble mevastatin (3). Lovastatin, pravastatin, and simvastatin are type 1 statins. Type 2 statins are classically lipophobic and are distinguished from type 1 by the replacement of the butyryl side chain with a fluorophenol group and typically possess larger side chains than type 1 statins (3). Atorvastatin, cerivastatin, fluvastatin, pitavastatin, and rosuvastatin are type 2 statins.

Statins exert their cholesterol-lowering effect by binding to the active site of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (HMGR), a rate-limiting enzyme involved in cholesterol biosynthesis (3). HMGR is an integral part of the mevalonate pathway, which not only is essential for cholesterol biosynthesis but also contributes to the production of isoprenoids, lipid compounds that are essential for cell signaling and structure. As well as inhibition of cholesterol, statins have also been found to have a number of cholesterol-independent, so-called "pleiotropic" effects. Statins have been reported to confer anti-inflammatory, immunomodulatory, and anticancer effects on host cells, and these effects are well characterized (5–9). Furthermore, several studies have explored the pleiotropic effects of statins in combating multisystem microbial infections, such as sepsis and pneumonia, and a growing number of studies are demonstrating that statins can directly influence the growth and virulence of bacterial pathogens. With the global increase in antibiotic resistance to existing antibiotics and the search for new antimicrobial strategies reaching a critical stage, there is increasing interest in the possibility of repurposing existing drugs that have already been approved to treat different clinical conditions but that also possess antimicrobial activity. The repurposing of these drugs would significantly reduce the lead time from bench to bedside. Given their pleiotropic activities, statins are strong potential candidates to be repurposed as novel antimicrobial agents. However, the evidence for this remains controversial owing to the number of apparently contradictory studies. This review evaluates and discusses the effects of individual statins on bacterial growth and virulence and bacterial infections in the context of pathogen-host interactomes (summarized in Fig. 2).

CLINICAL EVIDENCE THAT STATINS INFLUENCE MORBIDITY AND MORTALITY OF PATIENTS WITH MICROBIAL INFECTIONS

The clinical potential of statins as antimicrobial agents has been the subject of several studies and reviews. A number of meta-analyses of cohort studies on the impact of overall statin use on different infection outcomes showed positive findings, albeit while highlighting the limitations and heterogeneity of the studies (10–13). These reviews included studies on infections such as bacteremia, pneumonia, sepsis, and some acute infections, and the patient populations received several different statins. For instance, two single-center retrospective studies showed that patients with bacteremia who had undergone prior statin treatment have significantly decreased risks of in-hospital mortality of 6% versus 28% ($P = 0.002$) and 13% versus 24% ($P = 0.001$), respectively (14, 15). The latter study also showed that there was an inverse correlation between the duration of statin treatment and the risk of mortality in comparisons of statin use ≥12 and <12 weeks prior to infection (11% versus 14%, $P = 0.04$) (15). A meta-analysis of available published data found that the use of statins was specifically associated with a reduced risk of morbidity and mortality resulting from pneumonia (12). A retrospective study of patients in the United Kingdom found that current statin treatment (i.e., treatment administered within the previous 30 days) reduced pneumonia-associated mortality (adjusted odds ratio [OR], 0.47; 95% confidence interval [CI], 0.25 to 0.88) (16), while prior statin treatment also reduced mortality rates in patients in the United

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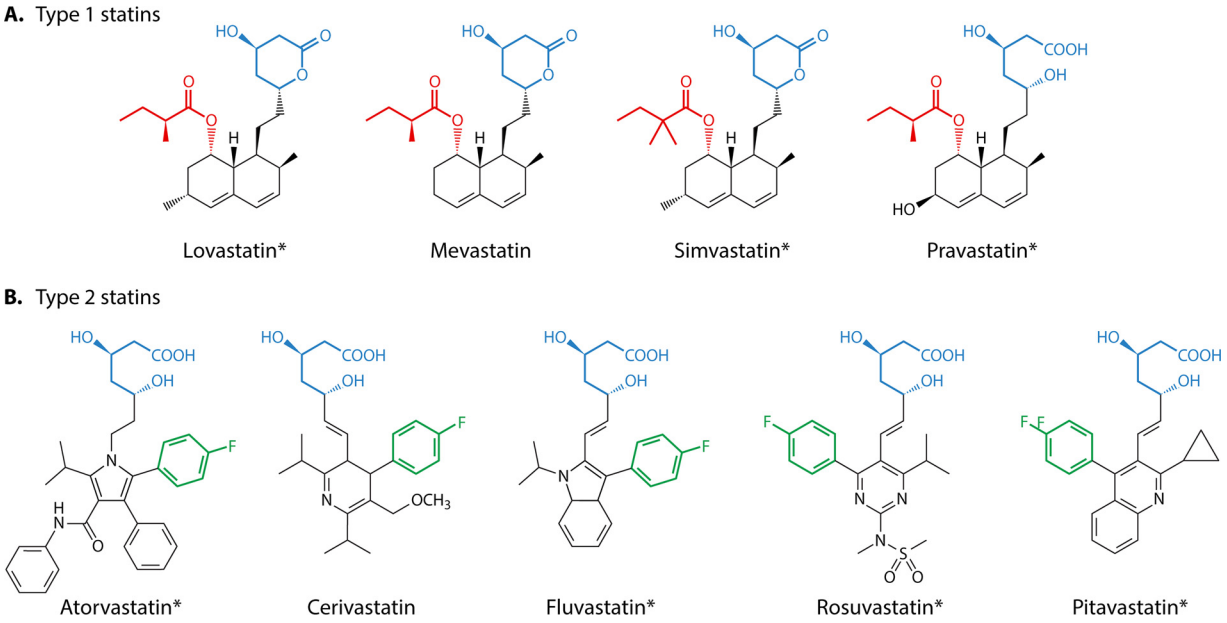

FIG 1 Chemical structures of statins. (A) Type 1 statins are characterized by a conserved lactone ring (blue), a decahydronaphthalene (Decalin) structure (black), and a butyryl side chain which is different in each statin (red). (B) Type 2 statins differ from type 1 statins due to the replacement of the butyryl side chain with a fluorophenyl group (green), and although the lactone ring structure is conserved in all statins, the decahydronaphthalene group of type 1 statins is replaced by a longer distinct side chain. Statins marked with an asterisk (*) are licensed for treatment of high cholesterol.

States with community-acquired pneumonia (CAP) (adjusted OR, 0.36; 95% CI, 0.14 to 0.92) (17). Furthermore, data from the Justification for the Use of Statin in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) study, which was initially undertaken to determine whether rosuvastatin could reduce the risk of cardiac disease in people without hyperlipidemia (18), were retrospectively analyzed in 2012. The results of the analysis suggested that rosuvastatin treatment may decrease the occurrence of pneumonia before (hazard ratio [HR], 0.81; 95% CI, 0.67 to 0.97) or after (HR, 0.83; 95% CI, 0.69 to 1.00) a cardiac event (19). In contrast, however, an earlier prospective cohort study which examined adults in six Canadian hospitals had concluded that, after adjusting for confounding factors such as the "healthy user effect," prior statin treatment does not result in reduced mortality from pneumonia (20). The latter study encompassed 3,415 patients >17 years of age with pneumonia admitted to a hospital, while the JUPITER randomized, double-blind, placebo-controlled trial of 17,802 healthy patients was restricted to men >50

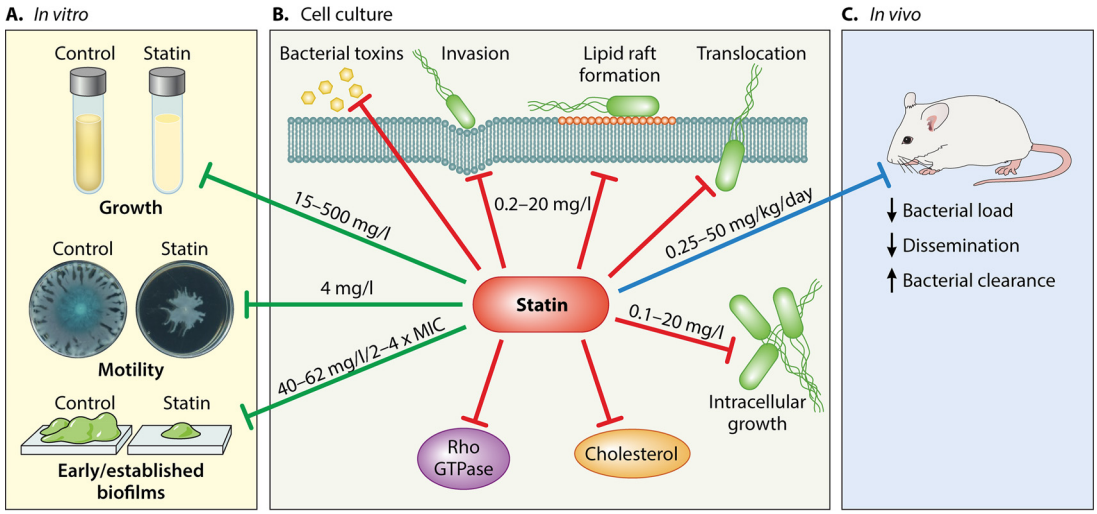

FIG 2 Statins modulate bacterial growth and virulence. (A) *In vitro* effects of statins on bacterial species. Statins reduce *in vitro* bacterial growth, motility, and attachment. (B) Key antivirulence mechanisms of statins. At physiological concentrations, statin treatment can reduce bacterial invasion and translocation in addition to inhibiting lipid raft production. The inhibition of Rho GTPase activity and cholesterol production by statins contributes to reduced bacterial virulence, decreased toxicity, and impaired intracellular survival. (C) At physiological concentrations, statin treatment can reduce bacterial load and dissemination and increase bacterial clearance in mouse models of infection.

and women >60 years of age. Indeed, the JUPITER study was designed to address the healthy user effect, suggesting that the differences in study design, in addition to age differences between the cohorts, may underpin the contrasting observations.

Sepsis is a serious infection-induced whole-body inflammatory state, and, due to the immunomodulatory activity of statins, several studies have been carried out to evaluate the benefit of statin therapy in the prevention or treatment of the disease. While the type, design, size, and measured outcomes of the studies have been varied and overall results conflicting, in recent years extensive reviews evaluating these clinical studies have been published (21–27). The majority of clinical studies to date have been retrospective cohort studies evaluating the impact of prior treatment with statins on disease progression and mortality. Many of these, plus several meta-analysis reviews, showed promising results in which prior use of statins significantly reduced disease progression and/or mortality associated with sepsis (25, 28–32). For instance, studies by Almog et al. and Martin et al. demonstrated a reduced risk of developing severe sepsis in patients pretreated with statins (2.4% versus 19% [$P < 0.001$] and 56% versus 86% [$P < 0.02$], respectively), while Mortensen et al. showed a reduced risk of 30-day mortality in patients using statins (OR, 0.48; 95% CI, 0.36 to 0.64). One of the main limitations attributed to these studies was limited sample size, and, to address this limitation, a recent population-based, propensity score-matched analysis of the effects of low and high doses of statins on sepsis outcomes was performed that involved a cohort of 27,792 statin users compared with an equal number of nonusers (33). That extensive study demonstrated a significant reduction of 1-year mortality (HR, 0.83; 95% CI, 0.81 to 0.85) and of adverse consequences of sepsis such as in-hospital death (OR, 0.86; 95% CI, 0.83 to 0.89) and intensive care unit (ICU) admission (OR, 0.95; 95% CI, 0.92 to 0.98) in patients pretreated with statins. The results also showed that the benefits of pretreatment with statins increased significantly with higher doses.

Therefore, several studies have shown the promising potential of the prior use of statins in reducing the initiation or progression of infections. Nevertheless, it is difficult to draw conclusions on whether the effects of these statins were directly antibacterial/anti-inflammatory or were due to pleitropic effects on comorbidities associated with the infections. For example, it is estimated that cardiovascular events account for up to 30% of deaths in patients with CAP and it could therefore be argued that prior statin use could improve cardiovascular health and thus reduce mortality rather than having any direct effect on the infection. Against this, a study indicated that, while prior statin use was significantly associated with decreased 90-day mortality in CAP patients, there was no significant association with cardiovascular events (34). In order to fully understand the mechanistic effects of prior statin use on infections, similar studies targeting, for example, specific comorbidities and/or inflammatory markers would be required.

In contrast to prior use of statins, however, studies investigating the benefits of *de novo* treatment of infections with statins have generally not shown favorable results. A recent randomized control trial (RCT) investigating the effect of rosuvastatin on the clinical outcome of patients with sepsis-associated acute respiratory distress syndrome was discontinued because of futility (35). Moreover, a number of recent meta-analyses of RCTs suggested that there is no significant evidence to suggest that statin use improves the mortality outcome of patients with sepsis (25–27).

Further large-scale RCT research is also recommended to evaluate the efficacy of using *de novo* statin therapy to treat specific infections. Of particular note is that the majority of the studies reviewed so far did not adjust for the type of statin used or the type of bacteria causing the infection. An interesting study of the effect of prior statin use on mortality in patients with bloodstream infections found a significant reduction in 90-day mortality in statin users with Gram-negative infections (adjusted OR, 0.38; 95% CI, 0.20 to 0.72; $P = 0.003$) but no significant difference in statin users with Gram-positive infections (adjusted OR, 1.22; 95% CI, 0.69 to 2.17; $P = 0.49$) (36), suggesting that the type of bacterial infection may be a significant factor.

EFFECTS OF STATINS ON *IN VITRO* BACTERIAL GROWTH

There is a large body of evidence demonstrating that statins have direct antibacterial effects on the *in vitro* growth of both Gram-positive and Gram-negative bacterial pathogens responsible for a wide range of infections (Table 1), although there have been conflicting reports on MICs (ranging from 15 mg/liter to 500 mg/liter), and strain specificity may be a factor (Table 1). The growth of the Gram-positive nosocomial pathogens *Staphylococcus aureus* and *Streptococcus pneumoniae* has been shown to be inhibited by atorvastatin, rosuvastatin, and simvastatin (37–43), while fluvastatin has also been reported to inhibit the growth of *S. aureus* (37). In addition, both type 1 (simvastatin) and type 2 (atorvastatin, fluvastatin, and rosuvastatin) statins have demonstrated a bacteriostatic effect against other Gram-positive cocci, notably, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, *Enterococcus*, and *Bacillus* spp. (37–39, 41). Promisingly, simvastatin, lovastatin, and rosuvastatin have also been shown to have antibacterial effects on the growth of antibiotic-resistant species such as methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *S. aureus* (VRSA), and vancomycin-resistant enterococci (VRE), although the MICs are typically higher than against antibiotic-sensitive strains (Table 1) (37–42).

Both type 1 and type 2 statins have also been found to inhibit the growth of a number of clinically important Gram-negative species, including several respiratory pathogens. The growth of the nosocomial respiratory pathogens *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* is inhibited by atorvastatin, rosuvastatin, and simvastatin (MICs ranging from 15 to 333 mg/liter) (38, 39), and simvastatin was reportedly bactericidal against *Moraxella catarrhalis* (MIC, 15 mg/liter) (43). In addition to respiratory pathogens, statins have also been reported to inhibit other Gram-negative nosocomial pathogens. Masadeh et al. reported that atorvastatin, rosuvastatin, and simvastatin have bacteriostatic effects on a range of pathogens, including *Citrobacter freundii*, *Enterobacter aerogenes*, *Haemophilus influenzae*, and *Proteus mirabilis* (MICs ranging from 15 to 166 mg/liter) (38). Simvastatin and lovastatin (10 mg/liter) are also reportedly bactericidal against the spirochete *Borrelia burgdorferi* (the causative agent of Lyme disease) (45), and atorvastatin, rosuvastatin, and simvastatin were found to inhibit the growth of *Escherichia coli*, a prominent cause of gastroenteritis and urinary tract infections (38). In contrast, however, Bergman et al., using a maximum concentration of 250 mg/liter, observed that simvastatin did not inhibit the growth of *H. influenzae* (43), while Graziano et al. found that simvastatin, atorvastatin, and pravastatin at concentrations up to 250 mg/liter did not inhibit the growth of *P. aeruginosa*, *E. coli*, or *Enterococcus faecalis* (42). Furthermore, a study by Thangamani et al. (41) indicated that, while the growth of Gram-

TABLE 1 MIC of statins against Gram-positive and Gram-negative bacteria

Bacterium	Statin MIC (mg/liter)[a]					Reference(s)
	Sim	Fluv	Ator	Ros	Prav	
Gram positive						
S. aureus						
MSSA	16 to 63	~200	42 to >250	208 to 342	>250	37–42
MSSA clinical isolate	60.42	nt	52.08	341.67	nt	38
MRSA	32 to 167	~250 to >1,024	83 to >1,024	100 to >1,024	>250 to >1,024	37–39, 41, 42
MRSA clinical isolate	116.67	nt	108.33	500	nt	38
VISA group of strains	32	nt	nt	nt	nt	41
VRSA group of strains	32 to 64	nt	nt	nt	nt	41
S. epidermidis						
Type strains	26 to 32	nt	21	167	nt	38, 41
Clinical isolate	35	nt	20	233	nt	38
S. pneumoniae						
Type strains	16 to 167	>123	104	333	>50	38, 41, 43
Clinical isolate	292	nt	229	417	nt	38
Enterococci						
VSE	50 to 52	300	83 to 250	100 to 333	nt	37–39
VSE clinical isolate	292	nt	96	333	nt	38
VRE	30 to 104	500	167 to 250	100 to 500	nt	37–39
VRE clinical isolate	292	nt	217	500	nt	38
E. faecalis group of strains	32	nt	nt	nt	nt	41
S. pyogenes						
ATTC19615	62.5	nt	83.33	166.67	nt	38
Clinical isolate	146	nt	133.33	275	nt	38
L. monocytogenes						
Group of strains	32	nt	nt	nt	nt	41
B. anthracis						
Type strains	16	nt	nt	nt	nt	41
Gram negative						
H. influenzae						
Clinical isolate	146 to >250	nt	104	367	nt	38, 43
ATTC29247	52	nt	83	167	nt	38
M. catarrhalis						
Clinical isolate	16	nt	nt	nt	nt	43
E. coli						
Type strains	52 to >250	nt	26 to >250	104	>250	38, 39, 42
O157:H7 ATCC 700728	>256	nt	nt	nt	nt	41
Clinical isolate	112	nt	100	125	nt	38
P. aeruginosa						
Type strains	166 to >1,024	>1,024	83 to >1,024	100 to >1,024	>250 to >1,024	38, 39, 41, 42, 67
Clinical isolate	121	nt	96	292	nt	38
K. pneumoniae						
Type strains	167 to >256	nt	167	333	nt	38, 41
Clinical isolate	242	nt	217	258	nt	38
A. baumannii						
Type strains	104 to >256	nt	16	333	nt	38, 41
Clinical isolate	32	nt	22	300	nt	38
C. freundii						
ATTC 8090	52	nt	83	167	nt	38
Clinical isolate	133	nt	108	333	nt	38
E. aerogenes						
ATTC 29751	26	nt	16	104	nt	38
Clinical isolate	33	nt	20	183	nt	38
P. mirabilis						
ATTC 12459	167	nt	63	250	nt	38
Clinical isolate	146	nt	133	275	nt	38
S. Typhimurium						
ATCC 700720	>256	nt	nt	nt	nt	41

[a] Sim, simvastatin; Fluv, fluvastatin; Ator, atorvastatin; Ros, rosuvastatin; Prav, pravastatin; VISA, vancomycin-intermediate *Staphylococcus aureus*; VSE, vancomycin-sensitive enterococci; nt, not tested.

positive species was inhibited by statins, the growth of *P. aeruginosa* ATCC 15442 was not inhibited by simvastatin, atorvastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, or rosuvastatin. They also reported that simvastatin did not inhibit the growth of a range of other Gram-negative pathogens, including different strains of *P. aeruginosa*, *K. pneumoniae*, *A. baumannii*, *E. coli*, and *Salmonella enterica* serovar Typhimurium. Interestingly, they did show that, in combination with subinhibitory concentrations of colistin, which compromises outer membrane integrity, simvastatin had antibacterial activity against the range of Gram-negative pathogens at MICs of 8 to 32 mg/liter. While the activity shown by simvastatin against *E. coli* ATCC 35218 (38) is in direct contrast to the lack of activity of simvastatin against *E. coli* ATCC 35150, ATCC 700728, ATCC 25922, and ATCC 10536 (41, 42), it is worth noting that the *E. coli* ATCC 3218 assays were performed on solid agar while the other studies were performed using the broth microdilution method, perhaps explaining the apparent differences in activity.

Taken together, the data suggest that the antibacterial activity of statins may be statin specific and/or strain/species specific or both. Simvastatin and atorvastatin generally appear to be more effective against *S. aureus*, *S. pneumoniae*, and enterococci than other statins (37–39, 43), while three distinct simvastatin MICs were reported against *S. aureus* clinical isolates from the United Kingdom and Jordan as well as against typed reference strains (Table 1) (37, 38, 41, 42). It is also noteworthy that, while the MICs of statins varied according to the statin and pathogen tested, the *in vitro* MICs ranged from circa 15 to 400 mg/liter, far exceeding the range of typical peak plasma concentrations of patients on oral statins, which generally range from circa 10 to 300 μg/liter. Moreover, in the majority of cases, the *in vitro* statin MICs against multidrug-resistant pathogens were even greater than those against equivalent antibiotic-susceptible strains. As such, at these MICs, it is unlikely that they would qualify as lead molecules in drug discovery programs. This variability in MICs could be considered somewhat unexpected for what is essentially a novel antibiotic compound being administered to a naive population. However, recent studies have reported significant phenotypic and genotypic diversity within clinical populations, suggesting that adaptation to environmental or host-related factors may be widespread (46–48). While the mechanism of action of statin antimicrobial and antivirulence activity remains to be elucidated, some reports suggest the involvement of isoprenoids and membrane integrity (49). Further deciphering the interaction between statins and the microbial membrane may provide answers to explain this apparent heterogeneity, although other targets within the microbial cell must also be considered.

However, two studies have recently demonstrated the *in vivo* clinical efficacy of locally high concentrations of statins, whereby topical applications of simvastatin at MIC/sub-MICs significantly enhanced bacterial clearance and healing of methicillin-sensitive *S. aureus* (MSSA)- and MRSA-contaminated wounds in mouse wound models (40, 41). Wang et al. showed that application of simvastatin (62.5 mg/liter) reduced the MSSA wound size by over 50% at day 7 and significantly reduced (>60% reduction) the bacterial load visible in the wound histology (40), while Thangamani et al. showed that topical simvastatin at concentrations of 1% and 3% significantly reduced the bacterial load in MRSA wounds by 75% and 90%, respectively (41). The latter study also showed that this topical application of simvastatin had an additive healing effect and that it reduced the production of proinflammatory cytokines (interleukin-6 [IL-6], tumor necrosis factor alpha [TNF-α], and IL-1β) in MRSA-infected wound lesions.

The mechanism by which statins inhibit bacterial growth is unclear. As previously described, statins inhibit the mevalonate pathway in human cells. This pathway is present in higher eukaryotes, as well as in several bacterial species, including staphylococci and streptococci. However, not all bacteria possess a mevalonate pathway, and in these species (and in plants), isoprenoid metabolism is mediated through the 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate (MEP-DOXP) pathway, which is mevalonate independent (50, 51). The MEP-DOXP and mevalonate pathways both feed into the production of isoprenoid intermediates. Generally, it appears that Gram-positive bacteria tend to possess a mevalonate pathway, while Gram-negative species utilize mevalonate-independent isoprenoid biosynthesis, although there are some exceptions to this observation. Statins have been shown to inhibit the growth of *S. aureus* by binding to and inhibiting the activity of its HMGR enzyme (52), and this may to some extent explain why Gram-positive bacteria tend to be more sensitive to statins. However, statins can attenuate the growth of bacteria irrespective of the presence of HMGR, although the mechanism is unknown and studies have reported equivalent statin MICs in species with and without HMGR (38, 39).

EFFECTS OF STATINS ON INTRACELLULAR GROWTH OF BACTERIA

The effects of statins on the intracellular growth of pathogens have also been studied, and, at drug concentrations closer to physiological levels, they have been shown to reduce the growth of several obligate intracellular bacterial pathogens. Recent reports demonstrated that lovastatin at 0.4 mg/liter (53) and both atorvastatin and simvastatin, in a dose-dependent fashion (0.08 to 0.8 mg/liter), reduced the survival of the leprosy-causing species *Mycobacterium leprae* (by up to 90% and 75%, respectively) in *in vitro* macrophage models, but in a cholesterol-dependent manner (54), suggesting an indirect effect on cholesterol levels, as the intracellular growth of these pathogens requires cholesterol. Prior but not concurrent treatment of murine fibroblast (L929) cells with lovastatin at 0.4 mg/liter also reduced both the intracellular growth of the respiratory pathogen *Coxiella burnetii* (which causes Q fever) (by 43%, $P = 0.064$) (55) and plaque formation by the causative agent of Rocky mountain spotted fever, *Rickettsia conorii* (by 64%, $P = 0.003$) (56). Interestingly, in *in vivo* studies, administration of the hydrophobic statin simvastatin, at a physiological concentration (0.5 mg/kg of body weight), but not administration of the hydrophilic statin pravastatin, significantly decreased (up to 83%) the levels of the respiratory pathogen *Chlamydiae pneumoniae* in lung cells of infected mice (57, 58). It was also found that cerivastatin (0.1 mg/liter) reduced the cross-infection of vascular smooth muscle cells (VSMC) by *C. pneumoniae*-infected macrophages (57, 59). In those studies, the authors also suggested that the reduced growth may have been an indirect effect due to cholesterol inhibition.

A number of studies have resulted in reports of inhibition of the nonobligate intracellular growth of *Mycobacterium tuberculosis* in peripheral blood mononuclear cells (PBMCs) and macrophages. Parihar et al. demonstrated that *M. tuberculosis* growth was significantly reduced (circa 2-fold, $P < 0.05$) in human mononuclear cells and macrophages taken from atorvastatin-treated

patients with familial hypercholesterolemia compared with the results seen with healthy donors while also showing that treatment with simvastatin (20.6 mg/liter) significantly reduced (circa 3-fold, $P < 0.01$) *M. tuberculosis* growth in murine macrophages and that treatment with both simvastatin and rosuvastatin significantly decreased (circa 2-fold to 10-fold, $P < 0.05/0.01$) the bacterial load in the liver, spleen, and lungs of infected mice (20 mg/kg) (60). The study further demonstrated that the simvastatin-mediated decrease in bacterial growth was reversed by the presence of mevalonate, the product of HMG-CoA reductase, and suggested that statins control infection by phagolysosomal arrest of *M. tuberculosis*. These results were corroborated by those from a study by Lobato et al. in which they showed that atorvastatin and simvastatin (2 μM) significantly inhibited *M. tuberculosis* growth (circa 60% reduction) in macrophages and that this was again reversed by the presence of mevalonate (54). A previous study by Parihar et al. also demonstrated that simvastatin treatment (20.6 mg/liter) could significantly reduce, by up to 4-fold ($P < 0.001$), the ability of the foodborne pathogen *Listeria monocytogenes* to grow inside mouse and primary macrophages, in a cholesterol-dependent manner, and could significantly reduce bacterial burden and dissemination (by 100-fold) to the liver ($P < 0.001$) and spleen ($P < 0.05$) in infected mice (61). The intracellular growth of another foodborne bacterium, gastroenteritis-causing *Salmonella enterica* serovar Typhimurium, was also attenuated more than 10-fold by lovastatin (50 nM and 30 μM) treatment of murine macrophages, at least in part due to attenuation of the mevalonate pathway (62). A key mechanism behind the attenuation of internalized bacterial infections by statins appears to be the statin-mediated inhibition of lipid raft formation. Lipid rafts are glycoprotein domains present in the cell membrane, which are formed as a result of cholesterol spontaneously interacting with sphingoglycolipids. Bacteria can manipulate lipid rafts in order to invade and survive within cells and induce apoptosis (63). However, statins are known to inhibit the formation of lipid rafts due to inhibition of cholesterol biosynthesis (44). Two studies investigating the effects of statins on intracellular growth of *L. monocytogenes* and plaque formation of *R. conorii* suggest that their findings were due to the inhibition of lipid raft formation by statins (56, 61).

As well as inhibiting intracellular growth, statin treatment at physiological concentrations promotes increased bacterial killing in host cells. Simvastatin treatment significantly reduced the burden of *S. pneumoniae* in the lungs of infected mice (dose = 1/10 mg/kg/day, 50-fold/100-fold reduction, $P = 0.02/0.002$) (64) and significantly increased bacterial clearance (65% reduction, $P = 0.01$) and reduced dissemination (90% reduction, $P = 0.01$) of *S. aureus* in a mouse model of pneumonia (dose = 0.25 mg/kg/day) (65). Simvastatin (~41.7 mg/kg/day) treatment also reduced *S. aureus* recovery by circa 35% from mouse peritoneal cells ($P < 0.005$) and by 2-fold in lung cells ($P < 0.05$), and mevastatin (50 μM) treatment significantly reduced (40% reduction, $P < 0.005$) the amount of *S. aureus* recovered from intracellular infection of human neutrophils and mouse macrophages (66). In the latter study, evidence suggested that there was no direct effect on bacterial viability but that statins promoted bacterial killing by inducing the formation of phagocyte extracellular traps.

Therefore, the evidence indicates that, while the mechanisms by which physiological concentrations of statins influence intracellular or *in vivo* bacterial infections are not fully understood, most studies suggest a mechanism of indirect action mainly due to pleiotropic effects of modulating the mevalonate pathway in the host.

EFFECTS OF STATINS ON BACTERIAL VIRULENCE

An interesting development in the field of statins and bacterial infection is the discovery that sublethal doses of statins may influence bacterial virulence, raising the possibility that statins may be repurposed as specific antivirulence therapeutics. A number of studies have investigated the impact of statin treatment on *in vitro* bacterial virulence (Table 2). Wang et al. and Graziano et al. both showed that *S. aureus* biofilm formation is inhibited by simvastatin treatment (40, 42), while Hennessy et al. demonstrated that both the *in vitro* motility and early biofilm formation of the predominant cystic fibrosis-associated pathogen *P. aeruginosa* are attenuated by statin concentrations subinhibitory to growth (4 and 40 mg/liter, respectively) (67). Graziano and colleagues also showed that simvastatin (4× MIC) treatment could disrupt established *S. aureus* biofilms, and Thangamani et al. demonstrated that simvastatin treatment at 2× and 4× MICs reduced the levels of established biofilms of both *S. aureus* and *S. epidermidis* by approximately 40% (41, 42). The latter study by Thangamani et al. also showed that simvastatin treatment suppressed the production of the *S. aureus* toxins Panton-Valentine leucocidin (PVL) and α-hemolysin (Hla) produced by MRSA. They also showed that simvastatin inhibited bacterial protein synthesis and suggested that the reduction in toxin production may be a reflection of this.

In cell culture studies, simvastatin (4 mg/liter) treatment significantly ($P \leq 0.05$) increased the adhesion of *P. aeruginosa* to lung cells (68) but the translocation of *P. aeruginosa* across the apical membrane of kidney cells was significantly ($P < 0.05$) inhibited by simvastatin treatment (5 μM [2 mg/liter]) (70). Neither of these studies showed an alteration in the invasive potential of *P. aeruginosa* in the presence of statin; however, the invasion of other pathogens is inhibited by statins. Horn et al. demonstrated reduced invasion of *S. aureus* into vascular epithelial cells in the presence of physiological concentrations of simvastatin (0.04 to 0.4 mg/liter) (69), while mevastatin (4 mg/liter) treatment completely inhibited the internalization of group B *Streptococcus*, a common cause of meningitis, in HeLa cells (71) and attenuated the invasion of *E. coli* into bladder epithelial cells (72). In the latter studies, inhibition of bacterial invasion was proposed to be due to the ability of simvastatin and mevastatin to inhibit the activation of Rho GTPase proteins as a result of the inhibition of the production of the isoprenoid intermediates farnesyl-pyrophosphate and geranylgeranyl-pyrophosphate, which are required for the prenylation and activation of Rho GTPases (73).

Therefore, there is promising evidence that statins may influence the invasiveness and/or biofilm formation of some pathogens; however, a number of studies have observed the absence of statins affecting other bacterial virulence factors (Table 2). Bacterial cell-cell communication may not be impacted by statins, as simvastatin, lovastatin, and mevastatin failed to alter N-acyl-homoserine lactone (AHL) or *Pseudomonas* quinolone signal (PQS) quorum sensing by *P. aeruginosa* and mevastatin failed to alter AHL signaling by *Burkholderia cenocepacia*, both prominent causes of respiratory infections in cystic fibrosis patients (67, 74). In the same studies, transcription of the *exoS* type three secretion toxin and protease production, respectively, were not altered by the statins tested. Furthermore, an in-depth study carried out using *S. pneumoniae* demonstrated that subinhibitory concentra-

tions of simvastatin (1 mg/liter) did not directly influence the activity of the pneumolysin toxin against red blood cells (75). However, the same study showed that simvastatin did protect vascular endothelial cells from pneumolysin-induced cytotoxicity *in vitro*. This protective effect was reversed by the presence of mevalonate, again suggesting an indirect effect. The protection was confirmed *in vivo*, with results showing that it extended to reduced lung damage and increased survival in a mouse model of infection.

Indeed, several studies have shown that statins can reduce the impact of bacterial toxins on host cells. In a study that utilized *S. aureus* alpha toxin, leukocyte recruitment and adhesion in mice were attenuated by simvastatin pretreatment (100 μg/kg) by >70% ($P < 0.01$) (76). This finding is significant as it suggests that statins may reduce alpha-toxin-mediated inflammation and cardiovascular damage. In addition, lovastatin (1 mg/liter) treatment improved the survival of mice exposed to another *S. aureus* toxin, enterotoxin B, by 50% (77) and the cytotoxicity of *Bacillus anthracis* lethal toxin against macrophages was reduced >60% by treatment with fluvastatin, mevastatin, and simvastatin (78).

The protective mechanism(s) of statins against bacterial virulence has not been established; however, the impact of statins on host cell isoprenoid metabolism appears to include regulation of at least some of the effects on bacterial virulence observed in cell culture and infection models. Several studies have shown that the observed effect of statin on bacterial virulence can be reversed by the addition of exogenous mevalonate (54, 59–61, 66, 69, 75, 77, 79), while statin-mediated cholesterol depletion is protective against bacterial toxins (61, 75) and contributes to the killing of intracellular bacteria (45, 54, 60–62, 66). In addition, the regulation of the inflammatory response by statins may account for some of these protective effects. For instance, cerivastatin treatment attenuated the production of proinflammatory mediators and superoxide in macrophages infected with *C. pneumoniae*, and this was associated with a reduced bacterial infection rate (79). The inflammatory response in lipopolysaccharide-treated mice was also reduced by cerivastatin treatment, leading to improved survival (80), while simvastatin treatment reduced both lung injury and the production of proinflammatory chemokines in a mouse sepsis model (81).

COPRESCRIPTION OF STATINS WITH ANTIBIOTICS

It has been hypothesized that physiological or subinhibitory doses of statins could be used in combination with antibiotics to increase the efficacy of treatment. Many researchers have proposed dual-action combinations that remove the virulence threat, i.e., the toxin or biofilm, facilitating clearance by the antibiotic. Indeed, the optimism prompted by the growing evidence for the effectiveness of novel antivirulence approaches has been tempered by the realization that conventional antibiotics will still be required to clear the infecting pathogen and resolve the infection. Current information on the synergistic relationship between statins and antibiotics is limited and conflicting (Table 3). A significant synergistic effect resulting in increased bacterial lysis has been reported with sublethal doses of penicillin and simvastatin (7.8 mg/liter) against pneumococcal growth *in vitro* (43), while atorvastatin and simvastatin (0.2 μM) treatment increased the efficacy of rifampin against *M. tuberculosis* and *M. leprae* infection *in vitro* by approximately 50% (54). In addition, *in vivo* mouse studies showed that atorvastatin (80 mg/kg/day) treatment increased the efficacy of rifampin against *M. leprae* infection ($P < 0.05$) (54) and that simvastatin (25 mg/kg) treatment increased

TABLE 2 Effect of statins on bacterial virulence

Bacterial species	Effect of the following statin(s) (mg/liter) on the indicated virulence trait[a]											
	Sim (mg/liter)	Sim (mg/liter)	Sim/Lov/Mev (mg/liter)				Sim (mg/liter)	Sim (mg/liter)	Mev (mg/liter)	Sim (mg/liter)	Sim (mg/liter)	
	Reduced biofilm formation	Disruption of established biofilm	Motility	QS	Protease	T3SS ExoS	Increased adhesion to human cells	Reduced invasion	Reduced invasion	Translocation	Toxin production	Reference
S. aureus	0.98–62.5	62.5										42
	62.5											40
		64								⇑ 40	40	41
								0.04–0.4				69
S. epidermitis		128										41
P. aeruginosa	4, 40		40	NC		NC	4	NC				67, 68
								NC		⇓ 2		70
Streptococcus									4			71
E. coli									4			72
B. cenocepacia				NC	NC							74

[a] Sim, simvastatin; Ator, atorvastatin; Prav, pravastatin; Fluv, fluvastatin; QS, quorum sensing; T3SS, type III secretion system; NC, no change.

TABLE 3 Effect of statins on antibiotic activity

Bacterial species	Antibiotic synergy[a]						Reference
	In vitro			*In vivo* (mice)			
	Antibiotic + statin	Statin concn	Effect	Antibiotic + statin	Statin concn (mg/kg/day)	Effect	
Pneumococci	Pen + Sim	7.8 mg/liter	⇑ Autolysis				43
MRSA/VRSA	Mup/Fus/Dap + Sim	<32 mg/liter	⇓ Growth				41
S. aureus	Van + Sim	?	NC				42
M. tuberculosis	Rif + Sim/Ator	0.2 μM	⇓ Viability				54
				Rif, Pyr, Iso + Sim	25	⇑ Bacillary killing	82
M. leprae	Rif + Ator	0.2 μM	⇓ Viability	Rif + Ator	80	⇓ Viability	54
A. baumannii	Ami/Imi/Min + Prav/Sim/Ator/Fluv		NC				83
P. aeruginosa	Cip/Cep/Pip + Ator/Fluv		NC				83
K. pneumoniae	Cip/Cep/Pip + Ator/Fluv		NC				83
E. coli	Cip/Cep/Pip + Ator/Fluv		NC				83

[a] Statins: Sim, simvastatin; Ator, atorvastatin; Prav, pravastatin; Fluv, fluvastatin. Antibiotics: Pen, penicillin; Mup, mupirocin; Fus, fusidic acid; Dap, daptomycin; Van, vancomycin; Rif, rifampin; Pyr, pyrazinamide; Iso, isoniazid; Ami, amikacin; Imi, imipenem; Min, minocycline; Cip, ciprofloxacin; Cep, cefepime; Pip, piperacillin. NC, no change. Arrows pointing up indicate increases; arrows pointing down indicate decreases.

the *in vivo* activity of first-line anti-TB antibiotics, reducing the lung bacillary burden by >1 $\log_{10}$ ($P < 0.01$) (82). Thangamani and colleagues demonstrated a positive synergistic effect of simvastatin on the antimicrobial effect of four topical antibiotics, mupirocin, fusidic acid, retapamulin, and daptomycin, against clinical isolates of multidrug-resistant *S. aureus*. However, Graziano et al. showed that there was no synergistic effect between simvastatin and vancomycin against *S. aureus* (42). A recent study which examined the *in vitro* effects of five statins, at concentrations equivalent to recommended physiological doses (for simvastatin, lovastatin, atorvastatin, and pravastatin, 0.01, 0.05, and 0.1 mg/liter; for fluvastatin, 0.1, 0.2, and 0.3 mg/liter), on the MICs of six antibiotics against four clinically important Gram-negative strains—*P. aeruginosa*, *A. baumannii*, *E. coli*, and *K. pneumoniae*—found that the statin treatments did not significantly change the susceptibility of any of those bacteria to any of the antibiotics tested (83). However, the results of that *in vitro* study may not reflect the true activity in an *in vivo* setting; therefore, further *in vivo* investigations are warranted. This is particularly relevant given that the majority of the studies reviewed here that looked at the mechanism by which statins influence bacterial growth or virulence *in vivo* suggest indirect effects as a result of interactions with host cells. In addition, the antibiofilm activity of statins toward Gram-negative pathogens, which would be expected to reduce the MIC of antibiotics in biofilm-forming populations (accounting for approximately 80% of all infections), would not be reflected in the planktonic *in vitro* MIC assays performed.

Note, however, that the repurposing of statins for use as combinatorial antibiotics would rely on their compatibility with currently administered antibiotics. While data concerning this aspect of antimicrobial therapy are limited, certain antibiotics may interfere with the metabolism of statins, which can lead to increased serum levels and thus to an increased risk of adverse effects (84). For instance, certain statins, including simvastatin, lovastatin, and atorvastatin, are metabolized by cytochrome P450 3A4 (CYP3A4) isoenzymes and studies have shown that coprescription with drugs that inhibit CYP3A, such as macrolide antibiotics, can lead to increased adverse effects, including rhabdomyolysis, in elderly patients (85–92). In light of this, the U.S. FDA has stated that "caution should be exercised when prescribing clarithromycin with statins" and, in particular, that "concomitant use of clarithromycin with lovastatin or simvastatin is contraindicated" (95). In contrast, they suggest that the concomitant use of statins not dependent on CYP3A metabolism (e.g., fluvastatin) could be considered. However, a recent study by Li and colleagues demonstrated significantly increased adverse effects when clarithromycin was coprescribed with statins not metabolized by CYP3A4 (93), suggesting additional mechanisms of drug interactions independent of the CYP3A4 pathway, possibly related to impaired hepatic uptake of statins. In contrast to studies on macrolide-statin interactions, no additive harmful effects have been attributed to the combined use of statins and the lipopeptide antibiotic daptomycin, despite both agents being associated with muscle injury (94).

SUMMARY

The repurposing of statins as antimicrobial agents was shown to hold potential promise when clinical studies revealed that patients on cholesterol-lowering statins showed improved outcomes from bacterial infections. However, as outlined in this review, the most convincing evidence of significantly improved infection outcomes is seen when patients are pretreated with statins, and the antimicrobial effect is probably indirect. There is little evidence of significantly improved outcomes when infections are treated with *de novo* statins. However, while the evidence for statin effectiveness thus far has been provided from prophylactic studies, the evidence of antivirulence activity emerging for statins, whereby pathogens may be silenced rather than killed, offers an alternative perspective on their potential clinical utility. In addition, statins may also offer selectivity in targeting pathogenesis rather than the microbial population or microbiome as a whole, which is a major factor in maintaining host homeostasis. This could have the added advantage of removing the selective pressure that underpins the continued spread of antibiotic resistance among populations. Thus, further RCTs and prospective studies have been recommended and, on the basis of this review, the design of these new studies will be crucial, as *in vitro* and mouse studies clearly show that the most gain may be achieved by matching particular statins with particular infecting pathogens. Moreover, one of the most limiting fac-

tors is the concentration of statins required for the inhibition of bacterial growth *in vitro*. In almost all cases cited, the *in vitro* MICs far exceed the general plasma levels found in patients receiving cholesterol-lowering statins, and the feasibility of raising the dose is questionable due to cytotoxicity and increased risk of debilitating side effects. One area where specific targeted studies may be particularly beneficial is in the treatment of infections caused by intracellular pathogens. Many of the *in vitro* cellular studies outlined here showed significant results by the use of statins at physiological concentrations, while again suggesting that the effect is indirect. It would be interesting to see if these beneficial effects could be mimicked in *in vivo* clinical studies.

The effect of statins on *in vitro* virulence of some pathogens is interesting but again is hindered by the high concentrations required for significant results. However, this hindrance may be overcome by using subinhibitory concentrations of statins in combination with existing antibiotics. The evidence presented here regarding the repurposing of statins in combination therapies is promising but again may be statin/pathogen specific. While the most significant results have again been against intracellular bacteria, there are few *in vivo*/clinical studies available describing such therapies against extracellular pathogens. In designing such studies, however, the possibility of adverse effects associated with drug-drug interactions should be an important consideration.

Therefore, while the results of clinical studies regarding the repurposing of statins as antimicrobials have been inconclusive overall, the evidence presented here suggests that further prospective studies focusing on statin and pathogen specificity, bacterial virulence, combinatorial therapy, and/or means of drug administration are warranted.

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