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**Proposed Title:** Statins as next generation anti-microbials: Is there potential for repurposing?

**Short Title:** Statins inhibit bacterial growth and virulence

**Authors:** Emma Hennessy[1§], Claire Adams[1§], F. Jerry Reen[1] and Fergal O'Gara[1,2*]

[1]BIOMERIT Research Centre, School of Microbiology, National University of Ireland, Cork, Ireland

[2]School of Biomedical Sciences, Curtin University, Perth, WA, Australia

§ Both authors contributed equally

[*]*Corresponding author*. Address: BIOMERIT Research Centre, Department of Microbiology, University College Cork, College Road, Cork, Ireland.

Email: f.ogara@ucc.ie; Phone: +353 21 4902861; Fax: +353 21 4903101.

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17 **Abstract**

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

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## Introduction

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 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, where the lactone ring is hydrolyzed in the liver (4). Statins can be divided into two broad classes (Figure 1). Type 1 statins are lipophilic, and possess a butaryl 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 butaryl 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-CoenzymeA reductase (HMGR), a rate-limiting enzyme involved in cholesterol biosynthesis (3). HMGR is an integral part of the mevalonate pathway, which is not only 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 the 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 anti-cancer effects on host cells, and these effects are well-characterized

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(5–9). Furthermore, several studies have explored the pleiotropic effects of statins in combating multi-system 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 anti-microbial 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 Figure 2).

**Clinical evidence that statins influence morbidity and mortality of patients with microbial infections.**

The clinical potential of statins as anti-microbial 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 bacteraemia, pneumonia, sepsis and some acute infections and patient populations received several different statins. For instance, two single centre retrospective studies showed that patients

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with bacteraemia who have undergone prior statin treatment have a significant decreased risk of in hospital mortality of 6% vs 28% (p = 0.002) and 13% vs 24% (p = 0.001) respectively (14, 15). The latter study also showed there was an inverse correlation between the length of statin treatment and risk of mortality when they compared statin use ≥12 and <12 weeks prior to infection (11% vs 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 UK found that current statin treatment (within last 30 days) reduced pneumonia-associated mortality (adjusted OR 0.47, 95% CI 0.25-0.88) (16), while prior statin treatment also reduced mortality rates in patients in the USA with community-acquired pneumonia (CAP) (adjusted OR 0.36, 95% CI 0.14–0.92) (17). Furthermore, data from the Justification for the Use of Statin in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER), which was initially undertaken to determine whether rosuvastatin could reduce the risk of cardiac disease in people without hyperlidemia (18) were retrospectively analysed in 2012. This analysis suggested that rosuvastatin treatment may decrease the occurrence of pneumonia before (HR 0.81, 95% CI 0.67–0.97) or after a cardiac event (HR 0.83, 95% CI 0.69–1.00) (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 yield reduced mortality from pneumonia (20). This latter study encompassed 3415 patients >17 yrs of age with pneumonia admitted to hospital, while the JUPITER randomized, double-blind, placebo-controlled trial of 17,802 healthy patients was restricted to men >50yrs and women >60yrs of age. Indeed, the JUPITER study was designed to address the 'healthy user effect' suggesting that

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93 study design in addition to age differences between the cohorts may underpin the contrasting
94 observations.

95 Sepsis is a serious infection-induced whole body inflammatory state, and due to the
96 immunomodulatory activity of statins, several studies have been carried out to evaluate the
97 benefit of statin therapy in the prevention or treatment of the disease. While the type, design, size
98 and measured outcomes of the studies have been varied and overall results conflicting, in recent
99 years extensive reviews evaluating these clinical studies have been published (21-27). The
100 majority of clinical studies to date have been retrospective cohort studies evaluating the impact
101 of prior treatment with statins on disease progression and mortality. Many of these, plus several
102 meta-analysis reviews, showed promising results whereby prior use of statins significantly
103 reduced disease progression and/or mortality associated with sepsis (25, 28 - 32). For instance,
104 studied by Almog et al. and Martin et al. demonstrated a reduced risk of developing severe sepsis
105 in patients pretreated with statins (2.4% vs 19%, $P<0.001$ and 56% vs 86%, $P<0.02$ respectively)
106 while Mortensen et al. showed a reduced risk of 30 day mortality in patients using statins (OR
107 0.48, 95% CI 0.36-0.64). One of the main limitations attributed to these studies was limited
108 sample size, and against this, a recent population-based, propensity score-matched analysis of the
109 effect of low and high doses of statins on sepsis outcomes involved a cohort of 27,792 statin
110 users compared with an equal number of non-users (33). This extensive study demonstrated a
111 significant reduction of 1-year mortality (HR 0.83, 95 % CI 0.81–0.85) and adverse
112 consequences of sepsis such as in-hospital death (OR 0.86, 95 % CI 0.83–0.89) and ICU
113 admission (OR 0.95, 95 % CI 0.92–0.98) in patients pretreated with statins. They also showed
114 that the benefits of pretreatment with statins increased significantly with higher doses.

115 Therefore, several studies have shown promising potential for the prior use of statins in the

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prevention / progression of infections. Nevertheless, it is difficult to draw conclusions on whether these statins effects were directly anti-bacterial / inflammatory or due to pleitrophic effects on co-morbitities associated with the infections. For example, it is estimated that cardiovascular events account for up to 30% of deaths in patients with CAP and therefore it could be argued that prior statin use could improve cardiovascular health and thus reduce mortaliry rather than having any direct efect on the infection. Against this a study has reported that while prior statin use was significantly associated with decreased 90-day mortality in CAP patients, there was no significant association with cardiocasular events (34). In order to fully understand the mechanistic effects of prior statin use on infections similar studies targeting for example specific co-morbidities 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 suggest 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 0.20–0.72, *P*=0.003) but no significant



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139 difference in statin users with Gram-positive infections (adjusted OR 1.22, 95% CI 0.69–2.17,
140 *P*=0.49) (36), suggesting that the type of bacterial infection may be a significant factor.
141

142 **Effects of statins on *in vitro* bacterial growth.**

143 There is a large body of evidence demonstrating that statins have direct anti-bacterial effects on
144 the *in vitro* growth of both Gram-positive and Gram-negative bacterial pathogens responsible for
145 a wide range of infections (Table 1), although there have been conflicting reports on MICs
146 (ranging from 15 mg/l to 500 mg/l) and strain specificity may be a factor (Table 1). The growth
147 of the Gram-positive nosocomial pathogens *Staphylococcus aureus* and *Streptococcus*
148 *pneumoniae* has been shown to be inhibited by atorvastatin, rosuvastatin and simvastatin (37–
149 43), while fluvastatin has also been reported to inhibit the growth of *S. aureus* (37). In addition,
150 both type 1 (simvastatin) and type 2 (atorvastatin, fluvastatin and rosuvastatin) statins have also
151 demonstrated a bacteriostatic effect against other Gram-positive cocci, notably *Streptococcus*
152 *pyogenes, Staphylococcus epidermidis, Enterococcus* and *Bacillus spp.* (37, 39, 40, 42).
153 Promisingly, simvastatin, lovastatin and rosuvastatin have also been shown to have anti-bacterial
154 effects on the growth of antibiotic-resistant species such as methicillin-resistant *S. aureus*
155 (MRSA), vancomycin-resistant *S. aureus* (VRSA) and vancomycin-resistant enterococci (VRE),
156 although the MIC concentrations are typically higher than against antibiotic sensitive strains
157 (Table 1) (37, 39 - 43).

158 Both type 1 and type 2 statins have also been found to inhibit the growth of a number of
159 clinically important Gram-negative species including several respiratory pathogens. The growth
160 of the nosocomial respiratory pathogens *Pseudomonas aeruginosa*, *Acinetobacter baumanii* and

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*Klebsiella pneumoniae* is inhibited by atorvastatin, rosuvastatin and simvastatin (MICs ranging from 15 - 333 mg/l) (39, 40) and simvastatin was reportedly bactericidal against *Moraxella catarrhalis* (MIC 15 mg/l) (38). 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 against a range of pathogens including *Citrobacter freundii*, *Enterobacter aerogenes*, *Haemophilus influenzae* and *Proteus mirabilis* (MICs ranging from 15 - 166 mg/l) (39). Simvastatin and lovastatin (10 mg/l) are also reportedly bactericidal against the spirochete *Borrelia burgdorferi* (the causative agent of Lyme disease) (44) and atorvastatin, rosuvastatin and simvastatin were found to inhibit the growth of *Escherichia coli*, a prominent cause of gastroenteritis and urinary tract infections (39). In contrast, however, Bergman et al., using a maximum concentration of 250 mg/L observed that simvastatin did not inhibit the growth of *H. influenzae* (38), while Graziano et al. found that simvastatin, atorvastatin and pravastatin at concentrations up to 250 mg/l did not inhibit the growth of *P. aeruginosa, E. coli* or *Enterococcus faecalis* (43). Furthermore, the study by Thangamani et al. (42) reported that while the growth of Gram-positive species was inhibited by statins, the growth of *P. aeruginosa* ATCC 15442 was not inhibited by the statins 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. baumanii*, *E. coli* and *Salmonella enterica* serovar Typhimurium. Interestingly, they did show that when combined with sub-inhibitory concentrations of colistin, which compromises the outer membrane integrity, simvastatin had anti-bacterial activity against the range of Gram-negative pathogens at MICs of 8 – 32 mg/l. While the activity shown by simvastatin against *E. coli* ATCC35218 (39) is in direct

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184 contrast to the lack of activity by simvastatin against *E. coli* ATCC35150, ATCC 700728,
185 ATCC25922, and ATCC10536 (42-43), it is worth noting that the *E. coli* ATCC3218 assays
186 were performed on solid agar while the other studies were performed using the broth
187 microdilution method, perhaps explaining the apparent differences in activity.

188 Taken together, the data suggest that the anti-bacterial activity of statins may be both statin
189 specific and / or strain/species specific. Simvastatin and atorvastatin generally appear to be more
190 effective against *S. aureus, S. pneumoniae* and enterococci than other statins (37–40), while three
191 distinct simvastatin MICs were reported against *S. aureus* clinical isolates from the UK and
192 Jordan as well as typed reference strains (Table 1) (37, 39, 42, 43). It is also noteworthy that
193 while the MICs of statins varied according to statin and pathogen tested, the *in vitro* MICs
194 ranged from circa 15 to 400 mg/l, which far exceeds the typical peak plasma concentrations of
195 patients on oral statins, which generally ranges from circa 10 to 300 µg/l. Moreover, in the
196 majority of cases the *in vitro* statin MICs against multi-drug resistant pathogens were even
197 greater than those against equivalent antibiotic susceptible strains. As such, at these MIC
198 concentrations, it is unlikely that they would qualify as lead molecules in drug discovery
199 programs. This variability in MICs could be considered somewhat unexpected for what is
200 essentially a novel antibiotic compound being administered to a naive population. However,
201 recent studies have reported significant phenotypic and genotypic diversity within clinical
202 populations suggesting that adaptation to environmental or host related factors may be
203 widespread (45 - 47). While the mechanism of action of statin antimicrobial and anti-virulence
204 activity remains to be elucidated, some reports suggest the involvement of isoprenoids and
205 membrane integrity (48). Further deciphering the interaction between statins and the microbial

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membrane may provide answers to 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-MIC concentrations significantly enhanced bacterial clearance and healing of MSSA and MRSA *S. aureus*-contaminated wounds in mice wound models (41, 42). Wang et al. showed that application of simvastatin (62.5 mg/l) reduced the MSSA wound size by over 50% at day seven and significantly reduced (>60% reduction) the bacterial load visible in the wound histology (41), 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 (42). The latter study also showed that this topical application of simvastatin had an additive healing effect and it reduced the production of pro-inflammatory cytokines (IL-6, 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 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 (49, 50). 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.*



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*aureus* by binding to and inhibiting the activity of its HMGR enzyme (51) 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 (39, 40) .

**Effects of statins on intracellular growth of bacteria**

The effect of statins on the intracellular growth of pathogens has 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/l (52) and both atorvastatin and simvastatin, in a dose-dependent fashion (0.08 – 0.8 mg/l), 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 (53), suggesting an indirect effect on cholesterol levels as the intracellular growth of these pathogens requires cholesterol. Prior but not concomitant treatment of murine fibroblast (L929) cells with lovastatin at 0.4 mg/l also reduced both the intracellular growth of the respiratory pathogen *Coxiella burnetti* (which causes Q fever) (by 43%, *P*=0.064) (54), and plaque formation by the causative agent of Rocky mountain spotted fever, *Rickettsia conorii* (by 64%, *P*=0.003) (55). Interestingly, in *in vivo* studies the hydrophobic statin, simvastatin, at a physiological concentration (0.5mg/kg), but not the hydrophilic statin pravastatin significantly decreased (up tp 83%) the levels of the respiratory pathogen *Chlamydiae pneumoniae* in lung cells of infected mice (56, 57). It was also found that cerivastatin (0.1 mg/l) reduced the cross infection of VSMC (vascular smooth muscle cells) by *C. pneumoniae* infected macrophages (56,



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252 58). In these studies the authors also suggest that the reduced growth may be an indirect effect
253 due to cholesterol inhibition.

254 A number of studies report inhibition of the non-obligate intracellular growth of *Mycobacterium*
255 *tuberculosis* in peripheral blood mononuclear cells (PBMCs) and macrophages. Parihar et al.
256 demonstrated that *M. tuberculosis* growth was significantly reduced (circa 2-fold, $P<0.05$) in
257 human mononuclear cells and macrophages taken from atrovastatin-treated patients with familial
258 hypercholesterolemia compared with healthy donors while also showing that simvastatin (20.6
259 mg/l) significantly reduced (circa 3-fold, $P<0.01$) *M. tuberculosis* growth in murine macrophages
260 and both simvastatin and rosuvastatin significantly decreased (circa 2 to 10 –fold $P<0.05$ /0.01)
261 the bacterial load in the liver, spleen and lungs of infected mice (20 mg/kg) (59). The study
262 further demonstrated that the simvastatin-mediated decrease in bacterial growth was reversed by
263 mevalonate, the product of HMG-CoA reductase and suggested that statins control infection by
264 phagolysosomal arrest of *M. tuberculosis*. These results were corroborated by the study by
265 Lobato et al. whereby they showed that atorvastatin and simvastatin (2 µM) significantly
266 inhibited *M. tuberculosis* growth (circa 60% reduction) in macrophages and again this was
267 reversed by mevalonate (53). A previous study by Parihar et al. also demonstrated that
268 simvastatin treatment (20.6 mg/l) could significantly reduce, by up to 4-fold, ($P<0.001$) the
269 ability of the food borne pathogen *Listeria monocytogenes* to grow inside mouse and primary
270 macrophages, in a cholesterol dependent manner and significantly reduce the bacterial burden
271 and dissemination (by 100-fold) to the liver ($P<0.001$) and spleen ($P<0.05$) in infected mice (60).
272 The intracellular growth of another food borne bacteria, the gastroenteritis-causing *Salmonella*
273 *enterica* serovar Typhimurium, was also attenuated more than 10-fold by lovastatin (50 nM & 30
274 µM) treatment of murine macrophages, at least in part due to attenuation of the mevalonate



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pathway (61). 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 (62). However, statins are known to inhibit the formation of lipid rafts due to inhibition of cholesterol biosynthesis (63). Two studies investigating the effects of statins on the intracellular growth of *L. monocytogenes* and plaque formation of *R. conorii* suggest their findings were due to the inhibition of lipid raft formation by statins (55, 60).

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

Therefore, evidence suggests that, while the mechanisms by which physiological concentrations of statins influence intracellular or *in vivo* bacterial infections are not fully understood, most



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studies suggest 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 sub lethal doses of statins may influence bacterial virulence, raising the possibility that statins may be repurposed as specific anti-virulence 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 (41, 43) 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 sub-inhibitory to growth (4 & 40 mg/l respectively) (67). Graziano and colleagues also showed that simvastatin (4x MIC) could disrupt established *S. aureus* biofilms and Thangamani et al. demonstrated that simvastatin at 2x and 4x MIC concentrations reduced established biofilms of both *S. aureus* and *S. epidermidis* by approximately 40% (42, 43). This latter study by Thangamani et al. also showed that simvastatin 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 suggest that the reduction in toxin production may be a reflection of this.

In cell culture studies, simvastatin (4 mg/l) significantly increased ($P \leq 0.05$) the adhesion of *P. aeruginosa* to lung cells (68) but the translocation of *P. aeruginosa* across the apical membrane of kidney cells was significantly inhibited ($P<0.05$) by simvastatin treatment (5 µM / 2 mg/l) (69). Neither of these studies observed an alteration in the invasive potential of *P. aeruginosa* in



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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 - 0.4 mg/l) (70), while mevastatin (4 mg/l) completely inhibited the internalization of Group B *Streptococcus*, a common cause of meningitis, into HeLa cells (71), and attenuated the invasion of *E. coli* into bladder epithelial cells (72). In these 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 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 sub-inhibitory concentrations of simvastatin (1 mg/l) 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 mevalonate, again suggesting an indirect effect. The

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343 protection was confirmed *in vivo* whereby it extended to reduced lung damage and increased
344 survival in a mouse model of infection.

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

353 The protective mechanism(s) of statins against bacterial virulence has not been established,
354 however, the impact of statins on host cell isoprenoid metabolism appears to regulate at least
355 some of the effects observed on bacterial virulence in cell culture and infection models. Several
356 studies have shown that the observed statin effect on bacterial virulence can be reversed by the
357 addition of exogenous mevalonate (53, 58-60, 66, 70, 75, 77, 79), while statin-mediated
358 cholesterol depletion is protective against bacterial toxins (75, 60) and contributes to the killing
359 of intracellular bacteria (44, 53, 59 - 61, 66). In addition, the regulation of the inflammatory
360 response by statins may account for some of these protective effects. For instance, cerivastatin
361 treatment attenuated the production of pro-inflammatory mediators and superoxide in
362 macrophages infected with *C. pneumoniae*, and this was associated with a reduced bacterial
363 infection rate (79). The inflammatory response in lipopolysaccharide-treated mice was also
364 reduced by cerivastatin treatment, leading to improved survival (80), while simvastatin treatment

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reduced both lung injury and the production of pro-inflammatory chemokines in a mouse sepsis model (81).

**Co-prescription of statins with antibiotics**

It has been hypothesized that physiological or sub-inhibitory 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, either toxin or biofilm, facilitating clearance by the antibiotic. Indeed, the growing evidence for the effectiveness of next generation anti-virulence approaches has been tempered by a 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 2). A significant synergistic effect resulting in increased bacterial lysis has been reported with sub-lethal doses of penicillin and simvastatin (7.8 mg/l) against pneumococcal growth *in vitro* (38), while atorvastatin and simvastatin (0.2 μM) increased the efficacy of rifampin against *M. tuberculosis* and *M. leprae* infection *in vitro* by approximately 50% (53). In addition, *in vivo* mice studies showed that atorvastatin (80 mg/kg/day) increased the efficacy of rifampin against *M. leprae* infection ($P<0.05$) (53) and simvastatin (25 mg/kg) increased 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 anti-microbial effect of four topical antibiotics, mupirocin, fusidic acid, retapamulin and daptomycin, against clinical isolates of multi-drug resistant *S. aureus*. However, Graziano et al. showed there was no synergistic effect between simvastatin and vancomycin against *S. aureus* (43). A recent study, which examined the *in vitro* effects of five statins, at



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388 concentrations equivalent to recommended physiological doses (simvastatin, lovastatin,
389 atrovastatin, pravastatin = 0.01, 0.05, 0.1 mg/l; fluvastatin = 0.1, 0.2, 0.3 mg/l), on the MICs of
390 six antibiotics against four clinically important Gram-negative strains – *P. aeruginosa, A.*
391 *baumanii*, *E. coli* and *K. pneumoniae* – found that the statins did not significantly change the
392 susceptibilty of any of these bacteria to any of the antibiotics tested (83). However, this *in vitro*
393 study may not reflect the true activity in an *in vivo* setting and therefore further *in vivo*
394 investigations are warranted. This is particularly relevant given that the majority of the studies
395 reviewed here that looked at the mechanism by which statins influence bacterial growth or
396 virulence *in vivo* suggest indirect effects as a result of interactions with host cells. In addition, the
397 anti-biofilm activity of statins towards Gram-negative pathogens, which would be expected to
398 reduce the MIC of antibiotics in biofilm forming populations (accounting for approximately 80%
399 of all infections), would not be reflected in the planktonic *in vitro* MIC assays performed.

400 It is important to note, however, that the repurposing of statins for use as combinatorial
401 antibiotics would rely on their compatibility with currently administered antibiotics. While data
402 in this aspect of antimicrobial therapy is limited, certain antibiotics may interfere with the
403 metabolism of statins which can lead to increased serum levels and thus an increased risk of
404 adverse effects (84). For instance, certain statins including simvastatin, lovastatin, and
405 atorvastatin are metabolised by cytochrome P450 3A4 (CYP3A4) isoenzymes and studies have
406 shown that co-prescription with drugs that inhibit CYP3A, such as macrolide antibiotics, can
407 lead to increased adverse effects including rhabdomyolysis in elderly patients (85-92). In light
408 of this the US FDA has stated that 'caution should be exercised when prescribing clarithromycin
409 with statins' and in particular 'concomitant use of clarithromycin with lovastatin or simvastatin
410 is contraindicated' (89). In contrast they suggest that the concomitant use of statins not

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411 dependent on CYP3A metabolism (e.g. fluvastatin) could be considered. However, a recent study
412 by Li and colleagues demonstrated significantly increased adverse effects when clarithromycin
413 was co-prescribed with statins not metabolized by CYP3A4 (94), suggesting additional
414 mechanisms of drug interactions independent of the CYP3A4 pathway, possibly related to
415 impaired hepatic uptake of statins. In contrast to studies on macrolide-statin interactions, no
416 additive harmful effects have been attributed to the combined use of statins and the lipopeptide
417 antibiotic daptomycin, despite both agents being associated with muscle injury (95).
418
419 **Summary**
420 The repurposing of statins as anti-microbial agents held promising potential when clinical studies
421 revealed that patients on cholesterol lowering statins showed improved outcomes from bacterial
422 infections. However, as outlined in this review the most convincing evidence of significantly
423 improved infection outcomes is when patients are pretreated with statins and the anti-microbial
424 effect is probably indirect. There is little evidence of significantly improved outcomes when
425 infections are treated with de-novo statins. However, while the evidence for statin effectiveness
426 thus far has been provided from prophylactic studies, the anti-virulence activity emerging for
427 statins, whereby pathogens may be silenced rather than killed, offers an alternative perspective
428 on their potential clinical utility. In addition, statins may also offer selectivity in targeting
429 pathogenesis rather than the microbial population or microbiome as a whole, which is a major
430 factor in maintaining host homeostasis. This could have the added advantage of removing the
431 selective pressure that underpins the continued spread of antibiotic resistance among populations.
432 Thus, further RCTs and prospective studies have been recommended and based on this review
433 the design of these new studies will be crucial as *in vitro* and mouse studies clearly show that the



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most gain may be achieved by matching particular statins with particular infecting pathogens. Moreover, one of the most limiting factors 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 *in vitro* cellular studies outlined here show significant results when using statins at physiological concentrations, while again suggesting 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 may be overcome by using sub-inhibitory 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 against extracellular pathogens. When designing these studies however, the possibility of adverse effects associated with drug-drug- interactions should be an important consideration.

Therefore, while overall clinical studies regarding the repurposing of statins as anti-microbials are inconclusive, the evidence presented here suggests 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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457 **References**


458 1. **Endo A, Kuroda M, Tanzawa K**. 1976. Competitive inhibition of 3-hydroxy-3-
459 methylglutaryl coenzyme A reductase by ML-236A and ML-236B fungal metabolites,
460 having hypocholesterolemic activity. FEBS Lett. **72**:323–326.

461 2. **Endo A, Kuroda M, Tsujita Y**. 1976. ML-236A, ML-236B, and ML-236C, new
462 inhibitors of cholesterogenesis produced by *Penicillium citrinium*. J. Antibiot. (Tokyo).
463 **29**:1346–1348.

464 3. **Istvan ES, Deisenhofer J**. 2001. Structural mechanism for statin inhibition of HMG-CoA
465 reductase. Science (80-. ). **292**:1160–1164.

466 4. **Hamelin BA, Turgeon J**. 1998. Hydrophilicity/lipophilicity: relevance for the
467 pharmacology and clinical effects of HMG-CoA reductase inhibitors. Trends Pharmacol.
468 Sci. **19**:26–37.

469 5. **Rodriguez AL, Wojcik BM, Wrobleski SK, Myers DD, Wakefield TW, Diaz JA**.
470 2012. Statins, inflammation and deep vein thrombosis: a systematic review. J. Thromb.
471 Thrombolysis **33**:371–82.

472 6. **Quist-Paulsen P**. 2010. Statins and inflammation: an update. Curr. Opin. Cardiol.
473 **25**:399–405.

474 7. **Chow SC**. 2009. Immunomodulation by statins: mechanisms and potential impact on
475 autoimmune diseases. Arch. Immunol. Ther. Exp. (Warsz). **57**:243–251.

Accepted Manuscript Posted Online

8. **Swanson KM, Hohl RJ**. 2006. Anti-cancer therapy: targeting the mevalonate pathway. Curr. Cancer Drug Targets **6**:15–37.

9. **Dulak J, Józkowicz A**. 2005. Anti-angiogenic and anti-inflammatory effects of statins: relevance to anti-cancer therapy. Curr. Cancer Drug Targets **5**:579–594.

10. **Tleyjeh IM, Kashour T, Hakim_FA, Zimmerman_VA, Erwin PJ, Sutton AJ, Ibrahim T.** 2009 Statins for the prevention and treatment of infections: a systematic review andmeta-analysis. Arch Intern Med **169:**1658–67.

11. **Janda S, Young A, FitzGerald JM, Etminan M, Swiston J.** 2010. The effect of statins onmortality from severe infections and sepsis: a systematic review and meta-analysis. J Crit Care **25**:656.e7-22.

12. **Chopra V, Rogers MAM, Buist M, Govindan S, Lindenauer PK, Saint S, Flanders SA.** 2012. Is statin use associated with reduced mortality after pneumonia? A systematicreview and meta-analysis. Am J Med **125:**1111–23.

13. **Ma J, Wen X, Peng J, Lu Y, Guo Z, Lu J.** 2012. Systematic review and meta-analysis on the association between outpatient statins use and infectious disease-related mortality. PLoS ONE **7**:e51548.

14. **Liappis AP, Kan VL, Rochester CG, Simon GL**. 2001. The effect of statins on mortality in patients with bacteremia. Clin. Infect. Dis. **33**:1352–1357.



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Antimicrobial Agents and Chemotherapy

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15. **Nseir W, Mograbi J, Khateeb J, Abu-Elheja O, Bishara J, Jihad B, Assy N**. 2012. The impact of prior long-term versus short-term statin use on the mortality of bacteraemic patients. Infection **40**:41–48.

16. **Schlienger RG, Fedson DS, Jick SS, Jick H, Meier CR**. 2007. Statins and the risk of pneumonia: a population-based, nested case-control study. Pharmacotherapy **27**:325–332.

17. **Mortensen EM, Restrepo MI, Anzueto A, Pugh J**. 2005. The effect of prior statin use on 30-day mortality for patients hospitalized with community-acquired pneumonia. Respir. Res. **6**:doi:10.1186/1465–9921–6–82.

18. **Ridker PM, Danielson E, Fonseca FAH, Genest J, Gotto AM, Kastelein JJP, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ**. 2008. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N. Engl. J. Med. **359**:2195–2207.

19. **Novack V, MacFadyen J, Malhotra A, Almog Y, Glynn RJ, Ridker PM**. 2012. The effect of rosuvastatin on incident pneumonia: results from the JUPITER trial. CMAJ **184**:E367–372.

20. **Majumdar SR, McAlister FA, Eurich DT, Padwal RS, Marrie TJ**. 2006. Statins and outcomes in patients admitted to hospital with community acquired pneumonia: population based prospective cohort study. BMJ **333**:999.

21. **Mermis JD, Simpson SQ**. 2012. HMG-CoA reductase inhibitors for prevention and treatment of severe sepsis. Curr. Infect. Dis. Rep. **14**:484–492.



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Antimicrobial Agents and Chemotherapy

Downloaded from http://aac.asm.org/ on November 5, 2019 at UNIV COLLEGE CORK

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514 22. **Dobesh PP, Swahn SM, Peterson EJ, Olsen KM**. 2010. Statins in sepsis. J. Pharm.
515 Pract. **23**:38–49.

516 23. **Kouroumichakis I, Papanas N, Proikaki S, Zarogoulidis P, Maltezos E**. 2011. Statins
517 in prevention and treatment of severe sepsis and septic shock. Eur. J. Intern. Med. **22**:125–
518 133.

519 24. **Dobesh PP, Olsen KM.** 2014. Statins role in the prevention and treatment of sepsis.
520 Pharmacol Res. **88**:31-40.

521 25. **Wan YD, Sun TW, Kan QC, Guan FX, Zhang SG.** 2014. Effect of statin therapy on
522 mortality from infection and sepsis: a meta-analysis of randomized and observational
523 studies. Crit Care. **18(2):**R71.

524 26. **Tralhão AF, Cés de Souza-Dantas V, Salluh JI, Póvoa PM.** 2014. Impact of statins in
525 outcomes of septic patients: a systematic review. Postgrad Med **126(7):**45-58.

526 27. **Thomas G, Hraiech S, Loundou A, Truwit J, Kruger P, McAuley DF, Papazian L,**
527 **Roch A.** 2015. Statin therapy in critically-ill patients with severe sepsis: a review and
528 meta-analysis of randomized clinical trials. Minerva Anestesiol Feb 18. [Epub ahead of
529 print]

530 28. **Almog Y, Shefer A, Novack V, Maimon N, Barski L, Eizinger M, Friger M, Zeller L,**
531 **Danon A**. 2004. Prior statin therapy is associated with a decreased rate of severe sepsis.
532 Circulation **110**:880–885.



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Antimicrobial Agents and Chemotherapy
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Antimicrobial Agents and Chemotherapy

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533 29. **Mortensen EM, Restrepo MI, Copeland LA, Pugh JA, Anzueto A, Cornell JE,Pugh**
534 **MJ**. 2007. Impact of previous statin and angiotensin II receptor blocker use on mortality
535 in patients hospitalized with sepsis. Pharmacotherapy **27**:1619–26.__

536 30. **Martin CP, Talbert RL, Burgess DS, Peters JI.** 2007. Effectiveness of statins in
537 reducing the rate of severe sepsis: a retrospective evaluation. Pharmacotherapy **27**:20–6._

538 31. **Schmidt H, Hennen R, Keller A, Russ M, Müller-Werdan U, Werdan K, Buerke M.**
539 2006. Association of statin therapy and increased survival in patients with multiple organ
540 dysfunction syndrome. Intensive Care Med **32**:1248–51._

541 32. **Dessap AM, Ouanes I, Rana N, Borghi B, Bazin C, Katsahian S, Hulin A, Brun-**
542 **Buisson C.** 2011. Effects of discontinuing or continuing ongoing statin therapy in severe
543 sepsis and septicshock: a retrospective cohort study. Crit Care **15**:R171.

544 33. **Ou SY, Chu H, Chao PW, Ou SM, Lee YJ, Kuo SC, Li SY, Shih CJ, Chen YT.** 2014.
545 Effect of the use of low and high potency statins and sepsis outcomes. Intensive Care
546 Med. **40(10)**:1509-17. doi: 10.1007/s00134-014-3418-1. Epub 2014 Aug 5.

547 34. **Wu A, Good C, Downs JR, Fine MJ, Pugh MJ, Anzueto A, Mortensen EM.** 2014. The
548 association of cardioprotective medications with pneumonia-related outcomes. PLoS One.
549 28;9(1):e85797.

550 35. **Papazian L, Roch A, Charles PE, Penot-Ragon C, Perrin G, Roulier P, Goutorbe P,**
551 **Lefrant JY, Wiramus S, Jung B, Perbet S, Hernu R, Nau A, Baldesi O, Allardet-**
552 **Servent J, Baumstarck K, Jouve E, Moussa M, Hraiech S, Guervilly C, Forel JM;**
553 **STATIN-VAP Study Group.** 2013. Effect of statin therapy on mortality in patients with



Antimicrobial Agents and Chemotherapy
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Antimicrobial Agents and Chemotherapy
Downloaded from http://aac.asm.org/ on November 5, 2019 at UNIV COLLEGE CORK

Accepted Manuscript Posted Online

554 ventilator-associated pneumonia: a randomized clinical trial. JAMA. **310(16):**1692-700.
555 doi: 10.1001/jama.2013.280031.

556 36. **Mehl A, Harthug S, Lydersen S, Paulsen J, Åsvold BO, Solligård E, Damås JK, Edna**
557 **TH.** 2014. Prior statin use and 90-day mortality in Gram-negative and Gram-positive
558 bloodstream infection: a prospective observational study. Eur J Clin Microbiol Infect Dis
559 **34(3):**609-17.

560 37. **Jerwood S, Cohen J**. 2008. Unexpected antimicrobial effect of statins. J. Antimicrob.
561 Chemother. **61**:362–364.

562 38. **Bergman P, Linde C, Putsep K, Pohanka A, Normark S, Henriques-Normark B,**
563 **Andersson J, Bjorkhem-Bergman L**. 2011. Studies on the Antibacterial Effects of
564 Statins - *In Vitro* and *In Vivo*. PLoS One **6**:e24394.

565 39. **Masadeh M, Mhaidat N, Alzoubi K, Al-Azzam S, Alnasser Z**. 2012. Antibacterial
566 activity of statins: a comparative study of atorvastatin, simvastatin, and rosuvastatin. Ann.
567 Clin. Microbiol. Antimicrob. **11**:doi:10.1186/1476–0711–11–13.

568 40. **Welsh A-M, Kruger P, Faoagali J**. 2009. Antimicrobial action of atorvastatin and
569 rosuvastatin. Pathology **41**:689–691.

570 41. **Wang CC, Yang PW, Yang SF, Hsieh KP, Tseng SP, Lin YC.** 2015. Topical
571 simvastatin promotes healing of Staphylococcus aureus-contaminated cutaneous wounds.
572 Int Wound J. 2015 Mar 8. doi: 10.1111/iwj.12431. [Epub ahead of print]



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42. **Thangamani S, Mohammad H, Abushahba MF, Hamed MI, Sobreira TJ, Hedrick VE, Paul LN, Seleem MN.** 2015. Exploring simvastatin, an antihyperlipidemic drug, as a potential topical antibacterial agent. Sci Rep. **5:**16407. doi: 10.1038/srep16407.

43. **Graziano TS, Cuzzullin MC, Franco GC, Schwartz-Filho HO, de Andrade ED, Groppo FC, Cogo-Müller K.** 2015. Statins and Antimicrobial Effects: Simvastatin as a Potential Drug against Staphylococcus aureus Biofilm. PLoS One. 10(5):e0128098. doi: 10.1371/journal.pone.0128098. eCollection 2015.

44. **Van Laar TA, Lin YH, Miller CL, Karna SL, Chambers JP, Seshu J.** 2012. Effect of levels of acetate on the mevalonate pathway of Borrelia burgdorferi. PLoS One. 2012;7(5):e38171.

45. **Fothergill JL, Mowat E, Ledson MJ, Walshaw MJ, Winstanley C.** 2010. Fluctuations in phenotypes and genotypes within populations of Pseudomonas aeruginosa in the cystic fibrosis lung during pulmonary exacerbations. J Med Microbiol. **59(4)**:472-81.

46. **Cullen L, Weiser R, Olszak T, Maldonado RF, Moreira AS, Slachmuylders L, Brackman G, Paunova-Krasteva TS, Zarnowiec P, Czerwonka G, Reilly J, Drevinek P, Kaca W, Melter O, De Soyza A, Perry A, Winstanley C, Stoitsova SR, Lavigne R, Mahenthiralingam E, Sá-Correia I, Coenye T, Drulis-Kawa Z, Augustyniak D, Valvano MA, McClean S.** 2015. Phenotypic characterization of an international Pseudomonas aeruginosa reference panel: strains of cystic fibrosis (CF) origin show less in vivo virulence than non-CF strains. Microbiology. **161(10):**1961-77.

**47. Hovey JG1, Watson EL, Langford ML, Hildebrandt E, Bathala S, Bolland JR, Spadafora D, Mendz GL, McGee DJ.** 2007. Genetic microheterogeneity and phenotypic variation of Helicobacter pylori arginase in clinical isolates.BMC Microbiol. **7:**26.



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Downloaded from http://aac.asm.org/ on November 5, 2019 at UNIV COLLEGE CORK

Accepted Manuscript Posted Online

596 **48.** **Haeri MR, White K, Qharebeglou M, Ansar MM.** 2005. Cholesterol suppresses
597 antimicrobial effect of statins. Iran J Basic Med Sci. 2015 Dec;**18(12)**:1253-6.
598

599 49. **Heuston S, Begley M, Gahan CGM, Hill C**. 2012. Isoprenoid biosynthesis in bacterial
600 pathogens. Microbiology **158**:1389–1401.

601 50. **Eisenreich W, Bacher A, Arigoni D, Rohdich F**. 2004. Biosynthesis of isoprenoids via
602 the non-mevalonate pathway. Cell. Mol. Life Sci. **61**:1401–1426.

603 51. **Wilding EI, Kim DY, Bryant AP, Gwynn MN, Lunsford RD, McDevitt D, Myers JE,**
604 **Rosenberg M, Sylvester D, Stauffacher C V, Rodwell VW**. 2000. Essentiality,
605 expression, and characterization of the class II 3-hydroxy-3-methylglutaryl coenzyme A
606 reductase of *Staphylococcus aureus*. J. Bacteriol. **182**:5147–5152.

607 52. **Mattos KA, Oliveira VCG, Berrêdo-Pinho M, Amaral JJ, Antunes LCM, Melo RCN,**
608 **Acosta CCD, Moura DF, Olmo R, Han J, Rosa PS, Almeida PE, Finlay BB, Borchers**
609 **CH, Sarno EN, Bozza PT, Atella GC, Pessolani MC V**. 2014. *Mycobacterium leprae*
610 intracellular survival relies on cholesterol accumulation in infected macrophages: a
611 potential target for new drugs for leprosy treatment. Cell. Microbiol.

612 53. **Lobato LS, Rosa PS, Ferreira Jda S, Neumann Ada S, da Silva MG, do Nascimento**
613 **DC, Soares CT, Pedrini SC, Oliveira DS, Monteiro CP, Pereira GM, Ribeiro-Alves**
614 **M, Hacker MA, Moraes MO, Pessolani MC, Duarte RS, Lara FA.** 2014. Statins
615 increase rifampin mycobactericidal effect. Antimicrob Agents Chemother **58(10):**5766-
616 74.



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Downloaded from http://aac.asm.org/ on November 5, 2019 at UNIV COLLEGE CORK

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54. **Botelho-Nevers E, Espinosa L, Raoult D, Rolain J-M**. 2008. Lovastatin, but not pravastatin, limits *in vitro* infection due to *Coxiella burnetii*. J. Antimicrob. Chemother. **62**:845–847.

55. **Botelho-Nevers E, Rolain JM, Espinosa L, Raoult D**. 2008. Statins limit *Rickettsia conorii* infection in cells. Int. J. Antimicrob. Agents **32**:344–348.

56. **Erkkilä L, Jauhiainen M, Laitinen K, Haasio K, Tiirola T, Saikku P, Leinonen M**. 2005. Effect of simvastatin, an established lipid-lowering drug, on pulmonary *Chlamydia pneumoniae* infection in mice. Antimicrob. Agents Chemother. **49**:3959–3962.

57. **Tiirola T, Jauhiainen M, Erkkilä L, Bloigu A, Leinonen M, Haasio K, Laitinen K, Saikku P.** 2007. Effect of pravastatin treatment on *Chlamydia pneumoniae* infection, inflammation and serum lipids in NIH/S mice. Int J Antimicrob Agents **29(6):**741-2.

58. **Dechend R, Gieffers J, Dietz R, Joerres A, Rupp J, Luft FC, Maass M**. 2003. Hydroxymethylglutaryl coenzyme A reductase inhibition reduces *Chlamydia pneumoniae*-induced cell interaction and activation. Circulation **108**:261–265.

59. **Parihar SP, Guler R, Khutlang R, Lang DM, Hurdayal R, Mhlanga MM, Suzuki H, Marais AD, Brombacher F.** 2014. Statin therapy reduces the mycobacterium tuberculosis burden in human macrophages and in mice by enhancing autophagy and phagosome maturation. J Infect Dis **209(5):**754-63.




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Downloaded from http://aac.asm.org/ on November 5, 2019 at UNIV COLLEGE CORK

Accepted Manuscript Posted Online

60. **Parihar SP, Guler R, Lang DM, Suzuki H, Marais AD, Brombacher F**. 2013. Simvastatin Enhances Protection against *Listeria monocytogenes* Infection in Mice by Counteracting *Listeria*-Induced Phagosomal Escape. PLoS One **8**:e75490.

61. **Catron DM, Lange Y, Borensztajn J, Sylvester MD, Jones BD, Haldar K**. 2004. *Salmonella enterica* serovar Typhimurium requires nonsterol precursors of the cholesterol biosynthetic pathway for intracellular proliferation. Infect. Immun. **72**:1036–1042.

62. **Riethmüller J, Riehle A, Grassmé H, Gulbins E**. 2006. Membrane rafts in host-pathogen interactions. Biochim. Biophys. Acta **1758**:2139–2147.

63. **Hansen GH, Niels-Christiansen LL, Thorsen E, Immerdal L, Danielsen EM**. 2000. Cholesterol depletion of enterocytes. Effect on the Golgi complex and apical membrane trafficking. J. Biol. Chem. **275**:5136–5142.

64. **Boyd AR, Hinojosa CA, Rodriguez PJ, Orihuela CJ**. 2012. Impact of oral simvastatin therapy on acute lung injury in mice during pneumococcal pneumonia. BMC Microbiol. **12**:doi: 10.1186/1471–2180–12–73.

65. **McDowell SA, Ma Y, Kusano R, Akinbi HT**. 2011. Simvastatin is protective during *Staphylococcus aureus* pneumonia. Curr. Pharm. Biotechnol. **12**:1455–1462.

66. **Chow OA, von Kockritz-Blickwede M, Bright AT, Hensler ME, Zinkernagel AS, Cogen AL, Gallo RL, Monestier M, Wang Y, Glass CK, Nizet V**. 2010. Statins enhance formation of phagocyte extracellular traps. Cell. Host. Microbe **8**:445–454.



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67. **Hennessy E, Mooij MJ, Legendre C, Reen FJ, O'Callaghan J, Adams C, O'Gara F**. 2013. Statins inhibit in vitro virulence phenotypes of *Pseudomonas aeruginosa*. J. Antibiot. (Tokyo). **66**:99–101.

68. **Hennessy E, O'Callaghan J, Mooij MJ, Legendre C, Camacho-Vanegas O, Camacho SC, Adams C, Martignetti JA, O'Gara F**. 2014. The Impact of Simvastatin on Pulmonary Effectors of Pseudomonas aeruginosa Infection. PLoS One **9**:e102200.

69. **Shibata H, Nishitani N, Yaohara S, Arakaki N, Higuti T, Kawazoe K, Minakuchi K**. 2012. Simvastatin represses translocation of *Pseudomonas aeruginosa* across Madin-Darby canine kidney cell monolayers. J. Med. Invest. **59**:186–191.

70. **Horn MP, Knecht SM, Rushing FL, Birdsong J, Siddall CP, Johnson CM, Abraham TN, Brown A, Volk CB, Gammon K, Bishop DL, McKillip JL, McDowell SA**. 2008. Simvastatin inhibits *Staphylococcus aureus* host cell invasion through modulation of isoprenoid intermediates. J. Pharmacol. Exp. Ther. **326**:135–143.

71. **Burnham CA, Shokoples SE, Tyrrell GJ**. 2007. Rac1, RhoA, and Cdc42 participate in HeLa cell invasion by group B streptococcus. FEMS Microbiol. Lett. **272**:8–14.

72. **Martinez JJ, Hultgren SJ**. 2002. Requirement of Rho-family GTPases in the invasion of Type 1-piliated uropathogenic *Escherichia coli*. Cell Microbiol. **4**:19–28.

73. **Goldstein JL, Brown MS**. 1990. Regulation of the mevalonate pathway. Nature **343**:425–430.




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673 74. **O'Grady EP, Viteri DF, Sokol PA**. 2012. A unique regulator contributes to quorum
674 sensing and virulence in *Burkholderia cenocepacia*. PLoS One **7**:e37611.

675 75. **Rosch JW, Boyd AR, Hinojosa E, Pestina T, Hu Y, Persons DA, Orihuela CJ,**
676 **Tuomanen EI**. 2010. Statins protect against fulminant pneumococcal infection and
677 cytolysin toxicity in a mouse model of sickle cell disease. J. Clin. Invest. **120**:627–635.

678 76. **Pruefer D, Makowski J, Schnell M, Buerke U, Dahm M, Oelert H, Sibelius U,**
679 **Grandel U, Grimminger F, Seeger W, Meyer J, Darius H, Buerke M**. 2002.
680 Simvastatin inhibits inflammatory properties of *Staphylococcus aureus* alpha-toxin.
681 Circulation **106**:2104–2110.

682 77. **Tilahun ME, Kwan A, Natarajan K, Quinn M, Tilahun AY, Xie C, Margulies DH,**
683 **Osborne BA, Goldsby RA, Rajagopalan G**. 2011. Chimeric anti-staphylococcal
684 enterotoxin B antibodies and lovastatin act synergistically to provide *in vivo* protection
685 against lethal doses of SEB. PLoS One **6**:e27203.

686 78. **deCathelineau AM, Bokoch GM**. 2009. Inactivation of rho GTPases by statins attenuates
687 anthrax lethal toxin activity. Infect. Immun. **77**:348–359.

688 79. **Kothe H, Dalhoff K, Rupp J, Muller A, Kreuzer J, Maass M, Katus H a.** 2000.
689 Hydroxymethylglutaryl coenzyme A reductase inhibitors modify the inflammatory
690 response of human nacrophages and endothelial cells infected with *Chlamydia*
691 *pneumoniae*. Circulation **101**:1760–1763.



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692 80. **Ando H, Takamura T, Ota T, Nagai Y, Kobayashi K**. 2000. Cerivastatin improves
693 survival of mice with lipopolysaccharide-induced sepsis. J. Pharmacol. Exp. Ther.
694 **294**:1043–1046.

695 81. **Zhang S, Rahman M, Zhang S, Qi Z, Thorlacius H**. 2011. Simvastatin antagonizes
696 CD40L secretion, CXC chemokine formation, and pulmonary infiltration of neutrophils in
697 abdominal sepsis. J. Leukoc. Biol. **89**:735–742.

698 82 **Skerry C, Pinn ML, Bruiners N, Pine R, Gennaro ML, Karakousis PC.** 2014.
699 Simvastatin increases the in vivo activity of the first-line tuberculosis regimen. J
700 Antimicrob Chemother **69(9):**2453-7.

701 83. **Farmer AR, Murray CK, Mende K, Akers KS, Zera WC, Beckius ML, Yun HC**.
702 2013. Effect of HMG-CoA reductase inhibitors on antimicrobial susceptibilities for gram-
703 negative rods. J. Basic Microbiol. **53**:336–339.

704 84. **Kellick KA, Bottorff M, Toth PP**. 2014. A clinician's guide to statin drug-drug
705 interactions. J. Clin. Lipidol. **8**:S30–46.

706 85. **Patel AM, Shariff S, Bailey DG, Juurlink DN, Gandhi S, Mamdani M, Gomes T,**
707 **Fleet J, Hwang YJ, Garg AX**. 2013. Statin toxicity from macrolide antibiotic
708 coprescription: a population-based cohort study. Ann. Intern. Med. **158**:869–76.

709 86. **Lee AJ, Maddix DS.** 2001. Rhabdomyolysis Secondary to a Drug Interaction between
710 Simvastatin and Clarithromycin. The Annals of Pharmacotherapy, **35**:26-31.



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87. **Pasqualetti G, Bini G, Tognini S, Polini A, Monzani F,** 2012. Clarithromycin-induced rhabdomyolysis: a case report. International Journal of General Medicine, **5**:283-285.

88. **Trieu J, Emmett L, Perera C, Thanakrishnan K, Van Der Wall H.** 2004. Rhabdomyolysis resulting from interaction of simvastatin and clarithromycin demonstrated by Tc-99m MDP scintigraphy. Clin Nucl Med **29**:803-4.

89. **Campbell G, Jayakumar U, McCracken S, Bene J.** 2007. A cautionary tale: delayed onset rhabdomyolysis due to erythromycin/simvastatin interaction. Age Ageing **36:**597.

90. **Mah Ming JB, Gill M.** 2003. Case report: drug-induced rhabdomyolysis after concomitant use of clarithromycin, atorvastatin, and lopinavir/ ritonavir in a patient with HIV AIDS Patient Care STDS **17:**207-10.

91. **Page SR, Yee KC.** 2014. Rhabdomyolysis in association with simvastatin and dosage increment in clarithromycin. Intern Med J. **44(7):**690-3.

92. **Cully M.** 2013. Dyslipidaemia: Risks of statin and antibiotic coprescription. Nature Reviews Cardiology **10:**432.

93. *Biaxin Filmtab (clarithromycin tablets, USP), Biaxin XL Filmtab (clarithromycin extended-release tablets), Biaxin Granules (clarithromycin for oral suspension, USP).* Silver Spring (MD): US Food and Drug Administration; 2015. Available: www.fda .gov/Safety/MedWatch/SafetyInformation/ucm258816.htm (accessed 2016 Jan. 15).

94. **Li DQ**, **Kim R**, **McArthur E**, **Fleet JL**, **Bailey DG**, **Juurlink D**, **Shariff SZ**, **Gomes T**, **Mamdani M**, **Gandhi S**, **Dixon S**, **Garg AX**. 2015. Risk of adverse events among older



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731 adults following co-prescription of clarithromycin and statins not metabolized by
732 cytochrome P450 3A4. CMAJ 187(3):174-80.

733 95. **Golightly LK, Barber GR, Barron MA, Page RL**. 2013. Statins and daptomycin: safety
734 assessment of concurrent use and evaluation of drug interaction liability. Drug Metabol.
735 Drug Interact. **28**:49–58.

736

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740 **Figure Legends**

741 **Figure 1: Chemical structures of statins. A)** Type 1 statins are characterised by a conserved
742 lactone ring (blue), a decalin structure (black) and a butaryl side chain (red), which is different in
743 each statin. **B)** Type 2 statins differ from type 1 statins due to the replacement of the butaryl side
744 chain with a flurophenyl group (green), and although the lactone ring structure is conserved in all
745 statins, the decalin group of Type 1 statins is replaced by a longer distinct side chain. Statins
746 marked with an asterisk (*) are licensed to treat high cholesterol.

747

748 **Figure 2: Statins modulate bacterial growth and virulence. A)** *In vitro* effects of statins on
749 bacterial species. Statins reduce *in vitro* bacterial growth, motility and attachment. **B)** Key anti-
750 virulence mechanisms of statins. At physiological concentrations statin treatment can reduce
751 bacterial invasion and translocation, in addition to inhibiting lipid raft production. The inhibition
752 of Rho GTPase activity and cholesterol production by statins contribute to reduced bacterial
753 virulence, decreased toxicity and impaired intracellular survival. **C)** At physiological
754 concentrations statin treatment can reduce bacterial load and dissemination and increase bacterial
755 clearance in mouse models of infection.

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762 **Table 1:** MIC of statins against Gram-positive and Gram-negative bacteria

| Bacteria | Gram | Statin MIC mg/l | | | | | Ref |
|---|---|---|---|---|---|---|---|
| | | Sim | Fluv | Ator | Ros | Prav | |
| ***S. Aureus*** | pos | | | | | | |
| MSSA | | 16 - 63 | ~200 | 42 - >250 | 208 - 342 | >250 | 37, 39-43 |
| MSSA clinical isolate | | 60.42 | nt | 52.08 | 341.67 | nt | 39 |
| MRSA | | 32 - 167 | ~250 - >1024 | 83 - >1024 | 100 - >1024 | >250 - >1024 | 37, 39-40, 42-43 |
| MRSA clinical isolate | | 116.67 | nt | 108.33 | 500 | nt | 39 |
| VISA group of strains | | 32 | nt | nt | nt | nt | 42 |
| VRSA group of strains | | 32 - 64 | nt | nt | nt | nt | 42 |
| ***S. epidermidis*** | pos | | | | | | |
| Type strains | | 26 - 32 | nt | 21 | 167 | nt | 39, 42 |
| Clinical isolate | | 35 | nt | 20 | 233 | nt | 39 |
| ***S. Pneumoniae*** | pos | | | | | | |
| Type strains | | 16 - 167 | >123 | 104 | 333 | >50 | 38, 39, 42 |
| Clinical isolate | | 292 | nt | 229 | 417 | nt | 39 |
| ***Enterococci*** | pos | | | | | | |
| VSE | | 50 - 52 | 300 | 83 - 250 | 100 - 333 | nt | 37, 39, 40 |
| VSE clinical isolate | | 292 | nt | 96 | 333 | nt | 39 |
| VRE | | 30 - 104 | 500 | 167 - 250 | 100 - 500 | nt | 37, 39, 40 |
| VRE clinical isolate | | 292 | nt | 217 | 500 | nt | 39 |
| *E. faecalis* group of strains | | 32 | nt | nt | nt | nt | 42 |



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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***S. pyogenes*** | pos | | | | | | |
| ATTC19615 | | 62.5 | nt | 83.33 | 166.67 | nt | 39 |
| Clinical isolate | | 146 | nt | 133.33 | 275 | nt | 39 |
| ***L. monocytogenes*** | pos | | | | | | |
| Group of strains | | 32 | nt | nt | nt | nt | 42 |
| ***B. anthracis*** | pos | | | | | | |
| Type strains | | 16 | nt | nt | nt | nt | 42 |
| ***H. influenza*** | neg | | | | | | |
| Clinical isolate | | 146 - >250 | nt | 104 | 367 | nt | 38, 39 |
| ATTC29247 | | 52 | nt | 83 | 167 | nt | 39 |
| ***Moraxella catarrhalis*** | neg | | | | | | |
| Clinical isolate | | 16 | nt | nt | nt | nt | 38 |
| ***E. coli*** | neg | | | | | | |
| Type strains | | 52 - >250 | nt | 26 - >250 | 104 | >250 | 39, 40, 43 |
| O157:H7 ATCC 700728 | | >256 | nt | nt | nt | nt | 42 |
| Clinical isolate | | 112 | nt | 100 | 125 | nt | 39 |
| ***P. aeruginosa*** | neg | | | | | | |
| Type strains | | 166 - >1024 | >1024 | 83 - >1024 | 100 - >1024l | >250 - >1024 | 39, 40, 42, 43, 63 |
| Clinical isolate | | 121 | nt | 96 | 292 | nt | 39 |
| ***K. pneumoniae*** | neg | | | | | | |
| Type strains | | 167 - >256 | nt | 167 | 333 | nt | 39, 42 |
| Clinical isolate | | 242 | nt | 217 | 258 | nt | 39 |



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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***A. baumannii*** | neg | | | | | | |
| Type strains | | 104 - >256 | nt | 16 | 333 | nt | 39, 42 |
| Clinical isolate | | 32 | nt | 22 | 300 | nt | 39 |
| ***C. freundii*** | neg | | | | | | |
| ATTC 8090 | | 52 | nt | 83 | 167 | nt | 39 |
| Clinical isolate | | 133 | nt | 108 | 333 | nt | 39 |
| ***E. aerogenes*** | neg | | | | | | |
| ATTC 29751 | | 26 | nt | 16 | 104 | nt | 39 |
| Clinical isolate | | 33 | nt | 20 | 183 | nt | 39 |
| ***P. mirabilis*** | neg | | | | | | |
| ATTC 12459 | | 167 | nt | 63 | 250 | nt | 39 |
| Clinical isolate | | 146 | nt | 133 | 275 | nt | 39 |
| ***S. Tphimurium*** | neg | | | | | | |
| ATCC 700720 | | >256 | nt | nt | nt | nt | 42 |

763

764 Key: Sim, Simvastatin; Fluv, Fluvastatin; Ator, Atorvastatin; Ros, Rosuvastatin; Prav,

765 Pravastatin; pos, Gram-positive; neg, Gram-negative; nt, not tested.

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766 Table 2. Effect of statins on bacterial virulence and antibiotic activity

| | Virulence trait | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Reduced biofilm formation | Disrupt established biofilm | Motility | QS | Protease | T3SS ExoS | Increased adhesion to human cells | Reduced Invasion | | Trans-location | Toxin prodn | |
| | Statin (mg/l) | | | | | | | | | | | |
| Bacteria | Sim | Sim | Sim/Lov/Mev | | | | Sim | Sim | Mev | Sim | Sim | Ref |
| *S. aureus* | 0.98 - 62.5 | 62.5 | - | | | | - | - | - | - | - | 43 |
| | 62.5 | - | - | | | | - | - | - | - | - | 41 |
| | - | 64 | - | | | | - | - | - | ⇑ 40 | 40 | 42 |
| | - | - | - | | | | - | 0.04 - 0.4 | - | - | - | 66 |
| *S.epidermitis* | - | 128 | - | | | | - | - | - | - | - | 42 |
| *P. aeruginosa* | 4 & 40 | - | 40 | NC | | NC | 4 | NC | - | - | - | 63,64 |
| | - | - | - | | | | - | NC | - | ⇓ 2 | - | 65 |
| *Streptococcus* | - | - | - | | | | - | - | 4 | - | - | 67 |
| *E.coli* | - | - | - | | | | - | - | 4 | - | - | 68 |
| *B. cepacia* | - | - | - | NC | NC | | - | - | - | - | - | 70 |



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| | Antibiotic synergy | | | | | | |
|---|---|---|---|---|---|---|---|
| | *In vitro* | | | In vivo (mice) | | | |
| Bacteria | Antibiotic + statin | Statin conc. | Effect | Antibiotic + statin | Statin conc. | Effect | Ref |
| Pneumococci | Pen + Sim | 7.8 | ⇑ Autolysis | - | - | - | 38 |
| MRSA/VRSA | Mup/Fus/Dap + Sim | <32 | ⇓ Growth | - | - | - | 42 |
| *S. aureus* | Van + Sim | ? | NC | - | - | - | 43 |
| *M. tuberculosis* | Rif + Sim/Ator | 0.2microM | ⇓ Viability | - | - | - | 49 |
| | | | | Rif, Pyr, iso + sim | 25 mg/kg/d | ⇑ bacillary killing | 78 |
| *M. leprae* | Rif + Ator | 0.2microM | ⇓ Viability | Rif + Ator | 80 mg/kg/d | ⇓ Viability | 49 |
| *A. baumanii,* | Ami/Imi/Min + Prav/Sim/Ator/Fluv | - | NC | - | - | - | 79 |
| *P. aeruginosa* | Cip/Cep/Pip + Ator/Fluv | - | NC | - | - | - | 79 |
| *K. pneumoniae* | Cip/Cep/Pip + Ator/Fluv | - | NC | - | - | - | 79 |
| *E. coli* | Cip/Cep/Pip + Ator/Fluv | - | NC | - | - | - | 79 |

Key: Statins: Sim, Simvastatin; Ator, Atorvastatin; Prav, Pravastatin; Fluv, Fluvastatin. Antibiotics: Pen, penicillin; Mup, mupirocin; Fus, fusidic acid; Dap, daptomycin; Van, vancomycin; Rif, rifampicin; Pyr, pyrazinamide; Iso, isoniazid; Ami, amikacin; Imi,



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770 imipenem; Min, minocycline; Cip, ciprofloxacin; Cep, cefepime ; Pip, piperacillin



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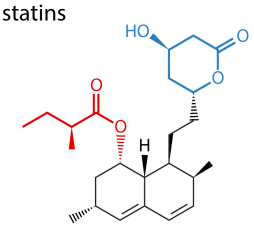
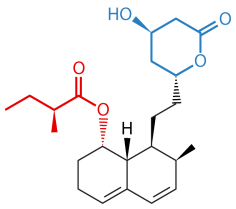
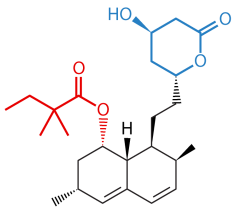
**A.** Type 1 statins



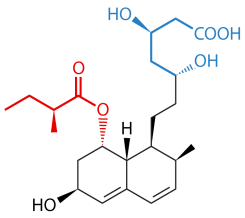
Lovastatin*



Mevastatin

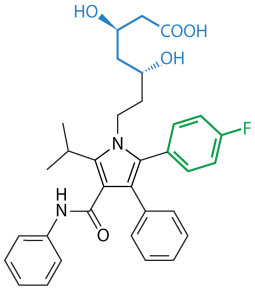


Simvastatin*

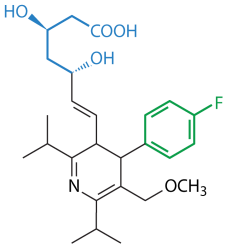


Pravastatin*

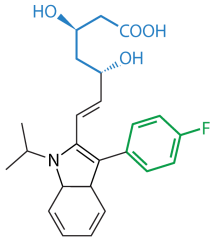
**B.** Type 2 statins



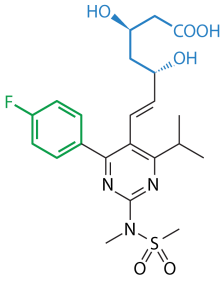
Atorvastatin*



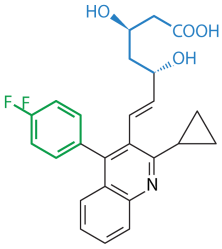
Cerivastatin



Fluvastatin*



Rosuvastatin*



Pitavastatin*

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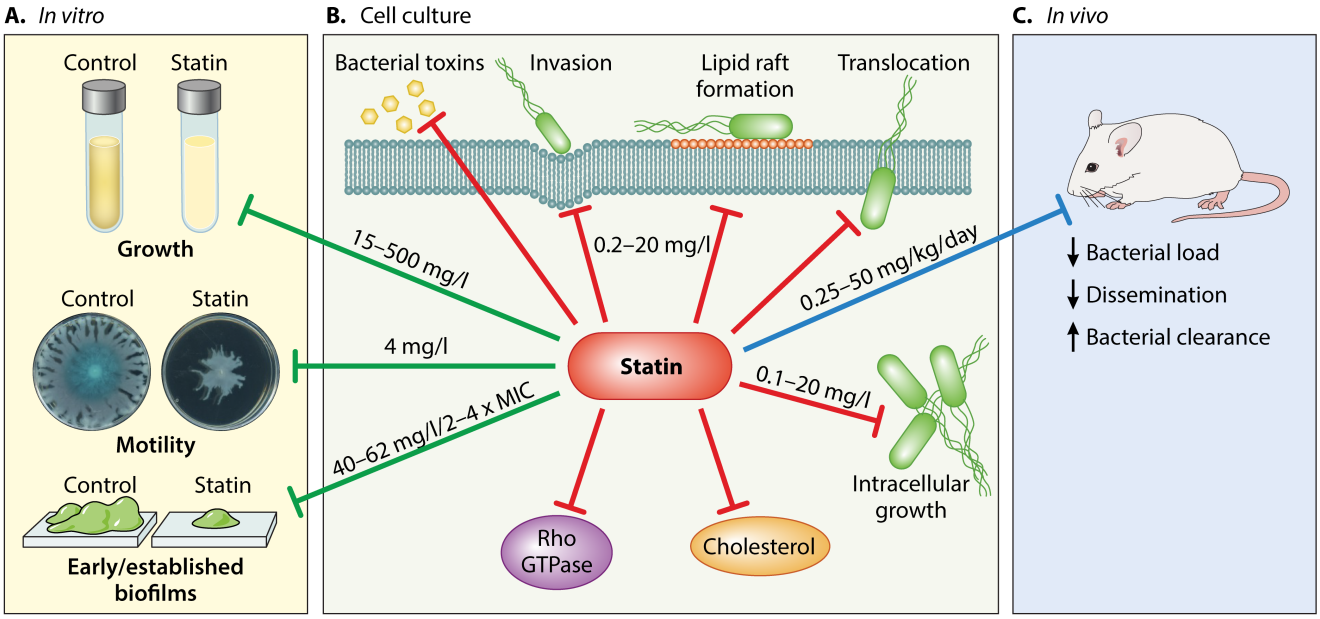


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