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EDITORIAL

Human immunodeficiency virus, atherosclerosis and *Chlamydophila pneumoniae*

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Abstract

Chlamydophila pneumoniae (*C. pneumoniae*) is an obligate, intracellular bacterium associated with a wide variety of acute and chronic diseases. *C. pneumoniae* infection is characterized by persistence and immunopathological damage to host target tissues, including the lung. Over the past 20 years, a variety of studies have investigated a possible link between *C. pneumoniae* infection and atherosclerosis, because of its role in all stages of atherosclerosis, from initial inflammatory lesions to plaque rupture. In the current highly active antiretroviral therapy (HAART) era, many human immunodeficiency virus (HIV)-infected patients are experiencing health problems that accompany the aging process, mainly the risk of cardiovascular disease (CVD). There is renewed interest in a link between atherosclerotic CVD and as yet poorly defined environmental exposures, including infectious agents. On the one hand, the patient with HIV and lipodystrophy caused by HAART and exacerbated by *C. pneumoniae* infection could be a factor of risk for atherosclerosis. An assessment of the therapy against *C. pneumoniae* and HAART should always be conducted. It is advisable that HIV-acquired immune deficiency syndrome patients undergo a serological test to determine exposure to *C. pneumoniae* and to assess treatment options. On the other hand, in patients with a positive serology to *C. pneumoniae*, an increment of the body mass index has been found; therefore, it is probable that the recurrent infection may play an important role in creating adverse endothelial conditions allowing the infection by *C. pneumoniae* in its chronic form, to damage the endothelial surface. Vascular studies would be necessary for corroboration.

Key words: Acquired immune deficiency syndrome; Atheroma; *Chlamydophila pneumoniae*; Immunobiology of infection; Obesity; Inflammation

Peer reviewer: Andrés Moya, Professor, Department of Genomics and Health, University of Valencia and Public Health Research Center of Valencia, Catedrático José Beltrán 2, Paterna 46980, Spain

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INTRODUCTION

According to World Health Organization estimates, 16.7 million people around the globe die of cardiovascular disease (CVD) each year[1]. Of the total annual CVD deaths, about 8.6 million occur in women. Heart attack and stroke deaths are responsible for twice as many deaths in women as all cancers combined[2]. In 2001, CVD contributed to nearly one-third of global deaths. Low and

middle income countries contributed to 85 percent of CVD deaths[3].

On the other hand, antiretroviral therapy has dramatically improved the life expectancy of patients with human immunodeficiency virus (HIV)[4]. In the present highly active antiretroviral therapy (HAART) era, many HIV-infected patients are experiencing health problems that accompany the aging process, mainly the risk of CVD. There is renewed interest in a link between atherosclerotic CVD and as yet poorly defined environmental exposures, including infectious agents. If epidemiologic and laboratory evidence eventually supports this association, atherosclerosis could emerge as another non-communicable chronic condition related to infection. *Chlamydophila pneumoniae* (*C. pneumoniae*) has been one of the most important agents associated with atherosclerosis. Two types of observational studies are used to study an association between *C. pneumoniae* and atherosclerosis: seroepidemiology and the demonstration of the organism in atherosclerotic tissue. The first suggestion that *C. pneumoniae* might be associated with atherosclerosis was in 1988 when Saikku *et al*[5] showed that persons with coronary artery disease (CAD) more frequently had *C. pneumoniae* antibody than population controls. These seroepidemiologic findings have now been confirmed by many investigators around the world, and several studies with similar findings have been reported. Mainly, there are three types of studies on the etiologic role for *C. pneumoniae* in atherosclerosis: animal models, possible mechanisms and clinical treatment trials. The animal model studies focus on determining if *C. pneumoniae* can initiate or accelerate the atherosclerotic process. Clinical trials will provide evidence for the role of *C. pneumoniae* in complications of atherosclerosis, usually myocardial infarction (MI) and related syndromes.

C. PNEUMONIAE AND ATHEROSCLEROSIS

C. pneumoniae is an important cause of pneumonia. Its prevalence was determined in community-acquired pneumonia during a period of 7 years in Italy. Serum samples from patients with pneumonia were evaluated using microimmunofluorescence (MIF) assays to detect *C. pneumoniae*-specific IgG and IgM antibodies. 12.5% patients complied with the diagnostic criteria of acute *C. pneumoniae* infection[6].

Implicating *C. pneumoniae* in the etiology of atherosclerosis is quite a complicated process because the Koch postulates should be fulfilled before concluding that this pathogen is causally involved in human atherosclerosis.

Lines of evidence associating *C. pneumoniae* with atherosclerosis include seroepidemiologic studies, direct detection of bacterial components in atherosclerotic lesions, occasional isolation of viable organisms from coronary and carotid atheromatous tissue, and *in vitro* and animal experiments (previewed in[7-9]). Most cross-sectional and prospective studies have correlated seroprevalence with MI, chronic coronary heart disease (CHD), or stroke[7]. My colleagues and I evaluated the association between CVD and antibodies against *Chlamydophila* in a Mexican population. Study subjects included 70 CVD hospitalized patients. Serum IgG and IgM antibodies against *C. pneumoniae* were determined by MIF and compared with those from 140 healthy individuals, matched by age and sex. IgG antibodies against *C. pneumoniae* were found in 94.3% patients, as compared to only 37% of healthy individuals ($P < 0.001$); therefore, an association between IgG antibodies against *C. pneumoniae* and CVD was found[10]. Likewise, we determined antibodies against *C. pneumoniae* in patients with acute MI and coronary risk factors. We studied 100 patients hospitalized in the Coronary Unit of La Raza Medical Center, Mexico. The patients had increased seropositivity for *C. pneumoniae* since 70% presented antibodies[11].

The strongest evidence associating *C. pneumoniae* with atherosclerotic CVD has been detection of bacterial components in atherosclerotic lesions. *C. pneumoniae* appears to display tropism for atheromas[9].

Detection of *C. pneumoniae* antigens or DNA in intimal thickened tissue and fatty streaks of young adults and Alaskan Natives (the latter group at low risk for coronary atherosclerosis) supports an early microbial role in its pathogenesis[12]. Postmortem, the Alaskan retrospective study also positively correlated with prior systemic infection due to the presence of *C. pneumoniae* in atherosclerotic lesions. Studies from Seattle reported a slightly higher detection rate in late stage lesions[9].

Detection of *C. pneumoniae* in plaques has not correlated well with serology[7,11], so investigators have attempted to predict endovascular infection through polymerase chain reaction (PCR) recognition of microbial DNA in peripheral blood monocytes. The prevalence of *C. pneumoniae* DNA in these mononuclear cells has varied between studies (perhaps due to differences in both assay sensitivity and extraction procedures), but was 59% in coronary angiography patients compared with 44% in blood donors in one series; the rate appears to increase with age[13,14]. However, some authors have not detected circulating *C. pneumoniae* in CAD[15].

In vitro studies support hypothesis that *C. pneumoniae* might directly promote atherosclerosis. Infection of human endothelial cells augments the production of inflammatory cytokines and modulates expression of adhesion molecules, enhancing recruitment of inflammatory leukocytes to the vessel wall[7]. Animal experiments have also explored the link between *C. pneumoniae* and atherosclerosis. Intranasal or intratracheal *C. pneumoniae* inoculation of New Zealand white rabbits fed a normal diet produced inflammatory changes of the aorta[16].

Inflammatory changes without foam cells and typical atheromas have also been observed in *C. pneumoniae* infected mice fed a normal chow diet[9,17]. However, inoculation of *C. pneumoniae* in mouse models led to aggravation of experimental atherosclerosis induced by a cholesterol-enriched diet[18].

C. PNEUMONIAE ANTIBODIES, ELEVATED BODY MASS INDEX AND CVD

The relationship between seropositivity for IgG antibod-

ies of *C. pneumoniae* and lipid levels has been studied[19]. The presence of IgG antibodies to *C. pneumoniae* is evidence of exposure to the organism but a single measurement will not distinguish between a primary infection, a repeat infection or a chronic infection. Previous studies showing an association between *C. pneumoniae* infection and lipid levels[20,21] largely ignored the role that confounding factors may have played in their findings. Several factors may confound this association: for example, low socioeconomic status, has been related to both elevated total cholesterol[22] and *C. pneumoniae* infection[23], although these are not consistent findings[24,25]. The date at which blood was collected may also have confounded the association. Seasonal variation in total cholesterol and high-density lipoprotein (HDL) cholesterol is a recognized phenomenon, with a total cholesterol peak[26,27] and HDL cholesterol trough[28] usually occurring during the winter months. The findings of the study[19] are consistent with the observation by Laurila *et al*[20], in a cross sectional study of male reindeer herders in Northern Finland, of decreased HDL, and HDL/total cholesterol ratio in subjects with serological evidence of *C. pneumoniae* infection. However, as in other studies of *C. pneumoniae* infection in which total cholesterol was measured[24,25,29], Laurila *et al*[21] did not observe an association between seropositivity and cholesterol levels, though in a follow up study, total cholesterol was increased in subjects with evidence of chronic infection.

The most commonly reported infection-induced lipid abnormalities in man and experimental animals have been decreased HDL, cholesterol and elevated triglycerides and very low density lipoprotein[30,31]. These effects are part of the acute phase response, appear to be mediated by cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1, and IL-6[31], and have been observed in persons with serological evidence of *C. pneumoniae* infection[20]. The acute phase response to infection may therefore explain the association observed in the study[19], with *C. pneumoniae* seropositivity and lowered HDL cholesterol but does not convincingly explain the elevated total cholesterol. The lipid response to chronic infection may be different from that which occurs in acute infection.

This was the first study[19] to examine the relation between infection with *C. pneumoniae* and lipid levels in both men and women. In both sexes, an effect on total cholesterol in the same direction was documented, but infected men had lower HDL cholesterol concentrations while infected women had higher concentrations. An epidemiological robust association[19] was demonstrated between an atherogenic lipid profile and evidence of infection with *C. pneumoniae* and although the possible mechanism of action may not be clear, this finding may have substantial public health implications. If treatment of *C. pneumoniae* infection resulted in a sustained reduction in total cholesterol similar in magnitude to the increase seen in infected subjects in this study, data from meta-analyses of cholesterol lowering trials[32] would suggest an expected decrease in the risk of ischemic heart disease in treated individuals (potentially 70% of the population) of at least 20%.

Manifestations of CVD have been also associated with chronic infection by *Helicobacter pylori* (*H. pylori*). This was investigated in subjects classified according to serology titers due to infection with *C. pneumoniae* and *H. pylori*, association between seropositivity and the degree of obesity and fasting insulin levels, as well as social factors. Frozen samples from serum of 310 middle-aged treated hypertensive and 288 age-matched and gender-matched normotensive controls from a defined population were analyzed. The body mass index (BMI) was calculated as kg/m^2. Fasting blood samples were drawn for measurement of serum lipid levels, blood glucose, plasma insulin and serum lipids, including total cholesterol and triglycerides. The titers for *C. pneumoniae* were determined by MIF. Subjects with combined positive serology for *H. pylori* and *C. pneumoniae* are characterized by greater age, lower socioeconomic class and higher BMI, as well as higher fasting levels of insulin compared to seronegative subjects. Obesity might be a marker not only for lower socioeconomic class but also for a greater than normal susceptibility to such infections[33].

C. pneumoniae infection has been linked to the development of CHD, but its relationship to CHD risk factors is less clear. The relation between past infection with *C. pneumoniae* and risk factors for CHD, including body weight amongst subjects with and without CHD was determined. Antibodies to *C. pneumoniae* and a range of CHD risk factors were measured in 170 subjects, of whom 43 had recent onset angina. The prevalence of seropositivity was similar for subjects with and without CHD and for those with or without hypertension. However on multivariate analysis, only BMI remained significant ($P < 0.05$). Although the study failed to find a greater prevalence of antibodies to *C. pneumoniae* amongst subjects with recent onset angina, there were associations with a number of cardiovascular risk factors. An increase in body weight appears to underscore these relationships[34,35].

Whether serum chlamydial lipopolysaccharide (cLPS), *C. pneumoniae* antibodies and high-sensitivity C-reactive protein (hsCRP) levels are associated with BMI was explored. The study population consisted of 174 patients with symptomatic carotid stenosis, abdominal aortic aneurysm or occlusive aortic disease. Information on BMI, diabetes, smoking, hypercholesterolemia, and statin therapy was available. Serum *C. pneumoniae* IgG and IgA antibodies, cLPS, hsCRP and total endotoxin acitivity (totLPS) were measured. BMI correlated with cLPS ($r = 0.197$, $P < 0.01$) and with hsCRP (rho = 0.195, $P < 0.01$); in addition, there was a positive correlation between cLPS and hsCRP (rho = 0.499, $P < 0.01$). A trend of an increasing proportion of *C. pneumoniae* IgG positivity (titer $\geqslant 64$, $P = 0.018$) and higher serum cLPS ($P = 0.01$) concentrations was observed across the BMI range. Elevated serum cLPS levels were associated with an elevated BMI. This is a novel finding and it strengthens the link between

chlamydial infection and obesity. A lack of association between totLPS and BMI suggests that the association between infection and an elevated BMI may be specific to certain pathogens[36].

C. PNEUMONIAE INFECTION AND HIV

In 1991, the case of an HIV infected adult with *C. pneumoniae* was reported. The patient presented with a clinical picture suggestive of *Pneumocystis carinii* pneumonia (PCP) but did not respond to standard anti-PCP therapy. The diagnosis was eventually confirmed by bronchoscopy and serology. *C. pneumoniae* pneumonia should be considered in the differential of pathogens that cause interstitial infiltrates in HIV infected individuals[37].

Seven hundred and sixty-four healthy subjects, 96 HIV infected and 50 children with vertically transmitted HIV infection were studied. In the HIV infected population, *C. pneumoniae* seroprevalence was higher than in immunocompetent controls (children, 26% *vs* 11%; adults, 60% *vs* 40%). HIV infected subjects seem to be at higher risk of developing *C. pneumoniae* infections. Further studies are needed to elucidate fully the pathogenic role of *C. pneumoniae* in HIV infected subjects, because this high antibody prevalence could be the result of either a greater rate of infection in immunocompromised subjects or a polyclonal immunoglobulin activation commonly found in HIV patients[38].

The significance of chlamydia serum IgG and IgA antibodies was studied by immunoperoxidase assay in 210 homosexual men at various stages of HIV infection. Cross-sectional analysis of chlamydia IgG antibodies indicated a significantly higher prevalence rate among acquired immune deficiency syndrome (AIDS) patients (27%) as compared to asymptomatic HIV seronegative subjects (6.0%) (P = 0.022). The geometric mean titer of IgG antibodies to chlamydia was also significantly higher in AIDS patients (106.4) as compared to HIV seronegative subjects (58.2) (P = 0.022)[39]. The prevalence of *C. pneumoniae* antibodies was evaluated in an Italian population of HIV infected and uninfected individuals in relation to the presence of HIV risk factors. A statistically significant higher *C. pneumoniae* seroprevalence was found to be related, by multivariate analysis, to sex, age, and presence of HIV risk factors but not to the presence of HIV infection itself. Likewise, HIV infected subjects seem to have progressively lost their *C. pneumoniae* IgG antibodies in mid and advanced stages of HIV infection. High *C. pneumoniae* IgG titers are rarely found in advanced stage HIV infected patients[40]. Later, the same authors described *C. pneumoniae* infection in patients seropositive for the HIV. They concluded that *C. pneumoniae* is a possible cause of severe respiratory infection in Italian HIV infected immunocompromised patients, and its presence must be suspected when patients do not respond to therapy with beta lactam agents or to anti-*Pneumocystis carinii* treatment[41].

The incidence of *C. pneumoniae* respiratory tract infection was determined in HIV positive or AIDS patients. Twenty of 82 patients were found to have IgG antibodies to *C. pneumoniae* at titers ranging between 1:16 and 1:1024. Seven of the patients had evidence of acute *C. pneumoniae* infection. Results of this study indicate that *C. pneumoniae* may play a role in the etiology of respiratory tract infections in HIV positive patients; this fact should affect empirical antibiotic therapy[42].

On the other hand, *C. pneumoniae* DNA was investigated by PCR, in cerebrospinal fluid (CSF) specimens from patients suffering from HIV-associated dementia complex (HADC). Four (17.3%) cases of *C. pneumoniae* infection were identified among 23 HADC individuals with DNA amplification of the major outer membrane protein gene and 16S rRNA gene sequences. Sequence analysis revealed significant homologies with *C. pneumoniae* compared to *Chlamydia trachomatis* (*C. trachomatis*) and *Chlamydophila psittaci* (*C. psittaci*). High mean levels of CSF specific anti-*C. pneumoniae* antibodies and *C. pneumoniae* antibody specific index values were significantly elevated by enzyme-linked immunosorbent assay in these patients. The results suggest a hypothetical role for *C. pneumoniae* in the pathogenesis or progression of HADC[43].

C. pneumoniae seropositivity is associated with CVD and HIV infection. Cell-mediated immune responses are important in the control of *Chlamydia pneumoniae*, and such responses may be impaired in HIV infected patients. An assay for detection of interferon (IFN)-γ in whole blood stimulated with *Chlamydia pneumoniae* antigen was developed and studied in HIV infected patients and uninfected controls. Among 34 HIV infected patients, none had an IFN-γ response to *C. pneumoniae* antigen, compared with five of 32 healthy controls ($P < 0.001$). Fewer HIV infected individuals elicited a serum IgG response when tested with a commercial enzyme immunoassay (P = 0.009), but this was not so for serum IgA (P = 0.12). Additionally, the IFN-γ and antibody assays showed a trend towards a bivariate response in normal controls. This indicates that cellular and antibody responses against *C. pneumoniae* may be mutually exclusive, with potential implications for the role of this organism in the genesis of CVD in both immunocompetent and HIV infected populations[44].

To better understand the possible role of *C. pneumoniae* infection in the pathogenesis of epi-aortic lesions in HIV positive patients, the presence of anti-*C. pneumoniae* antibodies was evaluated in a group of individuals subjected to ultrasonography of the epi-aortic vessels. The presence of specific antibodies in 129 subjects was determined; 59 patients were HIV positive, 30 had carotid plaques and 29 had no lesions. The control group was composed of 70 subjects. All were subjected to ultrasonography of the epi-aortic vessels. IgG, IgM and IgA anti-*C. pneumoniae* antibodies were measured with MIF and positive sera were tested for *C. trachomatis* and *C. psittaci*. No subjects were positive for IgM. Neither IgA nor IgG levels differed significantly in the three groups. The only highly significant variable was the use of protease inhibitors. Results suggest that the damage to the carotid wall in

HIV patients was not due to *C. pneumoniae*[45]. In contrast, our team determined whether the infection by *C. pneumoniae* is a risk factor for atherosclerosis in patients with AIDS. A case-control study of 43 patients with AIDS under HAART (16 cases and 27 controls) was conducted. To document atherosclerosis, a carotid and transcranial Doppler ultrasound was performed. Anti-*C. pneumoniae* antibodies were determined using a MIF test for IgM and IgG levels. To study the atherosclerosis risk factors, odds ratios were calculated for each IgG anti-*C. pneumoniae* antibody titer. A titer of 1:64 significantly increased the risk of atherosclerosis. These results suggest that hypertriglyceridemia and *C. pneumoniae* infection coexistence significantly increases the risk of atherosclerosis. The inverse geometric average of the antibody titers against *C. pneumoniae* in individuals with atheromatous plaque fell to 64, (two titers above controls). This difference turned out to be statistically significant. Exposure to *C. pneumoniae* with positive antibody (IgG) titers should be considered in any HIV diagnosed patient as a risk factor for atherosclerosis, having found that the inverse geometric averages of antibody titers are significantly different when comparing cases and controls, especially in patients with dyslipidemia, hypertriglyceridemia or in patients whose treatments could cause these conditions. An assessment of the therapy against *C. pneumoniae* and HAART should always be conducted. When we studied patients with concomitant hypertriglyceridemia, we found that the association increased three-fold. It is advisable to serologically test HIV-AIDS patients to determine exposure to *C. Pneumoniae* and to assess treatment options[46].

ATHEROMAS IN HIV AND *C. PNEUMONIA* INFECTED INDIVIDUALS

The development of anti-HIV antiretroviral therapies has significantly prolonged the life span of infected individuals, leading to the need to further understand the virus' impact on chronic disease processes such as atherosclerosis[47].

HIV infection is associated to accelerated atherosclerosis and vasculopathy, and although the involved mechanisms have yet to be elucidated, there are observations substantiating it: (1) An increase in the prevalence of cardiac risk factors in HIV-infected individuals; (2) The dyslipidemia reported after therapy with certain anti-HIV antiretrovirals; and (3) The pro-inflammatory effects due to monocyte/macrophage infiltration in HIV-infected individuals[47].

It has not been determined whether HIV *per se* can infect smooth muscle cells (SMCs) and hence hastens the development of vascular disease[47].

Atherosclerotic lesions are characterized by thickening of the intima and plaque formation, consisting of fibrous tissue encapsulating a lipid core. These lesions have SMCs some of which contain phagocytosed lipids. The presence of abundant macrophages is also recognized, particularly surrounding the lipid core: this is known as an atheroma. Macrophages play a crucial role in lipid phagocytosis and their function is referred to as "scavenging" since they attempt to remove the fat particles infiltrating the intima. These macrophages may "superphagocytose" and lead to cell death and subsequent "fat spillover" thus leading to atheroma formation. In terms of pathogenesis, atherosclerosis has been mainly considered a lipid disorder whereby an excessive lipid influx within the arterial wall and macrophage activity eventually foment lesion development in an attempt to eliminate lipids. It is currently known that macrophages do not only act as scavenger cells but rather as veritable immunocompetent cells that interact with other inflammatory cells such as lymphocytes and mast cells[48].

Immunohistochemical analysis of atherosclerotic plaques reveal the presence of macrophages and T lymphocytes, frequently in close cell to cell contact[49,50]. Furthermore, a variable number of these T lymphocytes appear to be activated[51]. Aside from macrophages and T lymphocytes, the atherosclerotic plaque also appears to contain clusters of activated mast cells, particularly in the posterior area of the lesions[52]. These aforementioned observations have led to the understanding that an immune intraplaque mediating cellular function is present within the atherosclerotic plaque. Consequently, the release of inflammation mediators or cytokines such as growth factors and other proteins from these cells may regulate a vast variety of cellular processes affecting the components of the atherosclerotic plaque; this would lead to a fine balance promoting plaque instability and its complications or, its stabilization[53].

Cytokines tend to be Th2 cell dependent, such as IFN-γ that may inhibit smooth muscle proliferation and decrease its ability to synthesize extracellular matrix. IFN-γ also increases the recruitment of immunocompetent cells thus regulating the expression of adhesion molecules on endothelial cells and playing an important role in the perpetuation of the inflammatory process. Other cytokines such as IL-1 and TNF may induce SMC apoptosis[54].

Macrophages destabilize plaque by releasing matrix metalloproteinases (MMPs). The macrophage layer, particularly when foam-laden, produces several MMPs including interstitial collagenase (MMP-1), stromelysin (MMP-3) and gelatinases MMP-2 and MMP-9. The release of these proteinases is controlled by inflammatory cytokines such as TNF and IL-1. Mast cells may orchestrate this cytokine release since the severity of the coronary syndrome correlates directly with their activation and not with that of macrophages or T cells; mast cells may thus play a functional role since they can release neutral proteases such as tryptase and chymase, both capable of destroying components of the extracellular matrix[55].

Finally, we must mention another mechanism operating in the process of immune activation within the arterial wall and it relates to the influx of modified lipoproteins such as oxidized low-density lipoprotein (OxLDL) within macrophages; these can induce non-antigen specific immune activation, transforming monocytes into foam cells

that in turn, lead to functional changes such as the release of growth factors, cytokines and enzymes. The specific activation of the adaptive immune response may play an important role in atherosclerotic disease; some studies have even shown increased levels of anti-OxLDL antibodies as well as elevated titers against infectious agents. Some implicated microorganisms include *c, H. pylori* and *C. pneumoniae*, the most suspect. A relationship between MI and CAD has been established with antibody titers against *C. pneumoniae*, as first described by Saikku *et al*[5] and subsequently confirmed by other groups[56]. The fact that chronic or past exposure to the above mentioned microorganisms exacerbates inflammation and contributes to atherosclerosis has also been reported[57].

Chlamydia pneumoniae has been detected in atherosclerotic lesions *via* several methods such as electron microscopy, immunohistochemistry, PCR and the microorganism has also been cultured from atherosclerotic plaques[8].

HIV infection has been associated with abnormal lipid profiles[58] and HAART may also cause lipid abnormalities and insulin resistance, both risk factors for atherosclerosis[59]. However, the role of HIV infection *per se* and its effects on the vascular wall in atherosclerosis pathogenesis are not well understood, although the effect of HIV infection on endothelial dysfunction and HIV infiltration of macrophages and monocytes has been studied[60]. It has been shown that arterial SMCs express three relevant HIV receptors: CD4, CCR5 and CXCR4. They play a role in leukocyte viral entry[61]. SMCs are the predominant cell type in arteries.

One possibility correlating HIV infection and atheroma formation suggests that HIV can directly infect SMCs and if so, hasten vascular disease. SMC infection by HIV has been demonstrated *in vivo* and *in vitro*. HIV p24 protein has been detected by confocal fluorescence microscopy in SMCs of atherosclerotic plaques from HIV-infected individuals. Also, SMCs can be infected *in vitro via* CD4-dependent mechanisms, CXCR4 or CCR5 chemokine receptors and endocytosis; this leads to a marked secretion of CCl2/MCP-1 by SMCs, a critical mediator of atherosclerosis. SMC infection by HIV may be part of a multifactorial mechanism that could explain the exacerbated atherosclerosis and vasculopathy seen in HIV-infected individuals[47].

DYSLIPIDEMIA, METABOLIC INFLAMMATORY CHANGES AND HIV

CVD risk associated with the fat redistribution seen among HIV infected individuals remains unexplained, but may be increased due to associated hyperlipidemia, hyperinsulinemia, increased visceral adiposity, and the prothrombotic state associated with these metabolic abnormalities[62]. Increased tissue type plasminogen activator (tPA) antigen, a marker of impaired fibrinolysis, predicts increased risk of CAD mortality among patients with a history of angina pectoris and CVD[63], as well as cerebrovascular events among individuals without a prior history of CVD[64]. Further, there is a strong association between hyperinsulinemia and impaired glucose tolerance and levels of plasminogen activator inhibitor-1 (PAI-1) and tPA in otherwise healthy adults[65], suggesting a mechanistic link between insulin resistant states and increased CVD. PAI-1 and tPA markers of fibrinolysis were characterized and correlated with an increased CVD risk in HIV positive lipodystrophic patients compared with controls. The effect of treatment with metformin on PAI-1 and tPA antigen levels in patients with HIV associated fat redistribution was also analyzed. The data demonstrated impaired fibrinolysis in association with hyperinsulinemia and an increased waist to hip ratio in patients with HIV infection and fat redistribution. This suggests that metformin improves the overall cardiovascular risk profile in HIV infected patients with fat redistribution[66].

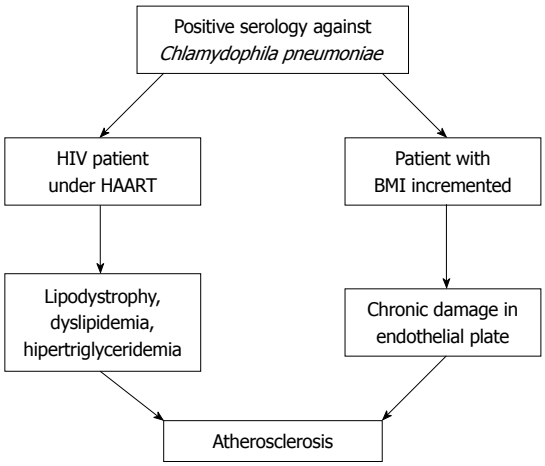

Figure 1 In the patient co infected with human immunodeficiency virus /chlamydia, under highly active antiretroviral therapy, a factor of risk can occur to develop lipodystrophy and other disorders of the metabolism of lipids as dyslipidemia or hipertriglyceridemia, conducting finally to atherosclerosis. Thus same, in patients with re infections and obesity, would be able to occur an activation in the mechanism of inflammation that has an important role in the pathogenesis of atherosclerosis resulting in a cycle of inflammation, activation and continuous cell recruitment.

We would suggest that (Figure 1) on the one hand, the patient with HIV and lipodystrophy caused by HAART and exacerbated by *C. pneumoniae* infection, could be a risk factor for atherosclerosis. An assessment of the therapy against *C. pneumoniae* and HAART should always be carried out. It is advisable that HIV-AIDS patients undergo a serological test to determine exposure to *C. pneumoniae*, and to assess treatment options. On the other hand, in patients with a positive serology to *C. pneumoniae,* an increase in BMI has been found; therefore, it is probable that the recurrent infection may play an important role in creating adverse endothelial conditions that allow the infection by *C. pneumoniae* in its chronic form to damage the endothelial plate. Vascular studies would be necessary for corroboration. In obesity, adipose tissue is inflamed and many inflammatory molecules, such as IL-6 and TNF, are produced. This low-grade inflammation in adipose tissue induces insulin resistance and obesity, which are linked to metabolic syndrome, type 2 diabetes and CHD. Slightly

elevated serum C-reactive protein (CRP) levels act as a marker of systemic inflammation, and have been shown to increase the risk of CHD. The synthesis of CRP is regulated by IL-6, which has been assumed to originate largely from adipose tissue.

CONCLUSION

C. pneumoniae is an obligate, intracellular bacterium associated with a wide variety of acute and chronic diseases. *C. pneumoniae* infection is characterized by persistence and immunopathological damage to host target tissues, including the lung. Over the past 20 years, a variety of studies have investigated a possible link between *C. pneumoniae* infection and atherosclerosis, because of its role in all stages of atherosclerosis, from initial inflammatory lesions to plaque rupture. In the present HAART era, many HIV-infected patients are experiencing the health problems that accompany the aging process, mainly the risk of CVD. There is renewed interest in a link between atherosclerotic CVD and as yet poorly defined environmental exposures, including infectious agents. In addition, there is an association between possible chronic *C. pneumoniae* infection and obesity.

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TOPIC HIGHLIGHT

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Transcribing virulence in *Staphylococcus aureus*

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Abstract

Staphylococcus aureus (*S. aureus*) is an important human pathogen capable of causing a diverse range of infections. Once regarded as an opportunistic pathogen causing primarily nosocomial infections, recent years have seen the emergence of *S. aureus* strains capable of causing serious infection even in otherwise healthy human hosts. There has been much debate about whether this transition is a function of unique genotypic characteristics or differences in the expression of conserved virulence factors, but irrespective of this debate it is clear that the ability of *S. aureus* to cause infection in all of its diverse forms is heavily influenced by its ability to modulate gene expression in response to changing conditions within the human host. Indeed, the *S. aureus* genome encodes more than 100 transcriptional regulators that modulate the production of virulence factors either directly *via* interactions with *cis* elements associated with genes encoding virulence factors or indirectly through their complex interactions with each other. The goal of this review is to summarize recent work describing these regulators and their contribution to defining *S. aureus* as a human pathogen.

Key words: *Staphylococcus aureus*; Regulatory elements; DNA-binding proteins; Two-component systems; Virulence factors

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a rapidly evolving human pathogen that is a leading cause of both chronic, biofilm-associated infections and acute, life-threatening toxemias. Its ability to cause these infections is dependent on its ability to coordinate the production of a myriad of virulence factors. These include exopolysaccharides, surface-associated protein adhesins, immune modulators, and extracellular proteins including a plethora of toxins. *S. aureus* employs an equally remarkable array of regulatory elements to coordinate the production of these virulence factors. These elements include (1) small, non-coding RNAs[1]; (2) alternative sigma factors (σ^B, σ^H, and σ^S) responsive to various stress conditions[2]; and (3) *trans*-acting transcriptional regulators. It is the latter two groups that is the focus of this review. Indeed, one of the first reports of a full *S. aureus* genome sequence identified 124

open-reading frames likely to encode transcriptional regulators, 89 of which had not been previously identified[3]. Generally speaking, these are DNA-binding proteins, although some have also been shown to modulate virulence phenotypes *via* direct interactions with mRNA[4]. In general, these factors, some upon activation, are capable of binding a specific sequence associated with their target genes and thereby either enhance or inhibit transcription, although in many cases it has proven difficult to identify a definitive recognition site. Many of these targets are themselves regulatory factors, thus creating a complex network of virulence gene expression.

Because DNA-binding proteins are located in the cytoplasm, it is imperative to have a mechanism of sensing the external environment and translating that information into an intracellular change in gene expression. As with other pathogens, this is often accomplished *via* two-component systems. In some cases, the activating signals for these systems are known, but in most cases they have not been defined. *S. aureus* also produces many transcriptional regulators that are not associated with a recognized two-component system, and the activating signals for most of these also remain undefined. Nevertheless, an important role in virulence has been established for many of these regulators, and summarizing these is the focus of this review.

TWO-COMPONENT SYSTEMS

Bacterial two-component systems create a communication bridge to the external environment, allowing the cell to translate an external stimulus into an intracellular change in gene expression. The defining components are a membrane-associated sensor histidine kinase (HK) and a cytoplasmic response regulator (RR). The genes encoding these components are often arranged in an operon, and therefore co-transcribed, with other genes involved in the same signaling pathway. After activation from an external signal, the HK typically dimerizes and trans-autophosphorylates[5]. This leads to phosphorylation of the RR, characteristically at a conserved aspartic acid residue. Phosphorylation of the RR induces a conformational change allowing it to bind DNA at a specific consensus sequence in a manner that alters transcription of the target gene. Based on homology with recognized sensors and response regulators, *S. aureus* has at least 16 two-component systems[6]. In addition to their role in pathogenesis, at least three of these systems have been shown to modulate resistance to antibacterial agents[7], thus further emphasizing their important role in pathogenesis of *S. aureus* infection.

AgrAC

The most definitively characterized two-component system in *S. aureus* is the accessory gene regulator (*agr*), which was first identified as a transposon-insertion mutant with a reduced capacity to produce multiple exotoxins[8]. AgrC is the sensor kinase, and it is responsive to the accumulation of an extracellular auto-inducing peptide (AIP) that is encoded by *agrD* and processed for export by AgrB. Induction occurs *in vitro* as cultures enter the post-exponential growth phase as the AIP accumulates, thus making *agr* a prototype quorum-sensing regulatory system. This peptide recognition is a unique feature of Gram-positive quorum-sensing systems, as Gram-negative organisms sense small molecules, typically homoserine lactones[9], rather than peptides. While *agr* itself is highly conserved, variation in the AIP and its AgrC receptor define interference groups, with the AIP of each group inducing the expression upon interaction with its cognate receptor but inhibiting induction upon interaction with the receptor from each of the other groups[10].

After induction by AIP, AgrC autophosphorylates[11] and then phosphorylates AgrA, which is the cytoplasmic response regulator that until recently was thought to bind and activate only the *agr*-associated P2 and P3 promoters. Induction of the P2 promoter leads to increased transcription of the *agr* operon (*agrABCD*), resulting in a positive feedback loop, while induction of the P3 promoter results in increased transcription of RNAIII, with the latter being a primary downstream effector of the *agr* system[12].

AgrA was also recently shown to bind the promoter region of the gene clusters encoding phenol-soluble modulins (PSMs)[13]. PSMs are small toxins that lyse human neutrophils[14], a key host defense against staphylococcal infection. While PSMs are found in virtually all *S. aureus* isolates, the levels in which they are produced vary widely among different strains due to differences in the level of *agr* expression. This has been correlated with increased virulence in several animal models of *S. aureus* infection, although not necessarily owing to the increased production of PSMs alone[15-17].

RNAIII is the effector molecule of the *agr* regulatory system. Although RNAIII includes the gene encoding delta-toxin, which is itself a PSM[18], its primary contribution to virulence is regulatory. It is a stable RNA characterized by 14 stem-loop structures and two long helices separating two independent domains[19], and it is these stem-loops that are responsible for its regulatory effects[1]. RNAIII production is induced by the binding of phosphorylated AgrA to the P3 promoter, thus accounting for its increased production *in vitro* as cultures enter the post-exponential growth phase and AIP accumulates to a critical threshold. In general, induction results in reduced production of surface-associated proteins and enhanced production of exotoxins[20].

The phenotype of an RNAIII mutant is characterized by major changes at the transcriptional level. However, RNAIII itself functions primarily at a post-transcriptional level to affect accessory transcription factors leading to changes in virulence gene expression. For instance, transcription of the gene encoding staphylococcal protein A (*spa*) is increased in the absence of RNAIII, but this is due to the fact that RNAIII normally represses production of other transcription factors (e.g., SarT, Rot,

and ultimately SarS) that would otherwise promote *spa* transcription[21]. Thus, in the absence of RNAIII, this repression does not occur, which results in the continued high level expression of *spa*. Additionally, RNAIII binds *spa* mRNA in a manner that both limits translation and promotes RNase III-mediated degradation[22].

This latter mechanism also plays a primary role in the RNAIII-mediated induction of toxin production[20]. This occurs *via* both direct and indirect pathways. For instance, in the case of *hla*, which encodes α toxin, the *hla* transcript forms a stem-loop structure that sequesters the Shine-Delgarno sequence, thus limiting translation. RNAIII overcomes this limitation by binding the *hla* transcript and relieving this stem-loop structure[23]. The translation of *hla* is thus upregulated in the presence of RNAIII *via* a direct interaction between RNAIII and *hla* mRNA. In other cases, the regulatory functions are mediated indirectly *via* the interaction between RNAIII and the *rot* transcript. Specifically, the regulatory functions of Rot (repressor of toxins) and Agr are antagonistic, with RNAIII limiting the production of Rot by binding to the Shine-Dalgarno sequence of the *rot* transcript and, as with the *spa* transcript, both inhibiting translation and targeting the existing transcript for degradation by RNaseIII[1]. In addition to its regulation of the genes encoding individual virulence factors, RNAIII also modulates the expression of other two-component systems including ArlRS, SaeRS and SrrAB, but the mechanism by which this occurs is not known[24-26].

Taken together, these results imply that *agr* plays a central role in *S. aureus* regulatory circuits. This is also reflected in the observation that mutation of *agr* has been consistently associated with a reduced capacity to cause infection[27]. Indeed, a primary determinant of the hypervirulence of isolates of the USA300 clonal lineage is their high level expression of *agr* and consequent high level production of critical exotoxins including α toxin and PSMs[27]. At the same time, this does not mean that *agr* expression is critical in all forms of *S. aureus* infection. One specific phenotype that may be particularly important in this regard is biofilm formation, with the high-level expression of *agr* generally being associated with a reduced capacity to form a biofilm[28-30]. It has been proposed that induction of *agr* expression may be important in promoting dispersal of *S. aureus* cells from an established biofilm, perhaps by inducing the production of extracellular proteases and/or nucleases[31]. This suggests that the expression of *agr* needs to be carefully controlled in the cells during biofilm development. However, several reports have documented the isolation of *agr* mutants directly from patients suffering from *S. aureus* infection[32,33], and it has even been suggested that *agr* dysfunction may be adaptive for survival within an infected host[34]. In fact, this is one specific context in which *agr* mutants have been shown to preferentially accumulate[34], perhaps owing to both the negative impact of *agr* on biofilm formation and the fact that its expression is metabolically expensive[35].

SaeRS

The *saeRS* two-component system was first identified as a transposon-insertion mutant deficient in exoprotein production[36]. *saePQRS* is transcribed as a 4-gene operon (*saePQRS*), with SaeS and SaeR being the sensor and response regulator respectively. A definitive role for SaeP and SaeQ has not yet been determined, although they may be involved in stabilization of SaeS in the membrane and/or modulating its return to the dephosphorylated state[37]. Once phosphorylated, SaeR binds to a specific target sequence ($GTTAAN_6GTTAA$) to activate transcription of *saePQRS* itself[38]. This is very similar, although not identical, to the AT-rich consensus binding site identified by Nygaard *et al*[39] based on alignments with additional SaeR-regulated target genes[39].

Several studies have demonstrated that *sae* also modulates the production of virulence factors other than toxins including surface proteins and capsule biosynthesis components[38,40-43]. Several experimental observations suggest that *saePQRS* is downstream of *agr*, as well as other regulatory loci. Transcription of *saePQRS* is activated by *agr* but is repressed by SigB, while SaeRS does not seem to affect transcription of *agr*, *sigB* or *sarA,* suggesting that SaeRS acts as an important downstream regulator within the *S. aureus* global regulatory network[36,42,44,45]. Genetic experiments on exoprotein production also suggest that *saePQRS* is downstream of and epistatic to *agr*[42]. Furthermore, inactivation of either *agr* or *sae* had a comparable impact on the virulence of a USA300 isolate in a murine pneumonia model[46]. However, while inactivation of *agr* or *sae* results in reduced production of extracellular proteins, the exoprotein profiles of the two mutants are not identical[36,47]. It is also clear from several studies that the two regulons are not equivalent[39,41,43]. For instance, inactivation of *sae* results in decreased transcription of the *fnbA* and *fnbB* genes[39,41], both of which encode fibronectin-binding proteins, while inactivation of *agr* has the opposite effect[48]. Thus, while *sae* seems to function downstream of *agr*, it is also capable of regulating its target genes independent of *agr*.

One of the most commonly studied strains of *S. aureus* is Newman, which has a naturally occurring point mutation in *saeS* resulting in substitution of a leucine with a proline (L18P). This results in increased kinase activity leading to constitutive activation of SaeR and increased transcription of the *saePQRS* genes[49]. However, only certain target genes within the SaeRS regulon are differentially regulated in Newman due to the polymorphism of SaeS. Class I target genes are sensitive to the $SaeS^P$ allele and Class II genes are not. Although the mechanistic basis for this difference is not clear, it does not appear to be due to a gene dosage effect[41]. When $SaeS^L$ is cloned into wild-type Newman, it is dominant over $SaeS^P$, suggesting instability of the system upon over-production, perhaps due to SaeS phosphatase rather than kinase activity[41].

Inactivation of *sae* is associated with increased transcription of several genes encoding extracellular proteases and increased accumulation of the corresponding

proteases themselves[50], and this may well have an indirect effect on other virulence phenotypes of *S. aureus*. For instance, Newman is one of the few strains in which inactivation of the staphylococcal accessory regulator (*sarA*) does not result in an α toxin-deficient phenotype, and it was recently demonstrated that this is due to the hyperactivity of $SaeS^P$ leading to the reduced production of extracellular proteases, and consequent reduced degradation of the toxin, rather than transcriptional changes associated with *hla*[51].

The environmental cues modulating SaeRS activity have not been clearly defined but are associated with stress conditions including high salt, low pH, and subinhibitory concentrations of antibiotic[44]. Because SaeRS is induced by hydrogen peroxide and α-defensins, and because many toxins are SaeRS-regulated, it has been hypothesized that this system could promote escape from polymorphonuclear leukocytes after phagocytosis[44]. Indeed, it has been demonstrated that an *saeRS* mutant strain has an impaired ability to survive in human neutrophils after phagocytosis[43].

ArlRS

Fournier *et al*[52] used transposon mutagenesis to identify genes involved in the regulation of the multidrug efflux pump NorA and identified *arlS*, inactivation of which resulted in increased resistance to quinolones. ArlS is the sensor and ArlR is the response regulator of this two-component system. A subsequent study confirmed that ArlRS also modulates the production of exoproteins, but in this case the phenotype was opposite to that of an *agr* mutant, with an *arlRS* mutant exhibiting increased production of multiple exoproteins[52]. Additionally, an *agr*/*arl* double mutant exhibited an exoprotein phenotype comparable to the isogenic *arl* mutant, suggesting that *arlRS* is upstream rather than downstream of *agr*. In contrast, *arlRS* induces expression of *sarA*. To the extent that *sarA* is a major repressor of protease production, this is consistent with the observation that protease activity is increased in an *arlRS* mutant[53]. Whether these effects are direct or indirect remains unclear.

Together, these results suggest that *arlRS* may be a key regulatory element that defines the "balance" between *agr* and *sarA*. Both of these regulatory elements have been implicated in biofilm formation, and *arlRS* has also been shown to have an impact in this regard. Specifically, inactivation of *arlRS* results in increased autolysis and an enhanced capacity to form a biofilm[52]. The fact that the biofilm phenotype appears to be independent of any effect on production of the *ica*-encoded poly-N-acetylglucosamine (PNAG)[54], together with the demonstration that extracellular DNA released from lysed *S. aureus* cells contributes to biofilm formation[55], suggest that increased autolysis may be responsible for the biofilm phenotype. However, the biofilm formed by an *arlRS* mutant is sensitive to exogenous proteinases, suggesting that this biofilm is also at least partially dependent on protein-protein interactions[54]. This is consistent with the observation that inactivation of *arlRS* results in dramatically increased amounts of extracellular and surface-associated protein A[53], both of which have been shown to contribute to *S. aureus* biofilm formation[56]. Finally, *arlRS* has been shown to promote the production of additional virulence factors including the exfoliative toxin and capsular polysaccharides, the latter being an indirect effect mediated through its positive regulation of MgrA production[57-59].

LytSR

Like *arlRS*, the *lytSR* two-component system is a negative regulator of *S. aureus* autolysis[60] and biofilm formation[61], and in fact *arlRS* is an activator of *lytSR* transcription[62]. These phenotypes are likely to be connected in that current models suggest that *lytSR*, together with CidR, collectively control the release of extracellular DNA (eDNA) by influencing expression of the *lrgAB* and *cidABC* operons, respectively, in modulating the production of murein hydrolases and consequently cell lysis[63]. Specifically, CidR activation of *cid* operon results in increased production of murein hydrolases, increased release of eDNA, and an increased capacity to form a biofilm, while activation of the *lrgAB* operon by LytSR has the opposite effects[55,64]. Although a cause-and-effect relationship between these phenotypes has not been proven, extracellular nuclease, whether applied exogenously or produced by *S. aureus*, has been shown to limit biofilm formation at least under certain *in vitro* conditions[29,64,65].

SrrAB

The *srrAB* two-component system was first identified based on homology with the ResDE two-component system in *B. subtilis*[66]. In response to oxygen stress, SrrAB represses expression of *agr* and the genes encoding certain exotoxins, including TSST-1[66]. However, it also represses transcription of *spa* and the production of protein A, which suggests that the impact of *srrAB* is not mediated directly through its regulation of *agr* but rather by direct interactions between the SrrA response regulator and the target genes themselves[67,68]. SrrAB also positively regulates expression of the *icaADBC* operon and production of PNAG, apparently by repressing transcription of the *ica*R-encoded repressor[67]. Whether the effect of SrrAB on the production of protein A or PNAG affects biofilm formation remains unknown, but the latter has been correlated with increased resistance to phagocytosis[47,69]. The link between oxygen availability, the activity of SrrAB, and the production of multiple types of virulence factors provides an important example of the link between central metabolic processes and virulence in *S. aureus*, a link that is also increasingly being made in the context of other *S. aureus* regulatory elements[70].

HssRS

The *hssRS* two-component system is an iron-responsive system that is highly conserved among Gram-positive pathogens including *B. anthracis*, *L. monocytogenes*, *S. epider-*

midis and *E. faecalis,* suggesting a conserved mechanism of iron acquisition among these organisms[71]. Iron is an essential nutrient for many bacterial species during infection[71,72]. However, free iron is severely limited in the human body but rather is complexed with a variety of iron-binding proteins. Therefore, in order for bacterial organisms to acquire iron they must have a mechanism for freeing complexed iron. *In vivo*, *S. aureus* can acquire iron in the form of heme, likely accessed *via* lysis of erythrocytes, using highly efficient transport systems that can move heme into the bacterial cytoplasm[73-75]. However, a high level of heme is toxic to the bacterial cell. To avoid toxicity, *S. aureus* senses heme by HssS resulting in HssR phosphorylation and binding to the promoter of *hrtAB*, which encodes an iron efflux pump that maintains intracellular heme homeostasis[76]. Whether there is cross-talk between this system and heme uptake systems, however, has not been demonstrated. In the absence of HrtAB, intracellular iron builds up causing a stress response characterized by the increased production of multiple virulence factors. Indeed, an *hrtAB* mutant is more virulent than the wild-type[71], likely due to the stress response induced by increasing intracellular heme.

Other two-component systems

The preceding discussion of two-component systems in *S. aureus* is by no means comprehensive, but it does summarize the impact of some of the best characterized systems. However, in the interest of inclusivity, we would note the existence of other, less well-characterized systems including KdpDE, which has been shown to link the AI-2/LuxS quorum-sensing system with capsule production[77]; VraSR, which induces a stress response to cell-wall inhibitors such as β-lactams and vancomycin[78]; GraSR, which aids in resistance to oxidative stress, heat stress, and vancomycin resistance[79]; BceAB, which is associated with altered susceptibility to bacitracin[7], and NsaRS, which plays a role in biofilm formation as well as cell envelope stability in response to cell wall and membrane disruption[80]. An additional two-component system that stands out from the others because it is the only one that is essential in *S. aureus* is WalKR (YycGF), which has been shown to be involved in peptidoglycan crosslinking and biofilm formation[81-83].

OTHER TRANSCRIPTIONAL REGULATORS

SarA-family

A primary class of transcriptional regulators that are not part of a two-component regulatory system, but do interact in multiple pathways with such systems, is the SarA family. The first gene encoding a member of this family, also identified in a screen of a transposon mutant library based on altered production of exotoxins, was designated the *sar*[84], which was subsequently changed to *sarA* based on identification of additional homologs now totaling 11[85]. Members of the SarA family have been shown to interact with each other forming part of a complex regulatory network controlling virulence factors (Figure 1). While all are winged-helix DNA-binding proteins, they can be divided into three structural families consisting of (1) relatively small, single-domain proteins (SarA, SarR, SarT, SarV, SarX and Rot); (2) larger, two-domain proteins (SarS, SarU, and SarY) in which each domain shares homology with the smaller homologs; and (3) small homologs with similarity to the MarR protein of Gram-negative bacteria (SarZ and MgrA)[85].

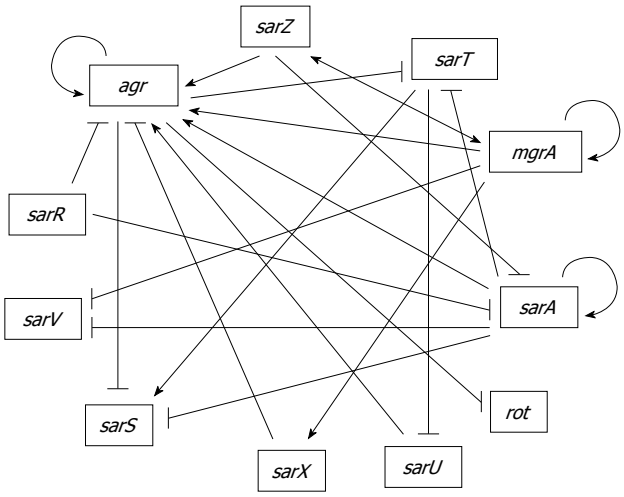

Figure 1 Proposed genetic regulatory network involving *sarA* family genes and *agr* in *Staphylococcus aureus*. The model is constructed from published studies[25,29,31,86,101,105,106,109,110,114,115,120-125,129,130,135,141,144] that are mostly based on a limited number of laboratory strains. Therefore, it may not be entirely applicable to all strains. Arrows indicate activation; blocked arrows indicate repression.

SarA

SarA, the prototype member of the SarA family, was identified in a screen of transposon-insertion mutants in the *S. aureus* strain DB based in part on its increased production of multiple exoproteins, a phenotype which clearly distinguished *sarA* from *agr*[84]. Based on this, it was proposed that *sarA* may function as a "counter-regulatory system to that of *agr*". This is consistent with the hypothesis that many regulatory functions in *S. aureus* are defined by the "balance" between *agr* and *sarA*, an issue that is discussed in more detail below in the specific context of biofilm formation. However, subsequent studies also confirmed that SarA binds to intergenic region between the *agr* P2 and P3 promoters[86,87] and is required for maximal transcription of *agr*[88]. Thus, it has become clear that SarA modulates the production of *S. aureus* virulence factors *via* both *agr*-dependent and *agr*-independent pathways.

The *sarA* locus is complex and includes three promoters (P1, P2, and P3) that drive the production of three transcripts (*sarB*, *sarC* and *sarA* respectively), with all three sharing the same termination site just downstream of the gene encoding SarA. The upstream *sarA* P2 and P3 promoters modulate the production of SarA by an unknown mechanism[89]. Specifically, inactivation of

P2 and P3 results in reduced production of SarA from the P1 promoter, and while the effect is relatively modest (approximately 2-fold), it appears to be functionally relevant with respect to both the *agr*-dependent and *agr*-independent pathways of SarA-mediated regulation[89]. This may account for the inability to demonstrate a difference between the three transcripts in complementation studies using a multi-copy vector targeting the *cna* promoter[90].

Both the signals that modulate SarA production and/or activity and the binding site for SarA are poorly defined. The DNA-binding activity of SarA is altered by redox state *in vitro*[91]. A recent report also demonstrated that SarA is phosphorylated by at least two serine/threonine kinases (Stk1/PknB and a poorly defined kinase encoded by SA0077) and that this also alters its DNA-binding capacity[92]. Two approaches have been taken to identifying the SarA binding site, with the first being alignment of the promoter regions of genes whose RNA products are altered in *sarA* mutants[93] and the second being the relatively unbiased approach of selective enhancement of systematic evolution of ligands by exponential enrichment (SELEX)[94], but generally speaking both approaches failed to define the characteristics of a definitive binding site beyond it being AT rich. It has been suggested on this basis that SarA may act as an architectural accessory protein rather than a classic transcription factor, a suggestion that is supported by the observation that SarA supports *E. coli* lambda phage integrase mediated site-specific recombination[91]. A recent report describing the interaction between SarA, SarR (see below) and *cis* elements within the *agr* promoter region also suggested that SarA binding may locally bend DNA in a fashion that brings AgrA into a favorable conformation to initiate transcription, particularly at the *agr* P2 promoter[87].

Irrespective of the mechanism involved, it is clear that inactivation of *sarA* results in major changes in the production and/or persistence of multiple RNA transcripts and that this has a global impact on the *S. aureus* virulon. Transcriptional profiling comparisons between the 8325-4 strain RN6390 and the clinical osteomyelitis isolate UAMS-1 demonstrate that this is somewhat strain-dependent, with the impact of mutating *sarA* in the latter being comparatively greater than the impact in the former[24]. RN6390 has defects in at least two genes that also have a major impact on global regulatory circuits. One of these is in *rsbU*, which results in reduced activity of the *sigB* regulon[47]. This is consistent with the observation that *sigB* has been shown to increase expression of *sarA* and reduce the level of RNAIII[95]. In this respect it is important to note that 8325-4 strains also have a mutation in *tcaR*, which results in reduced production of SarS[96], a SarA homolog also shown to influence the regulatory functions of SarA (see below).

As with *agr*, inactivation of *sarA* has been shown to attenuate virulence in multiple animal models of *S. aureus* infection including endopthalmitis, septic arthritis, osteomyelitis, and endocarditis[97-99]. Interestingly, all of these infections can arguably be said to be biofilm associated, an important observation given that inactivation of *sarA* has been consistently shown to result in a reduced capacity to form a biofilm[28]. In this respect it is also important to note that the impact of *sarA* on biofilm formation is opposite to that of *agr*[29], thus suggesting that the role of *sarA* in biofilm formation is independent of its regulation of *agr*. Indeed, in RN6390, a strain which expresses *agr* at high levels, inactivation of *agr* enhances biofilm formation in a manner that is reversed by concomitant inactivation of *sarA*, thus demonstrating that the impact of *sarA* is epistatic to *agr* in this context. Taken together, such results are consistent with the hypothesis that a primary determinant of the overall patterns of *S. aureus* virulence factor production is the "balance" between expression of *agr* and *sarA*[24], perhaps to the point of determining the relative capacity of different *S. aureus* strains to cause chronic, biofilm-associated infections *vs* acute, toxin-mediated disease.

There are several possible explanations for the biofilm-deficient phenotype of *sarA* mutants including the reduced expression of the *icaADBC* operon resulting in decreased production of PNAG, and the increased production of extracellular nucleases and proteases[29,31,64,65,100,101]. It seems unlikely that the decreased production of PNAG plays a predominant role given that inactivation of *sarA* has a greater impact on biofilm formation than inactivation of *icaADBC*[28]. Similarly, extracellular DNA has been shown to contribute to biofilm formation, but inactivation of the genes encoding *S. aureus* exonucleases has relatively little impact on the biofilm-deficient phenotype of *sarA* mutants[64,65]. In contrast, inactivation of the genes encoding extracellular proteases has a significant impact on the ability of *sarA* mutants to form a biofilm[24,29,102]. The effect of these proteases is presumably mediated *via* degradation of surface-associated proteins including FnbA, FnbB and protein A (Spa), all of which contribute to biofilm formation in *S. aureus* and have been shown to be produced in reduced amounts in *sarA* mutants owing to protease-mediated degradation[51,103,104].

Interestingly, the increased production of extracellular proteases has also been shown to result in the reduced accumulation of critical extracellular toxins in *sarA* mutants, at least under *in vitro* conditions[51]. These include α toxin and PSMs, both of which have been implicated as primary determinants of the hypervirulence of USA300 isolates[105]. Thus, while *agr* and *sarA* have opposite effects on the production of extracellular proteases, inactivation of either generally results in a toxin-deficient phenotype. This also suggests an alternative explanation for the reduced virulence of *sarA* mutants, although the relative contribution of these two *sarA*-dependent phenotypes in this respect remains to be determined.

Finally, the impact of SarA on exotoxin production has been shown to be heavily influenced by SaeRS, with the hyperactivity of SaeRS in Newman attenuating the increased production of extracellular proteases to a degree

that impacts the α toxin and PSM phenotypes of a Newman *sarA* mutant[51]. This, together with the impact of SarA on expression of *agr*, provides direct indications of the interactive role of SarA in *S. aureus* regulatory circuits. Additional interactions involving other SarA homologs are described below.

SarR

SarR was discovered based on its affinity for the *sarA*P2 promoter, with the binding of SarR repressing *sarA*[106]. In contrast, SarA binds its own promoters to enhance transcription, thus providing an example in which SarA and SarR serve competitive roles in modulating gene transcription. These two proteins also serve the opposing roles with respect to Agr since binding of SarA, together with AgrA, promote transcription from the *agr* P2 promoter whereas binding of SarR has the opposite effect[87]. It should be noted that SarR was originally shown to activate *agr* P2 promoter[107,108]. This discrepancy was attributed to the difference in *sigB*[87] but it is unclear how *sigB* reverses the effect. SarR promotes transcription of the proteases Aur and SspA, and may be involved in the SarA-dependent repression of these proteases, presumably also *via* competition for binding sites in the promoter regions[109]. In addition, SarR also binds to the *rot* promoter[107,108] but it is not known how SarR affects Rot. Thus, SarR plays an important but opposing role to SarA in both the *agr*-dependent and *agr*-independent pathways of SarA-mediated regulation. Based on these findings, SarR likely plays an important role in virulence due to its regulation of the well-characterized regulators SarA, Rot and Agr.

SarS

SarS (previously designated SarH1) was discovered using a search for proteins with affinity for the promoter region of *agr*-P3, *spa*, *hla* and *ssp*. However, SarS only affects expression of *spa* and *hla* but not RNAIII or *ssp*[110]. SarS is an activator of *spa* and a repressor of *hla* but it is repressed by Agr and SarA and activated by SigB and TcaR[96,111]. The fact that SarS is regulated by SigB and TcaR may explain why SarA affects *hla* transcription differently in RN6390, in which both SigB and TcaR are defective, than in other clinical isolates[111,112].

SarT/SarU

SarT was originally described by Schmidt *et al*[113] following a search for SarA homologs[113]. The *sarT* gene encodes a 118-residue protein and is present in certain strains of *S. aureus* including members of Clonal Complex 8 (CC8), to which CA-MRSA strains of the USA300 lineage and RN6390 belong, but absent in other clinically-relevant strains such as UAMS-1 (CC30 lineage)[113,114]. In the RN6390 background, *sarT* and *agr* are mutually repressive thus forming a negative feedback loop. In addition, *sarT* is also repressed by SarA[115]. Repression of *agr* by SarT was thought to explain the repression of *hla* by SarT in RN6390[115,116]. However, a later study shows that SarT represses *hla via sae* independently of *agr* and *rot* in strain COL[117]. A high level of *agr* in RN6390[118] may account for this difference. In RN6390, SarT also induces expression of protein A but indirectly through activation of *sarS*.

Adjacent to but divergently transcribed from *sarT* is *sarU*, whose expression is repressed by SarT[116]. Additionally, inactivation of *sarU* results in a reduction of both RNAII and RNAIII expression, suggesting a positive effect of SarU on *agr*. Because *sarT* has been shown to be repressed by *agr*, these relationships implicate a feedback loop involving SarT, SarU and RNAIII[116].

Recent studies have concluded that *sarT* and *sarU* are expressed at undetectable levels by northern blot[119]. However, deletion studies have revealed downstream effects of these genes, suggesting that they are expressed at very low but relevant levels[113,115,116]. For instance, it has been shown that a significant number of spontaneous non-hemolytic variants arise in biofilms that are phenotypically but not genotypically *agr* deficient. Transcriptional profiling of these variants found a 6-fold reduction in *sarU* suggesting SarU may be responsible for the *agr* deficiency[120]. These results imply that SarU may play a key role during biofilm-associated infections by modulating *agr*.

SarV

SarV was identified based on homology to SarA family. Both SarA and MgrA repress *sarV* gene expression. SarV is involved in regulation of autolysis, which may be part of the common pathway through which SarA and MgrA control autolysis[121]. Under laboratory conditions, *sarV* is poorly transcribed and the protein is not detectable in various strains in all phases of growth, likely due to repression by SarA and MgrA[119].

SarX

SarX was also identified based on sequence homology with the SarA family of transcription regulators. SarX has been shown to have maximal expression during the stationary phase of growth[122]. MgrA positively regulates *sarX* gene expression. SarX also acts as a repressor of the *agr* locus and can therefore regulate other genes *via* Agr[122]. SarX is highly expressed in RN6390 but is only expressed at very low levels in several tested strains[122] possibly due to difference in SigB in these strains. SarX has been shown to activate biofilm formation in *S. epidermidis*[123] raising the possibility that it may also have an effect on biofilm in *S. aureus*.

SarZ

SarZ was originally identified as a regulator of hemolysins and promotes virulence in both silkworm and mouse infection models[124]. SarZ positively regulates *agr* and *mgrA* expression but negatively regulates expression of *sarA*. SarZ affects surface proteins, toxins and biofilm through modulating the aforementioned global regulators as well as direct activation on SspA protease[125]. However,

SarZ binding to the promoters of various target genes is nonspecific[124,126]. In addition, SarZ activates SarS and is activated by MgrA[125]. SarZ and MgrA therefore interact in a positive feedback loop. SarZ expression is growth phase dependent, with maximum expression during early exponential phase[126]. Like MgrA, SarZ senses oxidative stress *via* a conserved cysteine residue providing another connection between metabolism and virulence[127,128].

MgrA

MgrA, also referred to as Rat and NorR, was identified in three independent laboratories[129-131]. MgrA regulates a multitude of virulence factors as well as antibiotic resistance[130,131]. Truong-Bolduc *et al*[130,132] initially described MgrA (NorR) as a regulator of NorA, which is a multidrug efflux pump providing quinolone resistance by direct DNA binding to the NorA promoter[130,132]. Binding of MgrA to the NorA promoter is dependent on phosphorylation by the kinase Stk1 (PknB), and RsbU is involved in dephosphorylation of MgrA leading to strain-dependent differences in MgrA function[133,134].

MgrA has been shown to up-regulate expression of 175 genes and down-regulate expression of 180 genes[59]. It was later shown that MgrA regulates *hla* and *spa* expression through *agr*-dependent and independent pathways[135]. In addition, MgrA has been found to repress biofilm formation, which is dependent on surface proteins, in part, through *agr*-dependent pathway and DNA release, probably by affecting LytSR and LrgAB[129,135,136].

MgrA has been shown to affect virulence in animal models of infection[59,137]. MgrA acts as a redox-switch as oxidation of the unique cysteine residue leads to its dissociation of MgrA from DNA[137]. Small molecules that disrupt the DNA-binding of MgrA had been shown to attenuate *S. aureus* virulence in mice[138] suggesting that MgrA could be a potential drug target.

Rot

Rot, repressor of toxins, is yet another SarA homolog[139]. It shares a high degree of sequence similarity to other members of the SarA family, but differs by its pI value (pI 5.0), which is more acidic than other SarA- homologs (pI values ranging from 8.5 to 10.7). Rot was first identified using transposon mutagenesis and screening for mutants capable of restoring the expression of α toxin and proteases in an *agr*-negative background[139]. Transcription of *rot* originates from at least three promoters and is growth-phase dependent[140,141]. Rot has an opposing effect on virulence gene expression by comparison to *agr*[139,142]. RNAIII blocks the translation of *rot* mRNA *via* an antisense mechanism, which explains why the regulatory function of Rot is only detected in *agr* deficient strains[20,143]. ClpX, a molecular chaperone, has also been shown to modulate Rot expression, likely by stimulating translation of the *rot* mRNA *via* a mechanism independent of RNAIII[144]. Rot has also been shown to repress α-toxin production by repressing the SaeRS two-component system[117]. In addition to toxins, Rot also positively regulates protein A[21,112,144,145].

AraC/XylS family transcription regulators

The *S. aureus* genome contains 6 ORFs with homology to the AraC/XylS family of transcriptional regulators. Of these, two have been characterized and demonstrated to play a role in biofilm formation[146,147]. Rbf was first identified using transposon mutagenesis in a screening for biofilm-deficient mutants and demonstrated to control biofilm in response to NaCl and glucose[147]. It was later determined that Rbf promotes biofilm formation *via* repression of *icaR*, a repressor of the *icaADBC* operon whose gene products synthesize PNAG. However, Rbf is unable to bind specifically at the *icaR* promoter[148,149] suggesting other regulators or post-translational modification of Rbf may be involved. Rbf has been shown to promote virulence in a murine foreign-body infection model[150].

Rsp, another AraC family regulator, has recently been characterized and shown to regulate biofilm formation[146]. However, Rsp differs from Rbf by repressing biofilm by negatively regulating surface proteins; in particular, FnbA, which has been shown to promote biofilm formation in the cell accumulation phase[151]. Interestingly, Rsp inhibits biofilm through repression of FnbA at the stage of primary attachment by direct binding to the promoter of *fnbA*[146].

CodY

CodY functions as a highly conserved regulatory transcription factor in low-GC Gram-positive bacteria and has recently been identified as a regulator of several virulence factors in *S. aureus*[152-154]. CodY acts in response to metabolite effectors such as GTP and the branched-chain amino acids isoleucine, leucine, and valine[153], all of which interact with a branched-chain amino acid domain on CodY, facilitating the direct binding of CodY to several promoters associated with virulence genes[155]. It is thought that CodY primarily acts as a negative regulator of virulence genes in *S. aureus*[156]. Microarray analysis and DNase footprinting of *codY* mutant clinical isolates have recently identified several negatively regulated targets of CodY including *agr*, *ica,* and *hla*[153,155]. The *agr* locus is responsible for regulation of many virulence factors and thus, the repression of this locus by CodY has profound phenotypic effects on the expression of virulence genes in *S. aureus*. For example, capsular polysaccharide production is repressed by CodY through *agr* as well as by direct promoter binding[155].

Apart from direct regulation of virulence genes, CodY also affects metabolic regulation in *S. aureus via* amino acid synthesis, carbon flow, nitrogen assimilation, and transport systems[153]. CodY is activated in nutrient-replete environments, repressing virulence factors and metabolic synthesis genes. For example, CodY is associated with repression of a lactate dehydrogenase (*ldh*1), which interconverts pyruvate and lactate[157]. *S. aureus* strains lacking *ldh*1 show significant attenuation of viru-

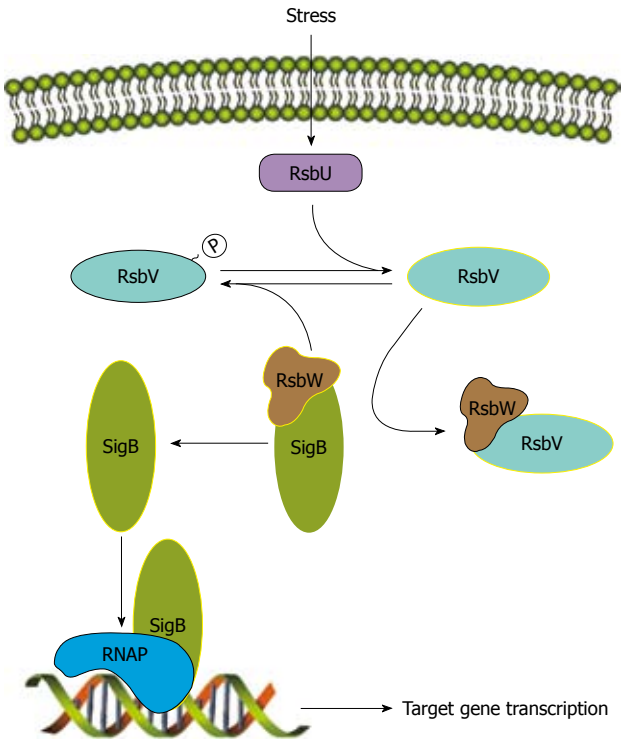

Figure 2 Post-transcriptional regulation of SigB. After stress-induction, RsbU de-phosphorylates RsbV, which can then bind specifically to RsbW thereby removing RsbW from SigB. Phosphorylated RsbV is inactive and therefore cannot bind RsbW. RsbW also promotes phosphorylation of RsbV to maintain its inactivity. RsbW binds to SigB to inhibit transcription by preventing SigB from complexing with the RNA polymerase (RNAP). Once SigB is free from inhibition by RsbW, it can complex with RNAP forming the holoenzyme and activate transcription of target genes. Active proteins are highlighted with yellow.

lence[157]. Thus, CodY is able to regulate virulence *via* direct binding of virulence gene promoters and *via* inhibition of metabolic regulatory pathways providing another regulatory link between metabolism and virulence[70,156].

CodY is repressed by the intracellular chaperone ClpC[40], possibly *via* ClpC-induced proteolytic degradation in association with ClpP. Although CodY acts as a repressor of virulence genes, it can also be negatively regulated under various environmental conditions, eliminating the repressive effect of CodY on virulence genes.

Sigma factors

Sigma factors are highly conserved among bacterial species. They provide promoter specificity to the RNA polymerase, and are highly regulated by anti-sigma factors *via* direct binding of the protein[158]. There are currently four identified Sigma factors in *S. aureus*: SigA, which is responsible for transcription of housekeeping genes; SigB, which is responsible for the transcription of stress-response genes; SigS, which controls expression of genes required for overall fitness and survival[2]; and SigH, which has a demonstrated involvement in competence and more recently, prophage integration and excision[159,160].

The most thoroughly studied of these is SigB, which is transcribed from the four-gene operon *rsbUVWsigB* that encodes an anti-sigma factor (RsbW), anti-anti-sigma factor (RsbV) and RsbU, an anti-RsbV phosphatase[95,161]. The regulation of SigB is very tightly controlled by RsbW, RsbV and RsbU (Figure 2). SigB controls expression of an array of genes responsible for the survival of hydrogen peroxide-induced stress and desiccation as well as production of the carotenoid staphyloxanthin and extracellular proteases[161-164]. SigB has also been demonstrated to aid in heat tolerance and resistance to cell-wall active antibiotics[165,166]. The repressive effect of SigB on V8 proteases positively regulates biofilm formation[162] because the presence of extracellular proteases has been correlated with the inability to form a biofilm[164]. SigB regulates its target genes either by recognizing a conserved sequence or by downstream regulators. For example, SigB effect on *sarA* or *agr* expression has been reported[95,167]. More recently, SigB has been shown to regulate several extracellular virulence factors and capsule through SpoVG[168,169], demonstrating a role for SigB in virulence as a response to stress.

CONCLUSION

In this review, we describe several regulators involved in virulence regulation. These represent only a fraction of all regulators encoded in the *S. aureus* genome. *S. aureus* is a pathogen that can cause a wide range of diseases and can infect almost every tissue. It is thus not surprising that a large number of regulators are needed to modulate the production of various virulence factors in different environmental conditions in the host. What is surprising is the high degree of complexity of the interactions among the regulators. Compounding the complexity is the finding that virulence genes in different strains often are regulated differently. The molecular mechanisms underlying some of the strain differences have been illustrated but most have not. Nonetheless, significant progress has been made toward understanding virulence gene regulation. However, most of the results have been obtained by *in vitro* studies. The big challenge that lies ahead would be to test the *in vitro* results in suitable animal models to better understand virulence gene regulation in pathogenesis. With the rise of antibiotic resistance and the prevalence of multi-drug resistant isolates, fully understanding the virulence regulation in pathogenesis may provide sound rationale for identifying regulators as potential targets for anti-staphylococcal drug therapies. Targeting a cellular factor not absolutely required for survival, such as a virulence regulator, may lessen selective pressures, and therefore resistance, while still attenuating virulence of the organism[170,171].

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REVIEW

Fighting nosocomial infections with biocidal non-intrusive hard and soft surfaces

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Abstract

Approximately 7 million people worldwide acquire a healthcare associated infection each year. Despite aggressive monitoring, hand washing campaigns and other infection control measures, nosocomial infections (NI) rates, especially those caused by antibiotic resistant pathogens, are unacceptably high worldwide. Additional ways to fight these infections need to be developed. A potential overlooked and neglected source of nosocomial pathogens are those found in non-intrusive soft and hard surfaces located in clinical settings. Soft surfaces, such as patient pyjamas and beddings, can be an excellent substrate for bacterial and fungal growth under appropriate temperature and humidity conditions as those present between patients and the bed. Bed making in hospitals releases large quantities of microorganisms into the air, which contaminate the immediate and non-immediate surroundings. Microbes can survive on hard surfaces, such as metal trays, bed rails and door knobs, for very prolonged periods of time. Thus soft and hard surfaces that are in direct or indirect contact with the patients can serve as a source of nosocomial pathogens. Recently it has been demonstrated that copper surfaces and copper oxide containing textiles have potent intrinsic biocidal properties. This manuscript reviews the recent laboratory and clinical studies, which demonstrate that biocidal surfaces made of copper or containing copper can reduce the microbiological burden and the NI rates.

Key words: Nosocomial infections; Health acquired infections; Copper; Copper oxide; Biocides; Surfaces; Microbiological burden

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INTRODUCTION

A nosocomial, or hospital-acquired, infection is a new infection that develops in a patient during hospitalization. Nosocomial infections (NI) are a worldwide problem that occur both in developed and in developing countries. For example, in the United States approximately 2 million patients annually contract an infection while being hospitalized[1], and it is the fourth among the causes of death in the United States only behind heart disease, cancer and stroke[2]; in Europe in 2007 there were about 3 million healthcare associated infections (HAI), of which approximately 50 000 resulted in death[3]; in Germany alone around 500 000 to 600 000 NI occurred during 2006[4]; methicillin-resistant *Staphylococcus aureus* (MRSA) infections alone are estimated to affect more than 150 000 patients annually in the European Union[5]; in Australia, more than 177 000 NI occur per year[6]; in the province of Quebec, Canada, the rate of NI is estimated to be

around 11%[7]; and the rates of NI in developing countries are even higher[8-11].

NI can be bacterial, viral, fungal, or even parasitic[12-15]. Some of the most common nosocomial pathogens are staphylococci (especially *Staphylococcus aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*), *Clostridium difficile* (*C. difficile*), *Streptococcus* species, Enterobacter species, Acinetobacter species, Klebsiella species, influenza virus and noroviruses[16-21]. The prevalence rates of pathogens that cause NI and have a high level of resistance to antibiotic treatments, such as multidrug-resistant (MDR) *P. aeruginosa*, extended-spectrum β-lactamase producing Enterobacteriaceae, MDR Acinetobacter baumannii, MRSA, and vancomycin resistant enterococci (VRE), are constantly increasing around the globe[22-28], creating a serious threat to the spread and treatment of infectious diseases, because the resistant pathogens are significantly more difficult to treat (e.g.,[29]).

Many measures to reduce the risk of pathogens transmission are sought by health care officials, physicians and scientists. These include improvement of national surveillance of NI, use of aggressive antibiotic control programs to reduce the spread of antibiotic-resistant strains, healthcare staff education for improved hygiene, isolation of infected patients, ultraviolet light sterilization, use of disposable equipment, development of patient care techniques to reduce risks of infection, improved cleaning techniques, improvement of cleaning equipment and sanitary facilities, increase in nursing and janitorial resources and better nutrition (e.g.,[30-34]). It is estimated that by using several of the above strategies simultaneously about one third of NI may be eliminated[35,36]. These measures are not the scope of this review and are widely described elsewhere in the literature (e.g.,[31,37-40]). But it is clear that even in clinical settings where all or most of these measures are implemented, the rates of NI are still too high, and thus new approaches to further fight these infections need to be explored.

NI may occur *via* several manners. It is recognized by the infection control community that the most important and frequent modes of transmission of nosocomial pathogens are through direct-contact between an infected or colonized person (e.g., health worker, visitor or patient) and a susceptible host[41-44], and indirectly *via* contaminated intrusive medical devices[44-49], from the patient's own flora from one part of the host's body to another[50], and *via* airborne particles[21,51-55].

In addition to the above well described modes of transmission of nosocomial pathogens, others[56] and we[57] hypothesised that contaminated textiles in hospitals might be an important source of microbes contributing to endogenous, indirect-contact, and aerosol transmission of nosocomial-related pathogens. Textiles are an excellent substrate for bacterial and fungal growth under appropriate moisture and temperature conditions, and it was shown that bacteria and fungi can survive for prolonged periods in hospital fabrics[58,59]. Microbial shedding from the body occurs continuously[60]. Microbial shedding is greater in patients[54,61]. Thus a bacterium, when shed into a textile fabric between the patient and the bed, either on his pyjama, pillowcase, sheet, or mattress, would readily proliferate since the moisture and temperature in the textile microenvironment would promote its proliferation. Others and we presented data that substantiate this premise[62-65]. Importantly, it was found by others that bed making releases large quantities of microorganisms into the atmosphere and the bacteria levels in the air fall back to background levels only after approximately 30 min[52,66-68]. The released bacteria were shown to contaminate adjacent surfaces, such as bed sheets, over bed tables, and patients' clothing, and even adjacent rooms *via* the air-conditioning systems. Similar results were reported following undressing and redressing of patients[69].

The contribution of contaminated hard surfaces, such as floors, bedrails, bedside tables and door knobs, to NI has been demonstrated too (e.g.,[70-80]). Similarly, contaminated textiles, such as contaminated sheets and pyjamas, in addition to being a source of aerosol transmission of microorganisms, can also directly contaminate the hospital personnel[56,76,81,82]. Hospital staff, even by using protective equipment such as gloves, can contaminate them by touching the contaminated textiles or contaminated surfaces and then transferring the microorganisms to other patients directly or indirectly by contaminating other surfaces, such as door knobs[76,83]. For example, it was found that 65% of the nurses who performed activities on patients with MRSA in wounds or urine, contaminated their nursing uniforms or gowns with MRSA. This in turn, can readily contaminate the clothing and hands of healthcare workers[54,76,83]. High similar contamination of gloves and gowns with MDR *Acinetobacter baumannii* by healthcare workers interacting with colonized patients has also been reported[84]. Furthermore, it was found that 42% of personnel with no direct contact with patients contaminated their gloves by touching contaminated surfaces[76].

Thus, we further hypothesized that use of antimicrobial textiles, especially in those that are in close contact with the patients, may significantly reduce bioburden in clinical settings and consequently reduce the risk of NI[57]. Being all surfaces biocidal in a hospital environment would further reduce the risk of pathogen transmission and NI since most common nosocomial pathogens can remain viable on surfaces for months[43,85]. Indeed, it has been shown that environmental disinfection interrupts the transmission of microbial pathogens[79,80,83,86,87]. However, there are increasing concerns that routine surface disinfection procedures in health care settings are frequently inadequate and possibly counterproductive[88,89]. Consequently, the notion that having potent safe biocidal non-intrusive hard and soft surfaces in medical settings, in direct or indirect contact with patients, capable of reducing the microbiological burden that would significantly contribute to reduction in transmission of nosocomial pathogens, is gaining recognition by the scientific community. This review focuses on the studies demonstrating that hard and soft surfaces containing copper reduce the

microbiological burden in clinical settings and the NI rates.

COPPER HAS POTENT BIOCIDAL PROPERTIES

Copper and copper compounds have a wide spectrum of antibacterial, antifungal and antiviral properties (reviewed in[90,91]). The wide range of microorganisms, including gram negative and gram positive bacteria, yeast, fungi and enveloped and non-enveloped viruses, that have been shown to be killed by copper or copper compounds, are summarized in Table 1. Importantly, copper surfaces or copper compounds have also been shown to be efficacious against hard-to-kill spores[92-98].

Copper exerts its toxicity to microorganisms through several parallel mechanisms, which eventually may lead to the microorganisms' death even within minutes of their exposure to copper[94,99-106]. These include plasma membrane permeabilization, membrane lipid peroxidation, alteration of proteins and inhibition of their biological assembly and activity and denaturation of nucleic acids[90,91]. In general, the redox cycling between Cu^{2+} and Cu^{1+}, which can catalyze the production of highly hydroxyl radicals, with subsequent damage to lipids, proteins, DNA and other biomolecules[90,107], makes copper further reactive and a particularly effective antimicrobial. Interestingly, two different "kill modes", under dry and wet conditions, have been attributed to copper surfaces[101,102,104,105].

BIOCIDAL SOFT SURFACES IN THE HEALTHCARE ENVIRONMENT

Copper oxide is a non-soluble form of copper that, similarly to other copper compounds, has potent wide spectrum biocidal properties[90]. It has, therefore, been chosen as the active copper form to be introduced into textile fibres from which woven and non-woven fabrics can be produced[64,108,109]. These copper-impregnated products possess permanent broad-spectrum anti-bacterial, antifungal and antiviral properties that are not affected by washings[64,91,99,108-112] (Table 1). This technology, for example, enables the production of biocidal fabrics (which inter alia kill antibiotic resistant bacteria)[64,91,108,109], antifungal socks (which inter alia alleviate symptoms of athlete's foot)[108,113], anti-viral masks and filters (which inter alia deactivate HIV-1, Influenza A and other viruses)[99,106,110,111], and anti-dust mite mattress-covers (which may reduce mite-related allergies)[108,114].

As explained in the previous chapter, we hypothesized that contaminated beddings may be an important overlooked source of nosocomial pathogens and therefore the use of potent biocidal beddings, especially pyjamas and sheets, that are in contact with the patients, may significantly reduce bioburden in clinical settings and consequently reduce the risk of NI[57]. Indeed, a pilot study with 30 patients, who slept overnight on regular sheets and then overnight on sheets containing copper-oxide demonstrated a statistically significant lower bacterial colonization on the copper-oxide containing sheets than on regular-sheets[64], clearly supporting our hypothesis.

Importantly, the development of biocidal textiles with the purpose of using them in clinical settings to reduce HAI is gaining momentum and other biocidal active ingredients have or are being explored. These include Cliniweave®[115], organofunctional silane[116], citric acid[117], silver[118,119], triclosan[120], quaternary ammonium compounds[121], chitosan and zeolite[122,123]. For biocidal textiles to be introduced into the hospital textiles they should have wide spectrum antimicrobial, antifungal and antiviral properties, be effective against the already existent antibiotic resistant microorganisms involved in NI, not allow for the development of microorganisms against the active component in them, be efficacious for the life of the material, not be affected by commercial washings, not cause skin irritation or sensitization and be safe to humans following continuous dermal exposure. Some of the above active ingredients have thus been found not to be appropriate for use in hospital related applications (e.g.,[120,124]).

Until recently, only a few trials in clinical settings have been performed with biocidal textiles. It was found that bioburden was significantly lower on garments worn by nurses when the garments were made from a silver and copper containing antibacterial fabric[125]. The antibacterial textiles were tested in two hospital units, an oncology surgery unit and an intensive care unit. Each garment was provided with a piece of test fabric sewed either on the right or left side of the garment, while the regular fabric of the garment on the other side was used as a control. Thirty garments were tested in each unit. They were all sterilized, so they would be free of bacteria at the beginning of the experiment. The nurses wore the same number of garments with the treated area on the left side 1 d and on the right side the following day. Both active and control sides of each garment were sampled simultaneously and the bioburden determined. The number of colony forming units (CFU) was significantly lower on the bioactive patches than on the control areas. The mean reduction rate was about 30% for the 60 garments tested. Reduction of about 50% of bioburden on sheets containing copper oxide compared to regular sheets, when used overnight by general ward patients, was demonstrated[64]. Similarly, reduction of bioburden on blankets containing a bound organofunctional silane was also reported[116]. Recently in a 16 wk, blinded cross-over clinical trial that compared levels of bacterial contamination, a significantly fewer MRSA colonies were detected on scrubs impregnated with nano-sized particles that increase the surface tension of the scrubs than on standard scrubs (http://www.vestexprotects.com/press/view/8-Vestagen-Announces-Completion-of-First-Clinical-Trial-of-Vestex). In contrast, a study that compared the contamination rates of silver containing jackets and pants

Table 1 Demonstrated biocidal efficacy of copper

	Hard surface	Soft surface	Other	Ref.
Bacteria				
Acinetobacter baumannii[1]	+	+	+	[130,164] UR[2]
Acinetobacter calcoaceticus/baumannii	-	-	+	[93,94,165]
Acinetobacter johnsonii	+	-	-	[105]
Acinetobacter lwoffii	-	-	+	[166]
Bacillus cereus	+	-	+	[101,167-169]
Bacillus globigii	-	-	+	[92]
Bacillus subtilis	-	+	+	[165,169-175]
Bacillus macerans	-	-	+	[176]
Brachybacterium conglomeratum	+	-	-	[105]
Brevibacterium	-	+	-	UR
Campylobacter jejuni	+	-	-	[129]
Citrobacter freundi	-	-	+	[165,177]
Clostridium difficile	+	-	+	[93,97,98]
Clostridium tyrobutyricum	-	-	+	[95]
Corynebacterium xerosis	-	+	-	UR
Deinococcus radiodurans	+	-	-	[101]
Desulfovibrio desulfuricans	-	-	+	[178]
Edwardsiella tarda	-	-	+	[179]
Enterobacter aerogenes	-	-	+	[168,180]
Enterobacter cloacae	+	+	+	[127,128,168,175]
Enterococcus sp.[1]	-	-	+	[93]
Enterococcus faecalis[1]	+	+	+	[64,108,112,136,137,168,180]
Enterococcus faecium[1]	+	-	-	[127,128,137,155,181]
Enterococcus gallinarum	+	-	-	[137]
Enterococcus hirae	+	-	-	[182]
Escherichia coli	+	+	+	[64,100,101,105,108,109,112,127,128,133,139,147,155,165, 168-172,181,183-193]
Klebsiella pneumoniae	+	+	+	[112,130,165,193-195]
Kocuria marina	+	-	-	[105]
Kocuria palustris	+	-	-	[105]
Legionella pneumophila	+	-	+	[93,140,159,196-198]
Listeria monocytogenes	+	+	+	[64,140,180,199,200]
Mycobacterium tuberculosis[1]	+	-	-	[130]
Micrococcus luteus	+	+	-	[105,127,128] UR
Morganella morganii	-	-	+	[177]
Pantoea stewartii	+	-	-	[105]
Photobacterium leiognathi	-	+	-	[112]
Proteus mirabilis	-	-	+	[194]
Proteus vulgaris	-	-	+	[168]
Pseudomonas aeruginosa	+	+	+	[112,127,128,130,144,164,167,168,171,172,175,201,202]
Pseudomonas fluorescens	+	-	-	[199]
Pseudomonas nitroreducens	-	-	+	[169]
Pseudomonas oleovorans	+	-	-	[105]
Pseudomonas putita	-	-	+	[203]
Pseudomonas striata	+	-	-	[176]
Salmonella spp.	+	+	+	[64,129,165,183]
Salmonella typhi	+	-	+	[141,174,177,190,194,203,204]
Salmonella typhimurium	+	-	-	[141,142,199,201]
Sarcina lutea	-	-	+	[167]
Serratia marcescens	-	-	+	[171]
Shewanella putrefaciens	+	-	-	[199]
Shigella dysenteriae	-	-	+	[194]
Shigella flexnerii	+	-	+	[165,174,177,204]
Sphingomonas panni	+	-	-	[105]
Staphylococcus aureus[1]	+	+	+	[64,93,94,105,108,109,112,127,128,130,131,134,138,165,167-172, 175,181,184,199,200,205,206]
Staphylococcus epidermidis	+	+	+	[105,168,191,195,207] UR
Staphylococcus haemolyticus	+	-	-	[105]
Staphylococcus hominis	+	-	-	[105]
Staphylococcus warnerii	+	-	-	[105]
Stenotrophomonas maltophilia	-	-	+	[164]
Streptococcus faecalis	-	+	-	[175]
Streptococcus pyogenes	-	-	+	[168]
Streptococcus sp.	-	-	+	[165,208]
Vibrio cholerae[1]	+	-	+	[141,190,209]
Yersinia pseudotuberculosis	-	-	+	[180]

Xanthomonas compestris	-	-	+	[202]
Fungi/Yeast				
Alternaria brassicae	-	-	+	[202]
Aspergillus bransilensis	-	+	-	UR
Aspergillus carbonarius	-	-	+	[210]
Aspergillus flavus	+	-	+	[96,172,203,204]
Aspergillus fumigatus	+	-	+	[96,211]
Aspergillus niger	+	+	+	[96,114,172,202,211-214]
Aspergillus oryzae	-	-	+	[212]
Candida albicans	+	+	+	[64,96,104,108,109,112-114,130,168,169,173,193,204,211,214,215]
Candida glabrata	-	-	+	[168,180,194,204]
Candida krusei	-	-	+	[168]
Candida parapsilosis	-	-	+	[168]
Candida tropicalis	-	-	+	[168,180]
Cronobacter sakazakii	-	-	+	[216]
Cryptococcus neoformans	-	-	+	[211]
Culvularia lunata	-	-	+	[195]
Epidermophyton floccosum	-	-	+	[211]
Fusarium culmonium	+	-	-	[96]
Fusarium oxysporium	+	-	+	[96,202]
Fusarium solani	+	-	+	[96,195,204]
Microsporum canis	-	-	+	[204,211]
Myrothecium verrucaria	-	-	+	[212]
Penicillium chrysogenum	+	-	-	[96]
Pleurotus ostreatus	-	-	+	[185]
Pycnoporus cinnabarinus	-	-	+	[185]
Rhizoctonia bataicola	-	-	+	[195,203]
Rhizoctonia solani	-	-	+	[213]
Rhizopus stolonifer	-	-	+	[203]
Saccharomyces cerevisiae	+	-	+	[103,104,169,217]
Torulopsis pintolopesii	-	-	+	[215]
Trichoderma viride	-	-	+	[212]
Trichophyton longifusus	-	-	+	[204]
Trichophyton mentagrophytes	-	+	+	[113,114,194,212]
Tricophyton rubrum	-	+	+	[113,211]
Tricophyton schoenleinii	-	-	+	[194]
Virus				
Avian influenza	-	+	+	[111,205]
Adenovirus type 1	+	+	-	[99,218]
Bacteriophages	-	-	+	[219-223]
Coxsackie virus types B2 and B4	+	-	-	[218]
Cytomegalovirus	-	+	-	[99]
Echovirus 4	+	-	-	[218]
Herpes simplex virus	-	-	+	[219,220]
Human immunodeficiency virus	-	+	+	[99,108,110,224]
Infectious bronchitis virus	-	-	+	[225]
Influenza A	+	+	-	[99,111,135]
Junin virus	-	-	+	[220]
Measles	-	+	-	[99]
Parainfluenza 3	-	+	-	[99]
Poliovirus	+	-	+	[222,226]
Pichinde	-	+	-	[99]
Punta Toro	-	+	-	[99]
Respiratory syncytial virus	-	+	-	[99]
Rhinovirus 2	-	+	-	[99]
Simian rotavirus SA11	+	-	-	[218]
Vaccinia	-	+	-	[99]
West nile virus	-	+	-	[108]
Yellow fever	-	+	-	[99]

[1]Tested also against antibiotic resistant pathogens; [2]Unpublished data.

and of standard textile clothing used by 10 emergency workers did not find any significant difference in the extent of microbial contamination between the textiles[119]. It may be that a larger sample size was required to prove the silver containing fabric efficacy. It should be taken into consideration that in contrary to *in vitro* conditions, a continual re-inoculation with pathogens occurs during real-life health care scenarios. In addition, the killing of the microorganisms is not on contact, as it takes time for the biocidal textiles to kill the exposed microorganisms. Thus, obtaining sterile hospital or health-care associated fabrics by biocidal textiles in a healthcare environment

cannot be expected. Obviously, trials demonstrating that the use of biocidal textiles does not only reduce bioburden in clinical settings, but also reduces NI rates, still need to be conducted.

BIOCIDAL HARD SURFACES IN THE HEALTHCARE ENVIRONMENT

On February 2008 the USA Environmental Protection Agency (EPA) permitted the USA Copper Association to make public health claims and state that copper alloy products kill 99.9% of disease causing bacteria within 2 h and continue to do so when re-exposed[126]. This approval has now been given to 355 different copper alloys (including brass and bronze) following many years of independent laboratory testing based on rigorous EPA approved protocols. Copper is the only hard surface metal that has received approval by the EPA to make antimicrobial public health claims. In addition to the tests conducted by the USA Copper Association in order to obtain the approval for the registered health claims, the biocidal properties of copper surfaces was demonstrated by many others as well[96-98,100-102,104,105,127-142]. As can be seen in Table 1, copper surfaces can be regarded as a wide spectrum biocidal surface, as it has been found to be efficacious against a wide array of gram positive and negative bacteria, fungi and viruses. The biocidal efficacy of copper surfaces increases with the copper concentration[97,101,104,127,128,130,133,134,137,139], exposure periods[96-98,100-102,104,127-130,133-135,137,139,140,143], humidity[127,128,131,136] and temperature[98,129,131,133,144]. The higher the microorganism inoculum load is the longer it takes to reach complete elimination of the exposed microorganisms[133,134,137]. In contrast to stainless steel, which is the metal most widely used in hospital care environments, copper surfaces are highly reactive, and thus residual soil and build-up of microbial cells is more likely to occur in copper surfaces than on stainless steel[145]. Different cleaning solutions or products may have different effects on the continual efficacy of the copper surfaces[145] and thus the right cleaning and appropriate cleaning protocols of copper surfaces need to be developed[102].

Importantly, the significant contribution of copper surfaces to the reduction of bioburden in clinical settings has recently been demonstrated[132,146,147]. One trial was conducted in the United Kingdom[146], one in South Africa[147] and one in Germany[132].

In the United Kingdom study[146] the efficacy of copper surfaces to reduce bioburden was examined in a busy acute medical ward, which included gastroenterology patients, and a cross-over model was utilized. A toilet seat, tap handles and a ward entrance door push plate each containing copper (60%-70% copper content) were sampled for the presence of microorganisms and compared to equivalent standard, non-copper-containing items in the same ward. The items were installed at least 6 mo prior to the commencement of the study to allow both healthcare workers and staff to become accustomed to the copper containing items. The hospital staff followed their standard cleaning routines, which included disinfection of both the control and test fixtures approximately every 2 h. The items were sampled once weekly for 10 wk at 07:00 and at 17:00 to determine the number of microorganisms present following quite and busy time periods, respectively. The following specific indicator bacteria were quantified: methicillin-sensitive *Staphylococcus aureus* (MSSA), MRSA, VRE, *C. difficile* and *E. coli*. After 5 wk, the copper-containing and non-copper-containing items were interchanged to exclude any possibility of bias according to preferential use of any particular item based on location. Median numbers of microorganisms harbored by the copper-containing items were between 90% and 100% lower than their control equivalents at both sampling time-points, the microbial loads being highly statistically significantly different between the matched tested items (P values ranging from < 0.05 to < 0.0001). Three of the indicator microorganisms (MSSA, VRE and *E. coli*) were only isolated from control items. MRSA and *C. difficile* were not isolated during this study.

In the South Africa study[147], a comparative controlled study was conducted at a busy walk-in primary healthcare clinic in a rural region. Two similar adjacent consulting rooms were chosen. One was fitted with copper sheets (99.9% pure copper) on desk and trolleys that were in constant contact with staff and patients and on top of cupboards and windowsills where contact was less frequent. The other room remained with its original surfaces that did not include any copper surfaces. Cleaning procedures were the same for both rooms and no disinfectants were used. Samples for microbiological determinations were taken from 5 equivalent touch surfaces from each room. Sampling was undertaken for a period of 4 and a half days every 6 wk by the same person for a period of 6 mo. Samples were taken before cleaning (at 7 am), post cleaning but pre consultation (at 8 am) and post consultation (at 4 pm). The temperature and humidity in both sampling rooms were comparable during the study period covered - winter, spring and summer. The average number of consultations in each room during each sampling series during the 6 mo study was similar (65 study and 68 control room). Statistically significantly lower overall mean total CFU for all copper surfaces, including those in constant contact with staff and patients and those with less frequent contact, were found ($P < 0.001$), being the mean reduction 71%.

In the German study[132], an oncological/pneumological and a geriatric ward was used to test the efficacy of copper surfaces in reducing bioburden. All touch surfaces in patient bed rooms, rest rooms and staff rooms were replaced with new surfaces composed of metallic copper-containing alloys, while matched rooms, where no changes were made in the touch surfaces, served as controls rooms. All surfaces were routinely cleaned each morning with a disinfectant. The trial lasted 32 wk, 16 in the summer and 16 in the winter. During both test periods of 16 wk, the total number of CFU on metallic cop-

per-containing surfaces was 63% of that on the control surfaces ($P < 0.001$). When analyzing per surface area, the differences were significant for door knobs, which had the highest overall microbial load. Bacterial loads in push plates and light switches were similar between the test and control samples. Interestingly, after disinfection of the copper and control surfaces, microbial repopulation of the surfaces was significantly delayed on copper alloys ($P < 0.05$).

In addition to the above studies, a clinical study was undertaken to compare the surface microbial contamination associated with pens constructed of either a copper alloy or stainless steel used by nurses on intensive care units. A significantly lower level of microbial contamination was found on the copper alloy pens[148].

Another study, conducted in the UK, investigated the efficacy of using biocidal hard surfaces impregnated with a silver based technology in reducing microbial contamination in a real-life hospital environment[149]. Two outpatient units were included in the 18 mo study. One unit was refurbished with the silver containing products, which included door knobs, blinds, tiles, sack holders and light switches. The other unit contained untreated items and served as a control. Both units were similar in terms of volume of people and layout and were subjected to similar standard cleaning practice. Both units were allowed to function for 12 mo before microbiological swabbing commenced. Swabs were collected over a 5-mo period from both units. The CFU counts in the unit containing the silver impregnated products were between 62% to 98% lower than the matched unit. CFU counts from the silver-treated materials were between 70% (fabrics) to 99% (laminates) lower than untreated equivalents. In addition, the bacterial contamination on untreated products in the ward containing the silver-impregnated products was on average 43.5% lower compared with untreated matched products in the control unit.

The above described trials clearly demonstrate that biocidal hard surfaces found in heath-care settings offer the potential to significantly reduce the number of microorganisms in the clinical environment and thus reduce the risk of HAI. However, the use of biocidal surfaces should not act as a replacement for cleaning in clinical areas, but as an adjunct in the fight against HAI.

IS MICROBIAL RESISTANCE TO COPPER A CONCERN?

Bacterial resistance is a major concern in infection control, as exemplified by the highly antibiotic resistant bacteria (with up to 2200-fold decreased sensitivity to the antibiotic (e.g.,[150]) that have evolved in less than 50 years of antibiotic usage, making infected patient treatment extremely difficult (e.g.,[29]). Thus, the possibility of development of resistance to biocides is a real concern[151,152]. Importantly, as opposed to antibiotics, in spite of copper being a part of the earth for millions of years, and being used by humans from the beginning of the civilization, no microorganisms that are highly resistant to copper have been found, but only microorganisms with reduced copper sensitivity (increased copper tolerance). For example, Enterococci bacteria isolated from the gut of pigs, which were fed for many months with high concentrations of copper in their diet, were 7 fold less susceptible to copper than Enterococci bacteria isolated from pigs not fed with copper[153,154]. The increased tolerance to copper is achieved by the induction of an efflux pump in the tolerant bacteria[154]. Outstandingly, the Enterococci and *E. coli* tolerant bacteria isolated from pig farms following the use of copper sulfate as feed supplement were rapidly killed when spread in a thin, moist layer on copper alloys with 85% or greater copper content or under dry conditions[155]. Tolerance, but not resistance, was found in nitrifying soil microorganisms exposed to Cu for nearly 80 years under field conditions[156]. Similarly, the spray of copper-containing compounds for years on vegetable and fruit crops to limit the spread of plant pathogenic bacteria and fungi, has favored the spread of copper tolerant genes among saprophytic and plant pathogenic bacteria[157]. The increased tolerance to copper was found to be associated with the amount of soluble copper and not with the total amount of copper[158]. Thus, even in soils where the concentration of copper was very high, but in a non-soluble form, no increase in tolerance to copper was observed[158]. The copper active ingredient used in the biocidal textiles is copper oxide, a non-soluble form of copper. Importantly, no resistant bacteria evolved *in vitro* when consecutively exposed to repeated fabrics containing 1% copper oxide[112]. Interestingly, bacteria were isolated from copper-containing surfaces and some exhibited prolonged (1 to 3 d) survival on dry but not on moist copper surfaces[105]. None of these isolates strains was copper resistant in culture[105]. Survival on copper-containing surfaces appeared to be the consequence of either endospore formation, survival on patches of dirt, or a special ability to endure a dry metallic copper surface.

The reason why no resistance to copper, but only tolerance, is found in microorganisms exposed to constant relatively high doses of copper, may be because copper exerts its biocidal/antimicrobial activity not through one mechanism (as most antibiotics), but through several parallel non-specific mechanisms[90,91]. As briefly mentioned previously, these mechanisms include: (1) denaturation of nucleic acids by binding to and/or disordering helical structures and/or by cross-linking between and within nucleic acid strands; (2) alteration of proteins and inhibition of their biological assembly and activity; (3) plasma membrane permeabilization; and (4) membrane lipid peroxidation. Furthermore, widespread appearance of bacteria tolerant or resistant to copper contact killing appears unlikely as plasmid DNA is completely degraded after cell death by contact killing, preventing the transfer of resistance determinants between organisms[137] and copper contact killing is very rapid precluding the acquisition of

Figure 1 Use of copper in the detailed products, which are in direct or indirect contact with patients, may significantly contribute to the reduction of nosocomial pathogen loads and nosocomial infections. HEPA:

resistance during cell division[102].

Thus, even though some organisms have mechanisms of tolerance to excess copper as described above, in general, all microorganisms cannot cope when exposed to high concentrations of copper and are irreversibly damaged. As a result, despite having been present throughout human history, and despite repeated historic use of copper as an antimicrobial agent over the centuries, copper was and remains a broad-spectrum biocidal/antimicrobial compound and yet no bacteria fully resistant to copper have been discovered.

CONCLUSION

Similar to the efficient control of Legionella infections and the reduction of molds and yeasts that has been achieved in hospital systems by simply incorporating copper-silver ionization devices into the hospital water distribution systems[159-161], the use of soft and hard surfaces containing biocidal copper in products such as those described in Figure 1, may play an important role in reduction of NI in hospital care environments. Furthermore, as NI are now spreading out from the hospital environment into the community (e.g.,[162,163]), the use of textiles, such as those impregnated with copper oxide, and hard surfaces containing a high percentage of copper, may not only significantly contribute to the reduction of HAI, but may also confer protection when used in homes for the elderly and in other environments where immune compromised individuals are at high risk of contracting infections.

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Events Calendar 2012

January 6-7, 2012
International Conference on Tuberculosis Therapy
Edinburgh, United Kingdom

January 15-20, 2012
Keystone Symposia: Drug Discovery for Protozoan Parasites
Santa Fe, New Mexico, United States

January 17-18, 2012
Cyber Security for Government Asia 2012
Kuala Lumpur, Malaysia

January 18-20, 2012
15th Bangkok International Symposium on HIV Medicine
Bangkok, Bangkok, Thailand

January 18-20, 2012
International Congress on Malaria Elimination (ICME)
Kish, Hormozgan, Iran

January 22-27, 2012
Keystone Symposia: Membranes in Motion: From Molecules to Disease
Tahoe City, CA, United States

January 25, 2012
Clinical Trials: ICH, GCP rules, regulatory (EMEA, FDA) GCP inspections.Key documents
Kiev, Ukraine

February 6-8, 2012
Mahidol International Conference on Infections and Cancers 2012
Bangkok, Thailand

February 10-11, 2012
30th Annual UC Davis Infectious Diseases Conference
Sacramento, CA, United States

February 17-18, 2012
National Seminar on Depletion of Forests and Livelihood Concerns
Bangalore, India

March 11-14, 2012
2012 International Conference on Emerging Infectious Diseases
Atlanta, United States

March 14-17, 2012
22nd Annual Meeting of the Society for Virology
Essen, Germany

March 21-25, 2012
Australasian Society for Infectious Diseases Scientific Meeting 2012
Fremantle, WA, Australia

March 22-23, 2012
Sexual Health 2012
London, United Kingdom

March 29-30, 2012
Modern methods of diagnosis and treatment of malignant tumors
Kiev, Ukraine

April 20-21, 2012
Diagnosis and treatment of advanced forms of prostate cancer, bladder cancer and kidney cancer
Kiev, Ukraine

May 10-13, 2012
American Conference for the Treatment of HIV
Denver, Colorado, United States

May 8-12, 2012
30th Annual Meeting of the European Society for Paediatric Infectious Diseases
Thessaloniki, Greece

May 19-22, 2012
New Perspectives on Immunity to Infection
EMBL, Heidelberg, Germany

June 16-17, 2012
Issues of Neurosurgery, Vascular Neurosurgery, Neurooncology, Spinal Surgery and Spinal Cord
Kiev, Ukraine

July 7-14, 2012
Infectious Disease Review
Seattle, Washington, DC, United States

July 22-27, 2012
XIX International AIDS Conference
Washington, DC, United States

August 20-22, 2012
2nd World Congress on Virology
Las Vegas, United States

September 1-2, 2012
Health Effects of Chernobyl Catastrophe - A Quarter of A Century
Kiev, Ukraine

September 15-16, 2012
Modern Principles of Treatment of Neurooncology Diseases. Prospects for Functional Neurosurgery
Yalta, Ukraine

November 10-17, 2012
Infectious Disease
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5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

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

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

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

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Personal author(s)

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

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

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

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

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

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

Patent (list all authors)

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

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Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom

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Italics

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