THE OCCURRENCE AND CONTROL OF PATHOGENS ON FOMITES

by

Jonathan D Sexton

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As members of the Dissertation Committee, we certify that we have read the dissertation

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_____Date:<u>3/29/13</u>

Charles P. Gerba, Ph.D.

_____Date: <u>3/29/13</u>

Kelly A. Reynolds, Ph.D.

_____Date: <u>3/29/13</u>

Kelly R. Bright, Ph.D.

_____Date: <u>3/29/13</u>

Ian L. Pepper, Ph.D.

Final approval and acceptance of this dissertation is contingent upon the

candidate's submission of the final copies of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and

recommend that it be accepted as fulfilling the dissertation requirement.

_____Date: <u>3/29/13</u>

Dissertation Director: Charles P. Gerba, Ph.D.

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SIGNED: Jonathan D Sexton

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TABLE OF CONTENTS

TABLE OF CONTENTS - *Continued*

TABLE OF CONTENTS - *Continued*

TABLE OF CONTENTS - *Continued*

LIST OF TABLES

ABSTRACT

Microbes survive for an extended period of time on fomites. With new strains of pathogens constantly emerging, it is important to understand their survival and spread and to evaluate the efficiency of new disinfection methods. The purpose of this study was to determine the occurrence of pathogens on fomites, and evaluate different disinfection methods (household bleach and steam vapor). Fomites were sampled in a variety of environments for the presence of pathogens including methicillin intermediate- and resistant- *Staphylococcus aureus*, *Clostridium difficile*, *Penecillium brevi-compactum, Alternaria alternata* and novel H1N1 influenza A. Samples were assayed using both cultural and genetic techniques to determine the microbial occurrence. In disinfection studies, samples were collected before and after disinfectant application. Steam vapor was effective at reducing microbial occurrence by >90% with a minimal contact time of 10-20 seconds on hospital fomites. The material and shape of the surface had an effect on the reduction rate, due to access of the disinfecting agent. In addition, low levels of initial contamination limited the reduction rate. Many surfaces had a starting contamination level of less than 3 $\log_{10}$ and after disinfection the contamination levels, in 69% of the samples, were at or near the limit of detection. This suggests a higher reduction is feasible. Household bleach reduced the presence of mold spores and mycelium by $\geq$99% on gypsum-wall board. Reduction rates were influenced by the growth stage of mold, with the mycelium requiring additional disinfectant application to achieve the 99% reduction rate. Novel H1N1 influenza A was not recovered on any surfaces in day care

facilities and elementary schools. This is consistent with previous studies looking at

H1N1 influenza occurrence in similar environments, though it is different from H3N2

influenza A studies. Survival differences among varying influenza strains are expected to

have an impact on pathogen spread and human health risks. Differences can be

quantitatively evaluated and used to develop more advanced risk assessment models.

Steam vapor and household bleach are effective at reducing risks of pathogens in the

environment and are critical interventions in an overall strategy to minimize exposure and

prevent disease.

CHAPTER 1: INTRODUCTION

Problem Definition:

Microbes can survive for an extended period of time on fomites, ranging from hours to months (Dietze et al., 2001; Kramer et al., 2006). Pathogen survival on fomites allows for potential transfer to other fomites and hands, increasing the risk of person-to-person transmission. Even after disinfection of surfaces it can be difficult to maintain a pathogen free environment, as microbes will settle out of the air or be recontaminated by touch (Boone and Gerba, 2007). Hospital patients that have an active *Clostridium difficile* infection can contaminate 49% of room surfaces compared to 29% of surfaces when the patient is only colonized (Gerding et al., 2008).

Pathogens are continuously emerging and gaining virulence factors. This is evident in the evolution of methicillin resistant *Staphylococcus aureus* (MRSA), which gained a resistance to commonly administered antibiotics (β-lactams) and the ability to infect people that are not considered immuno-compromised (Chini et al., 2008). In addition to MRSA, *C. difficile* has also gained the ability to infect non-immuno-compromised people (Kelly and LaMont, 2008). In 2009, a novel strain of influenza A emerged. This pathogen quickly spread to over 214 countries around the world and was a quadruple reassortment compared to the traditional triple reassortment of influenza A viruses (Hu, 2010; WHO, 2010).

In addition to the increased virulence of pathogens, certain popultaions have decreased their ability to fight off infections. This is due to $\geq 20\%$ of the United States population being immuno-compromised (Kendall et al., 2003; Flaherman et al., 2007). Immuno-compromised people include people with HIV/AIDS, elderly, young children, hospital patients and people with underlying conditions, such as cancer. The increased amount of immuno-compromised people, leads to larger importance of disinfecting surfaces. Exposure to a pathogen can cause illness resulting in a greater risk of hospitalization and death in this population. For example, exposure to mold is known to exacerbate symptoms for the 19% of the population that is estimated to have respiratory ailments, such as asthma, and allergic reactions (CDC, 2009a). Repeat exposure to certain pathogens can cause sensitization, which results in a larger immune response to a smaller number of microbes (Douwes and Pearce, 2003).

Horizontal transfer of genetic material between pathogens can create a pathogen with a greater resistance to antibiotics and more virulence factors. This allows a pathogen to spread readily though the hospital environment, which may contain immuno-compromised patients. For instance, a review of intensive care unit (ICU) admissions has shown that prior room occupation by patients carrying vancomycin-resistant enterocccus (VRE) increases the odds that the next room occupant will contract a VRE infection by 2.5 to 3.8 (hazard ratio) (Drees et al., 2008). This is due, in part, to contamination of fomites that are able to infect the next occupant.

A review of the literature suggests that conventional disinfection fails to reduce pathogens below detection limits. In a study completed by French et. al. (2004), it was

shown that conventional disinfection failed to reduce the presence of methicillin resistant *Staphylococcus aureus* (MRSA) on 66% (82/114) of the tested surfaces. Similarly, Byers et al. (1998) found that conventional disinfection failed to reduce the presence of vancomycin resistant *Enterococci* (VRE) contamination on 15.9% (60/376) of sampled sites. Nerandzic et. al. (2010) achieved an 80% reduction for *C. difficile* using UV radiation. This reduction was less than that achieved for MRSA and VRE, suggesting that more effective disinfection applications need to be evaluated.

Literature Review:

Nosocomial Pathogens

Clostridium difficile

Health Effects

Clostridium difficile is a major nosocomial pathogen and recently an increase in cases has been seen in the community. In the mid- to late 90's there was an incidence rate of 30-40 cases per 100,000 people. By 2005, this figure has increased to 50-84 cases per 100,000 people, with endemic outbreaks as high as 92.2 cases per 100,000 people (Kelly and LaMont, 2008). More than 300,000 cases of antibiotic-associated diarrhea each year are caused by *C. difficile* (Muto et al., 2005; Musa et al., 2010). The average cost of a case of *C. difficile* associated disease (CDAD) is $3,600 and in the United States, the total costs exceed $1 billion per year (McDonald, 2005). It is thought that antibiotic use is directly related to the spread of *C. difficile* as they help to disrupt the natural gut flora and allow *C. difficile* to proliferate (McDonald et al., 2005).

CDAD can be very difficult to treat. Even after a successful course of antibiotic treatment, there is an average recurrence rate of 20.2% (range of 6.7% to 28.6%) (Kelly and LaMont, 2008). The recurrence can be from the same strain as the initial infection or

caused by a different strain. After each recurrence, the risk of repeat infection increases

an additional 20% (Kelly and LaMont, 2008).

CDAD can be treated in multiple ways, including- the use of antibiotics or

probiotics (Kelly and LaMont, 2008). Antibiotics help to prohibit the growth of *C.*

difficile, while the probiotics regenerate the natural gut flora. Immunotherapy is another

common way to treat infections (Kelly and LaMont, 2008). This involves the intravenous

injection of immunoglobulins that are capable of neutralizing *C. diffifile* toxins A and B.

The third way that CDAD is treated is through the use of bacteriotherapy (Kelly and

LaMont, 2008). This involves introducing a nonpathogenic strain of *C. difficile* into the

gut in order to colonize the typical niche of the pathogenic strain. This type of therapy

also includes adding natural gut flora, normally from a close relative, in order to

reestablish colonization.

Pathogen Characteristics

Pathogenic strains of *C. difficile* are associated with the presence of two toxins,

enterotoxin A and cytotoxin B (Kuijper et al., 2006). These toxins bind to intestinal

epithelial cells and are then internalized. Once internalized, they are responsible for

catalyzing the glucosylation of cytoplasmic proteins which results in cell death (Kelly

and LaMont, 2008). Strains can produce either toxin (non-pathogenic) or a combination

of toxins A and B. Production of only toxin B is associated with pseudomembranous

colitis (Warny et al., 2005).

The genes for these toxins are carried on the pathogenicity locus (*PaLoc*) (Warny et al., 2005). Their production primarily occurs during the logarithmic growth phase. Higher production of these two toxins has been associated with more virulent strains of *C. difficile*. In addition to these two toxins, there is also a binary toxin that is produced by non-pathogenic and pathogenic strains (Kelly and LaMont, 2008). This toxin is not well understood and its role in infections is not defined, though it is thought to act synergistically with toxins A and B.

Evidence suggests that particularly virulent strains may spread more easily from one person to the next due to their higher rates of morbidity via contaminated surfaces. Studies have shown that contamination of the environment with *C. difficile* can result in the presence of the pathogen on the hands of healthcare workers (Hota, 2004) and hospital surfaces are routinely disinfected yet the pathogen still persists due to difficulties in its disinfection.

C. difficile is able to form endospores, which are resistant to environmental stresses (e.g., desiccation and temperature) and chemical disinfection. They also have the ability to survive on surfaces for up to five months (Hota, 2004). The surfaces that are most commonly contaminated with *C. difficile* include floors, bedrails, bed sheets, nurse call buttons, blood pressure cuffs, feeding tube equipment and intravenous catheters (Hota, 2004; Gerding et al., 2008). *C. difficile* has been isolated on hospital surfaces with frequencies as high as 58%. Surfaces are more contaminated in rooms in which the patient is colonized or infected by *C. difficile*. In a study by Gerding et. al. (2008), it was found that in rooms with an active infection of *C. difficile,* 49% of the surfaces were

contaminated, in comparison to 29% of surfaces in rooms where the patient was only colonized.

The type of disinfectant used to clean surfaces has an effect on the sporulation rate of *C. difficile*, in laboratory studies. Non-chlorine based disinfectants increase the sporulation rate of *C. difficile*, when used to disinfect surfaces that are contaminated with the pathogen (Kuijper et al., 2006). This suggests that the use of non-chlorine based disinfectants could enhance the spread of *C. difficile*.

Clostridium difficile can be divided into more than 150 different ribotypes and 24 different toxinotypes (Kuijper et al., 2006). Ribotype 027 was initially isolated in 1988 from a 28-year-old woman suffering from severe pseudomembranous colitis (Kuijper et al., 2006). This ribotype was rarely isolated in humans until the early 2000's, but has now become a more prevalent strain that is partly responsible for the increase in the number of *C. difficile* associated disease (CDAD) cases, the other factors being the increased use of antibiotics and an increase in the immuno-compromised population. In the mid to late 1990's, the U.S. had an average of 30-40 cases per 100,000 people. This increased to 84 cases per 100,000 people in 2005 (Kelly and LaMont, 2008). There was also a 26% increase in the number of discharge patients who were treated for CDAD from 2000-2001 in the U.S. (McDonald et al., 2005). The high rate of fluoroquinolone use has helped *C. difficile* ribotype 027 to spread more rapidly, as this strain is resistant to the antibiotic and other strains are not (McDonald et al., 2005).

One of the more prominent outbreaks associated with the 027 strain occurred in January of 2005 in Quebec, Canada. Thirty different hospitals experienced a spike in

CDAD cases that was five times the historical average. This was associated with an increased use of antibiotics and an increased virulence and/or resistance associated with this strain (McDonald et al., 2005; Kuijper et al., 2006). This strain is known to produce more than ten times the average concentration of toxins when compared to other non-ribotype 027 strains (Kelly and LaMont, 2008).

There are many ways in which the spread of *C. difficile* could be controlled besides disinfection. One is to limit the use of antibiotics that have been associated with *C. difficile* infection. This includes the use of flouroquinolones, particularly levofloxacin (Muto et al., 2005). This antibiotic was associated with 31% of all *C. difficile* infections at a hospital in Pittsburgh (Dial et al., 2004). In addition to antibiotics, proton pump inhibitors have also been associated with an increased prevalence of disease (Dial et al., 2004). Limiting the use of these inhibitors could also help to prevent infections.

Methicillin Resistant *Staphylococcus aureus*

Pathogen Characteristics

S. aureus is an opportunistic Gram positive bacterium that can be isolated on the epidermis and in the nasal passage of 32% (approximately 89.4 million people) of the United States population (Kuehnert et al., 2006). The symptoms associated with *S. aureus* include minor skin infections, life-threatening pneumonia, septicemia, and death (Klevens et al., 2007).

Some strains of *S. aureus* are resistant to many commonly administered β-lactam antibiotics (Naimi et al., 2003). These antibiotics contain a characteristic ring structure (three carbon atoms and one nitrogen atom) and include penicillin, amoxicillin, cephalosporin, and carbapenem and monobactam antibiotic groups. *S. aureus* strains that exhibit this resistance are known as methicillin resistant *Staphylococcus aureus* (MRSA).

Methicillin efficacy is achieved by binding to the penicillin binding proteins (PBP), that help to form the cross links in peptidoglycan. Once the PBPs are bound by the antibiotic they are unable to form these cross links, which renders the cell unable to reproduce (Stapleton and Taylor, 2002).

The defining feature of MRSA is the presence of the Staphylococcal cassette chromosome *mec* (SCC*mec*), which is a mobile element containing the methicillin resistance determinant *mecA* (Laplana et al., 2007). The *mecA* gene works by encoding for a new type of penicillin binding protein, PBP2a that has a low affinity for β-lactams. This allows the PBP to continue to form the cross links (Fan et al., 2007). The presence of the *mecA* gene allows for a minimal inhibitory concentration of oxicillin and other β-lactams of 4 mg/L, though there have been a few strains reported to be *mecA* positive and oxicillin sensitive (Hososaka et al., 2007). There are five different SCC*mec* variants (I-V) identified to date, with each variant carrying different genes and levels of resistance (Deurenberg et al., 2006). All of these variants possess the *mecA* gene, while variants II and III also carry non-β-lactam resistance genes on integrated plasmids and transposons.

To date, there are two main types of *S. aureus* that carry the *mecA* gene, hospital acquired-MRSA (HA-MRSA) and community acquired-MRSA (CA-MRSA). The first to

evolve was HA-MRSA, which was first reported in the United States in 1968 (Rice, 2006). HA-MRSA strains are resistant to β-lactam antibiotics and soon developed resistance to many non- β-lactam antibiotics as well (SCC*mec* variants II and III). The common antibiotic used to treat this form of infection is vancomycin (Rice, 2006). HA-MRSA is mainly associated with hospitals and long term care facilities.

CA-MRSA is primarily associated with the general population, or those who have had no contact with hospitals or long-term care facilities. HA-MRSA primarily infects the elderly or the immunocompromised. In contrast, the emergence of CA-MRSA has been associated with a worldwide increase in MRSA infections in young children and healthy adults (Tsuji et al., 2007). It is a unique form of MRSA, in that it carries additional genes and has different resistance factors. The most common virulence factor for CA-MRSA is the PVL exotoxin, which is the product of two genes, *LukS*-PV and *LukF*-PV (Sudagidan and Aydin, 2010). These genes have been proposed as CA-MRSA markers to distinguish it from HA-MRSA. CA-MRSA has a 96% prevalence of the PVL genes compared to a 25% for HA-MRSA (Chini et al., 2008). *LukS*-PV and *LukF*-PV encode for two subunits, LUKS-PV and LUKF-PV proteins, which together make up the Panton-Valentine leukocidin (PVL) exotoxin. PVL is a necrosis toxin in the synergohymenotropic toxin family. Its genes are encoded on a prophage, Φ-PVL, and integrated into the bacterial chromosome. The proteins, LukS-PV and LukF-PV, are secreted from the bacterium and assemble in the membrane of the host cell. After assembly, there is a pore formed between the two subunits, which allow the cell contents

to leak. The PVL toxin is especially lethal to human neutrophils, monocytes and macrophages (Gosbell, 2005).

The PVL gene evolved independently and prior to the *mecA* gene in community strains. The genomes of 100 methicillin sensitive *S. aureus* (MSSA) isolates collected from active skin and soft tissue infections were compared to the genome of a CA-MRSA strain. The CA-MRSA PVL genes closely resembled those of the PVL-positive MSSA strains (Monecke et al., 2007). This toxin was initially thought to be the virulence toxin that allowed individuals in the community to become infected. In a study done by Voyich et. al. (2006), mice infected with PVL positive and negative MRSA strains were examined and results demonstrated that the presence of the PVL genes does not increase MRSA virulence (Voyich et al., 2006). Due to the increased use of antibiotics, CA-MRSA is now filling the ecological niche of PVL-positive MSSA.

While the PVL genes are sometimes used to differentiate between HA-MRSA and CA-MRSA, there are also two other toxins believed to be involved in the virulence of CA-MRSA. The γ-hemolysin is a toxin that causes the breakdown of red blood cells. *LukE* and *lukD* each encode for a protein subunit of a separate toxin that looks and acts similar to the PVL toxin (Chambers, 2005). It works by inserting itself into the host membrane and creating a pore, which allows the internal contents to leak. These genes are thought to be partially responsible for the increased virulence of CA-MRSA.

S. aurues also contains the gene *mprF*. This gene is responsible for the resistance that *S. aureus* displays against host defensins and protegrins, preventing the formation of pores in the bacterial cytoplasmic membrane. It does this by modifying the membrane

phosphatidylglycerol with L-lysine. This modification reduces the negative charge of the membrane and helps to repel the cationic peptides of the defensins (Peschel et al., 2001).

Besides the PVL toxin, there are other factors that differentiate CA-MRSA from HA-MRSA. Most CA-MRSA strains have fewer antibiotic resistance factors than HA-MRSA. HA-MRSA is the form of *S. aureus* that can be found in hospitals and is known to be resistant to a wider range of antibiotics. It is believed that these two different types of MRSA evolved independently of each other, due to the presence and absence of various genetic factors (Chambers, 2001). This would support the theory of PVL evolution. There are many different strains of *S. aureus*. Some of these strains have the PVL genes and some do not, though strains with the genes are more commonly found in the community environment. It is thought that strains without the PVL genes gained resistance to β-lactams (HA-MRSA) before strains that encode for the PVL toxin (CA-MRSA).

CA-MRSA also has a faster doubling time than HA-MRSA (Gosbell, 2005). The community strains are able to double in 28 minutes compared to 39 minutes for nosocomial strains. This allows the community strain to establish an infection in the host more effectively.

There are many factors that are associated with a recent increase in the prevalence of MRSA infections. These include the presence of certain underlying diseases (e.g., AIDS and diabetes), different immune responses (neutrophil activation), and socioeconomic factors (limited access to healthcare, household crowding) (Fridkin et al.,

2005). Such underlying diseases are on the rise, so more infections in the community can be expected.

Previous antibiotic use also increases the risk of CA-MRSA infection (Lo et al., 2008). CA-MRSA also has the ability to infect healthy individuals. In the past, most MRSA infections arose in the immunocompromised populations or in people that have under gone an invasive procedure. CA-MRSA originally infected healthy populations, as it was able to out-compete the immune system, before moving into the nosocomial arena.

The dominate strain of MRSA that is circulating in both hospitals and the community is CA-MRSA clone USA300. This clonal strain has yet to become predominately multidrug-resistant, as HA-MRSA strains in the past have, although there have been cases of multidrug-resistance. Diep et al. (2008) found that men who have sex with men have been shown to be acquiring this type of infection. It is thought that this strain is circulating in those populations.

There are many hypotheses as to why USA300 has become the dominant strain of MRSA found in both the community and the hospital. USA300 carries the SCC*mec*IV element, which is smaller than the HA-MRSA SCC*mec* elements. This smaller element may enable more efficient horizontal transfer, as well as an increased growth rate (Seybold et al., 2006). This allows for a greater spread within the community and, the hospital. The rate of infections in the community is surpassing the rate of infections in the hospital, which has not always been the case (Liu et al., 2008).

USA300 has evolved to infect healthy individuals. This evolutionary trait makes it a perfect candidate to infect immunocompromised individuals. This, too, is a reason for the increased prevalence of CA-MRSA in the nosocomial setting.

The occurrence of CA-MRSA as the dominant type of MRSA in hospitals has lessened the environmental transmission distinction of CA-MRSA and HA-MRSA. USA300 will continue to be the dominant strain in and out of hospitals and will likely develop greater antibiotic resistances, as has been the case with *S. aureus* in the past.

Health Effects

Staphylococcus aureus can be both a commensal and a pathogen. It is found on the nares of 30% of the U.S. population (Wertheim et al., 2005). There are two distinct types of colonization, persistent and intermittent. Persistent colonization appears in 20% of the population and intermittent colonization in 30% (Wertheim et al, 2005; Gordon and Lowy, 2008). In one study, it was determined that 82% of patients with a *S. aureus* infection were infected with their native strain (Gordon and Lowy, 2008).

S. aureus has multiple survival strategies in the body. Biofilms can form on host tissues and prosthetic surfaces and can aid the organism in persistence and in evading the host's immune system.

MRSA can also survive in epithelial cells, which provide protection from the host's immune system. Small-colony variants (SCV) can be produced by MRSA. These SCV will invade a host cell only to emerge later, after reverting back to the virulent wild-

type phenotype. This strategy is important in recurring infections (Gordon and Lowy, 2008). In a 24-year study examining MRSA infections, Mulligan et al. (1992) found that the average number of strains isolated from an infected patient was 2.8. Differences in strains can lead to infections of varying sites and degrees of severity.

The rate of infection with invasive CA-MRSA varies between cities but is in the range of 18.0 to 31.8 cases per 100,000 people (Fridkin et al., 2005; Dancer, 2009). Males, blacks, the elderly, and children under two years old have the highest incidence rates. Also, inmates in correctional facilities, men who have sex with men, Native Americans, and Alaskan Natives are other groups of people that are more susceptible to infections with MRSA (Shukla, 2005). This is due to genetic and social determinates, such as a genetic predisposition to being colonized or personal and group hygiene. In a recent study, it was determined that an infection can occur with as few as 10 staphylococci, but may require as many as 1,000,000 in some cases (Dancer, 2009). The adverse health effects from MRSA infections have continued to rise, resulting in 18,650 deaths in 2005; this is a greater number of deaths than AIDS (Dancer, 2009).

Hospital Disinfection and Pathogen Control

The hospital environment allows for the growth and survival of nosocomial pathogens. Cleaning and disinfecting surfaces significantly disrupts the ecological niches of pathogens (Dancer, 1999). Thorough cleaning has resulted in a decrease in the number of infections. Florence Nightingale can be credited for first applying the idea of

environmental contamination with a high rate of infection in hospitals in the 1850s

(Dancer, 1999). She organized laundry services, the cleaning of equipment, and the

repairing of wards, which resulted in a decreased death rate (from 42% to 2%) from

cholera, typhus, and dysentery. In addition to a decreased death rate, the economic

burden of healthcare-associated infections (HAI) has dramatically decreased as well. In

2007, the estimated cost of HAI ranged from $28.4 billion to $45 billion and the use of

infection control interventions saved from $5.7 billion to $31.5 billion (CDC, 2009b).

Surface cleaning can be one of the first things cut during periods of decreased

economic stability in hospitals (Dancer, 1999, Griffith et al., 2000). Such relaxing of

cleaning practices can result in higher rates of secondary infections and a more general

distrust of the hospital's ability to treat patients due to the aesthetics. The responsibilities

of cleaning items are also divided in many hospitals, which can cause the cleaning of

some items to be overlooked or abandoned (Dancer, 2009). The delicate equipment and

items surrounding the patient are generally cleaned by the nurses, while other items are

decontaminated by the cleaning staff.

Outbreaks in hospitals can be best controlled by finding a discrete source of

contamination. Disinfecting the source of contamination is more effective than

disinfecting the general environment. For example, it is more efficient to disinfect a

contaminated air vent than all the rooms that the air vent services (Dancer, 1999). There

is an increased risk of a patient contracting MRSA when placed in a room in which the

previous occupant had a MRSA infection due to contamination of the near-patient hand

touch sites (Dancer, 2009; Andrade et al., 2009). In outbreaks of MRSA, it was found

that doubling the cleaning routine was effective at reducing the number of cases (Dancer, 2009). Hospitals are expected to benefit from the fomite decontamination guidelines developed for the food industry. These guidelines include the regular sampling of surfaces to monitor the cleaning efficacy before outbreaks occur (Dancer, 2009).

The amount and types of surfaces in the hospital environment limit the effectiveness of disinfectants. The surfaces are often irregularly shaped and rough, resulting in limited contact with the disinfectant. Bedrails are commonly made of plastic which over time can develop scratches, and if contaminated with pathogens, will offer some protection from disinfectants. There is also a large amount of electronic equipment, which traditional liquid chemical disinfectants may damage. In addition, many of these surfaces are very tedious to clean such as blood pressure cuffs and feeding tube equipment. The vast number of surfaces in the room that need to be disinfected creates a large work load for the environmental services department. This often leads to practices being unknowingly altered and can increase the rate of transmission (Gerding et al., 2008).

Liquid chemicals are most frequently used to disinfect surfaces in the healthcare environment and the CDC recommends that chlorine bleach be used during outbreaks of *C. difficile* (Gerding et al., 2008; Mayfield et al., 2000). Bleach has several disadvantages, including lengthy requisite contact times (5-10 minutes), potential reactivity with acids and other chemicals that produce toxic fumes, deactivation through contact with organic matter, and irritation to skin, eyes, and the respiratory tract (Tanner, 2009). Due to bleach being a respiratory irritant, it can be hazardous to workers or

visitors nearby. For this reason, rooms cannot be regularly disinfected while the patient or visitors are in the room. Bleach can also damage equipment (causing corrosion and pitting) (Gerding et al., 2008). In addition to chlorine, quaternary ammonium compounds are also commonly used.

Environmental decontamination is important to prevent the spread of nosocomial pathogens. Detergent sanitizers have been shown to decrease the presence of pathogens on 44-84% of surfaces (French et al., 2004; Byers et al., 1998). The use of unbuffered bleach solutions decreased the incidence of CDAD cases from 8.6 to 3.3 cases per 1000 patient-days compared to quaternary ammonium compounds, which had an incidence rate of 8.1 cases per 1000 patient days (Mayfield et al., 2000). The overall effectiveness of surface disinfection relative to public health benefits is still unknown and need to be evaluated further.

Nevertheless, a review of the literature suggests that conventional disinfection fails to reduce pathogens below detection limits. In a study completed by French et al. (2004), it was shown that conventional disinfection with a detergent sanitizer containing 5-15% non-ionic surfactant and 5-15% cationic surfactant failed to reduce the presence of MRSA on 66% (82/114) of the tested surfaces. Similarly, Byers et al. (1998) found that conventional disinfection failed to reduce vancomycin resistant *Enterococcus* (VRE) contamination in 15.9% (60/376) of sampled sites. These results are comparable to the disinfection of *C. difficile*, in that there is not a complete reduction. Nerandzic et al. (2010) achieved an 80% reduction for *C. difficile* using UV radiation and a 93% reduction for MRSA and VRE.

Hand and clothing hygiene are extremely important to limiting the spread of nosocomial pathogens. It is common, for multiple patients, to be in the care of a single nurse and doctor. Medical personnel are able to transmit pathogens on hands and clothing as they travel between patient rooms (Bhalla et al., 2004). Gloves are not mandatory in all cases and often are worn improperly. For example, gloves should be worn when dealing with MRSA or *C. difficile* patients, but do not need to be worn with patients who have broken limbs. Hands need to be washed immediately after the gloves have been removed, as well as when entering a patient's room (Goldmann et al., 1996). It is recommended to wash hands with soap containing chlorhexidine (Mayfield et al., 2000). Nurses should also have more training on enteric precautions when dealing with patients that have diarrhea to limit the spread of pathogens (Worsley, 1998).

In many hospitals, MRSA patients are isolated. Isolation might prove effective for *C. difficile* positive patients as well. If patients are isolated or kept only with other positive patients, the spread of *C. difficile* can be prevented.

Indoor Mold

Health Effects

Factors of Adverse Health Outcomes

Fungi (molds and yeast) have the largest biomass of all organisms and there is an estimated 1.5 million species, though only 7% have been identified (Maier et al., 2009). However, with less than 50 identified as human pathogens, the majority of molds are considered plant pathogens (Myrvik et al., 1974).

Adverse outcomes associated with mold exposure can be explained by a few factors including virulence, concentration, and host resistances (Genuis, 2007). Different species of mold cause variable health outcomes such as hay fever-type symptoms, sneezing, runny nose, red eyes, dermatitis, and cancer (EPA, 2009). This can be due to the allergenic properties of the mold, the presence of glucans, and the production of mycotoxins. *Alternaria alternata* is the main cause of allergies and asthma in children aged 6-11 years (Pieckova and Jesenska, 1999) due to its allergenic properties. Glucans are a component in the cellulose layer of many types of molds. They come in the form of β glucans and α glucans, with β glucans being more medically important (Maier et al., 2009; Iossifova et al., 2009). Low level exposure to β glucans, before the age of 1 year, can increase the risk of developing childhood asthma by seven times. In contrast, high level exposure can decrease the risk. Mycotoxins are a secondary metabolite that are

produced by molds and are able to cause adverse health effects in humans, animals and plants such as cancer, kidney damage, and damage to the immune system (Hussein and Brasel, 2001). Molds can produce multiple mycotoxins and some can be produced by multiple species of molds. Mycotoxins can also have different toxicities and work synergistically when present together as is the case of Ochratoxins A, B, and C (Muller et al., 2004). The end result of mycotoxin exposure can be cancer, as is the case with Aflatoxin B and Ochratoxins.

The exposure inoculum is related to the concentration of the mold, as well as the duration of the exposure. The inoculum can be a high concentration for a short period of time or a low level exposure for an extended period of time, as is the case with sick building syndrome (Rios et al., 2009). Sick building syndrome is associated with symptoms, such as headaches, eye, nose or throat irritations, and itchy skin and fatigue that are worsened the longer a person is in a mold contaminated building. The symptoms often disappear altogether once the person has been out of the building for a period of time (Rios et al., 2009). The exact cause of sick building syndrome cannot be determined, though it is thought that many of the cases are attributable to mold exposures.

Host resistances play a role in the health outcomes of mold exposure. Some people are more susceptible to develop adverse symptoms such as those with asthma or the immunocompromised (Genuis, 2007). There is also some evidence that exposure to molds early on in life will help to immunize one against the development of asthma and allergies (Liu and Murphy, 2003; Iossifova et al., 2009). This can be noted by comparing rural and urban communities. In the rural setting, there is a greater exposure to microbes

and a lower rate of asthma and allergies than in an urban setting (Liu and Murphy, 2003).

However, there is evidence that exposure to mold glucans before the age of three,

increases the risk to develop asthma by a factor of seven (Iossifova et al., 2009).

Pathogenesis

There are three basic types of pathogenesis that the body can experience after

exposure to mold. These include host hypersensitivity, harmful colonization (infection),

and the absorption and dissemination of mycotic biochemical products (Genuis, 2007).

Hypersensitivity results from both internal and external exposure to mold molecules that

induce a host immune response. Spores are considered the causative agent of mold-

induced allergies though previous studies show that fragments from the fungal mycelium

contain many of the same receptors and biomarkers as the spores and are also able to

cause adverse health effects (Gorny et al., 2002).

The exact mechanism of the mold-induced allergenic response is not well

characterized. Some theorize that the immuno-globulin E-inducing allergens, mycotoxins

and glucans, are primarily responsible (Douwes and Pearce, 2003). These are produced

by the organism and are found on the cell wall as well as excreted from the mold during

metabolism. It is also postulated that fungal proteases and surface antigens are able to

cause the immune response (O'Driscoll et al., 2005). The antigens and proteases are

related to human proteases and thus can illicit the adverse reactions. These are both found

in the cell walls of the spores and hyphae of molds.

The most common form of allergenic response is IgE allergic type reactions, though IgG may also be induced (Genuis, 2007). There are 70 distinct mold allergens identified that have been known to cause conjunctivitis, bronchitis, rhinitis and asthma. It is estimated that 35% of all newly diagnosed adult asthma cases can be attributed to occupational mold exposures (Genuis, 2007).

Mycotic infections are caused by the mold growing inside and colonizing the host. Sinus infections are generally thought to be caused by bacteria and viruses, though in many cases they have been attributed to molds, where the swelling and irritation of the sinus cavity is caused by the growing hyphae (Genuis, 2007). Once colonized in the sinus cavity, molds can produce symptoms such as headache, hearing loss, fatigue, central nervous system effects, and long and short term memory loss (Kilburn, 2009). *Aspergillus, Rhizopus* and *Alternaria* species have all been isolated as the cause of invasive mold sinusitis (Iwen et al., 1997).

Lung infections can also be caused by molds, with a majority of the infections caused by *Aspergillus, Scedosporium* and *Fusarium* species (Tarrand et al., 2003). In the southwest United States, a common lung infection, Valley Fever, is caused by the soil fungus *Coccidioides immitis* (Kolivras et al., 2001). This mold is able to cause infections in humans, as well as other mammals.

Nosocomial mold infections are also a cause for concern. Molds can be isolated in the hospital environment in the air, water, and on surfaces, despite routine disinfection and the use of water and air filters (Anaissie et al., 2003). Molds that are most commonly

isolated in hospitals include *Aspergillus, Paecilomyces, Alternaria,* and *Fusarium*

(Anaissie et al., 2003).

Exposure to biochemical products also includes exposure to mycotoxins. These

toxins can be airborne and found in food and drink. Exposure to these types of

compounds is able to cause sensitivity reactions or systemic effects (Genuis, 2007),

sometimes resulting in cancer. Multiple mycotoxins can be produced by a single species.

For instance, ochratoxin A, B and C can all be produced by multiple species of

Penicillium and *Aspergillus* (Heussner et al., 2007).

Health effects from mold exposures are thought to affect a large number of United

States citizens; 37 million people (18% of the population) suffer from nasal sinus disease.

The incidence of people suffering from chronic rhinosinusitis that is directly related to

mold is 93% (Ponikau et al., 1999), while one in seven (45 million) people suffer from

asthma. In addition, there is also decreased vitality of various organs caused by exposure

to molds and increased risk for certain cancers, such as liver and kidney cancers (Kilburn,

2009). These are all health effects that can be associated with mold exposure, in addition

to other microbes.

Pathogen Characteristics

Many factors contribute to the contamination of surfaces including the species

present, the surface materials, and the environmental conditions (e.g., temperature,

relative humidity, and water availability) (Pitkaranta et al., 2008). The variety of species

present is important due to competition and their ability to break down available

nutrients. The types of available nutrients present may select for or promote the growth of

one type of mold over another (Pitkaranta et al., 2008). Menetrez et. al. (2009) found that

Stachybotrys chartarum grows at a relative humidity as low as 64% but required some

additional surface moisture. The mold could also grow at 100% relative humidity, with

no additional moisture. Molds require water to grow, as well as a high relative humidity

of at least 60% (Silicato and Cannon, 2006), though the exact humidity and water

requirements vary based on the species.

In addition to fungal growth on surfaces, mold exposures may also occur from air

sources. Mold spores from different species and hyphal fragments are more frequently

detected in the air than on surfaces (Reboux et al., 2009). *Stachybotrys* species are more

commonly isolated on surfaces, while *Penicillium, Aspergillus,* and *Cladosporium*

species are more commonly isolated from the air. The latter three genera are the most

common indoor mold contaminants, though the levels reported vary (Reboux et al., 2009;

Pitkaranta et al., 2008; Koch et al., 2000; Scott et al., 2004).

Penicillium species are the most commonly isolated indoor mold with frequencies

varying from 68 to 90% of samples when isolated from the air and 2.8 to 63% from

surfaces (Gravesen et al., 1999; Reboux et al., 2009; Koch et al., 2000; Hyvarinen et al.,

1993; Reynolds et al., 2012). *Penicillium* species rely on the aerial dispersal of spores,

hence the high occurrence in indoor air. *Penicillium chysogenum* is the most common

mold found in food, and household dust and air (Scott et al., 2004). *Penicillium* is able to

grow in damp buildings and on indoor finishes. A teleomorph (sexual) stage has not been

identified to date for *Penicililum*. The genus *Penicillium* grows rapidly with loosely branched, smooth ellipsoidal conidia and produces yellow guttation droplets in the center of the colony. *P. chysogenum* is considered to be an important allergen, though it does not produce any mycotoxins (Scott et al., 2004), as do some species of *Penicillium*. This species is the only species used for the Skin Prick Allergen Test in France, even though there can be a wide variety of species present (Reboux et al., 2009) resulting in false negatives. *Penicillium* species also are commonly found outdoors, though in lower levels than indoors (Scott et al., 2004), suggesting that the mold is able to grow and produce spores in the indoor environment.

Cladosporium species are the second most commonly found indoor fungi with frequencies varying from 50 to 82% in the indoor air and as low as 31% on surfaces (Reboux et al., 2009; Koch et al., 2000; Reynolds et al., 2012). They too rely on the aerial dispersal of spores. Many *Cladosporium* species appear at specific times of the year, similar to *Penicillium* and *Aspergillus*. They are mainly found in the summer (Scott et al., 2004), though they have been isolated year-round from the indoor environment (Pitkaranta et al., 2008). Yeast-like organisms such as *Malassezia* and *Cryptococcus. Cladosporium* are mainly found in the winter.

Aspergillus species are also commonly isolated from the indoor air with frequencies ranging from 25 to 71% (Koch et al., 2000). They have a lower isolation rate for surfaces (<1 to 14%) (Reboux et al., 2009; Reynolds et al., 2012) and are similar to *Penicillium* in that they are also commonly isolated in the soil and outdoor environment, but at lower levels (Scott et al., 2004). In Denmark, *Aspergillus* has been isolated from up

to 55% of indoor dust samples (Koch et al., 2000). The average concentration of spores was 2.4×10^3 colony forming units (cfu) per gram of dust and ranged from $<10^2$ to $>10^6$ (Koch et al., 2000).

The wide range of isolation frequencies and concentrations can be attributed to a few factors. The first is temperature and climate, partly due to outdoor sources. Each organism grows more efficiently at a genus-specific temperature range. In the winter, there are different predominant genera, due to a shift in the range of temperatures. The amount of water in the air and on surfaces can also fluctuate with seasons. This will cause competition and new dominant species.

Another reason for this range is researcher bias. Researcher bias occurs as a result of media and growth condition choices. Each species of mold has its own specific optimal growth conditions (Pitkaranta et al., 2008). The media, as well as temperature choice, can cause one organism to out compete another. Also, some species have more rapid growth rates such as *Penicillium* species. This leads to some species being underrepresented in samples.

Molecular methods have the ability to identify mold species that cause adverse health effects in the indoor environment with greater precision than cultural methods (Pitkaranta et al., 2008). Cultural methods rely on mold viability and growth, while adverse health effects may also be caused by non-viable molds since individual protein markers can illicit an immune response (Genuis, 2007). Molecular methods also have the advantage of eliminating competition between mold species due to growth characteristics. DNA analysis showed that the majority of the species identified in indoor

air were not the most common ones found using cultural methods (Pitkaranta et al., 2008). The fungal communities were also more diverse than previously thought. The diversity was similar to that found in soil and plant rhizospheres, (>30 different species per 10 grams of soil) (Smit et al., 1999).

Molds can grow in a variety of locations in the home, due to the creation of moist microclimates (Portnoy et al., 2004). Some of the most common areas include indoor plants, carpets, bathrooms, firewood or newspaper piles, drip pans for the fridge and freezer, garages, and basements (Genuis, 2007). These locations can all be damp and provide the required moisture for mold growth. Growth leads to spore production and poor indoor air quality (Koch et al., 2000). *Alternaria* species are isolated indoors but at low concentrations suggesting that they do not reproduce indoors as readily as *Aspergillus* and *Cladosporium*. In many cases, the mold that is contributing to the increase of spores in the air is not even visible (Johnson et al., 2008). There may be mold growth in ceiling tiles, inside walls, in ventilation systems, under carpets or under counters. It is also thought that mold growth in one room can increase the number of airborne spores throughout the whole house due to ventilation. This could be why some homes with no visible mold growth have similar concentrations of spores in the air to those with visible mold growth (Reboux et al., 2009).

A study compared three different types of homes: 1) homes with visible molds and occupants that had adverse health effects, 2) homes with no visible molds and occupants that had allergies to molds and 3) homes with no visible molds and occupants with no allergies to molds. It was determined that the bedroom had the highest mold

concentration levels in homes with visible mold growth (Reboux et al., 2009). The

adverse health effects could stem from the length of time that occupants spend in the

bedroom. The bathroom had the highest concentration of mold spores in homes with no

visible mold growth, yet occupants still had allergies to molds. In each case *Penicillium*

was the most commonly isolated fungus (Reboux et al., 2009).

Regulations for Control

There are currently no air quality regulations regarding molds in indoor

environments (Johnson et al., 2008). This is due to the difficulty in developing a dose

response relationship with indoor mold exposures.

Assessing the quantitative levels of molds in indoor environments that cause

adverse health effects has proven difficult. Air quality in an indoor environment is

considered poor when air mold levels exceed the levels found (at the same time)

outdoors. Visible molds are commonly present with poor indoor air quality (Johnson et

al., 2008). Statistical tests have been used to compare the levels between indoor and

outdoor environments. Due to confounders, no statistical significance has been

demonstrated (Johnson et al., 2008).

The lack of data for the development of guidelines has led to an ongoing debate

over what levels of mold exposure are acceptable. Conflicting information on health

effects due to mold exposures has been reported over large concentration ranges. There

has been numerous court cases in which both sides have had an industrial hygienist

examine the scientific data, which resulted in conflicting recommendations (Johnson et al., 2008).

Scientists have proposed threshold levels for mold exposures. Reboux et al. (2009) developed four levels of concentration categories: 1) below 170 cfu/m^3, 2) 170-560 cfu/m^3, 3) 560-1000 cfu/m^3, and 4) greater than 1000 cfu/m^3. These concentration categories were based on *Penicillium* counts from previous studies. Each mold species would require a unique concentration category based on species virulence or associated toxicity. Applying a standard to encompass all mold species would be difficult since some species would pose a risk at concentrations at which others would not. However, having multiple standards based on individual mold species is not economical, as each mold would have to be identified rather than just monitoring the overall mold spore levels in the air.

Treatment Recommendations

Currently there are no quantitative standards for molds. The U.S. EPA does have strategies for reducing radon and lead levels in the home environment, which in 2003 were utilized in approximately 2 million homes (Wu et al., 2007). Radon and lead strategies could be modified for providing education and options to homeowners who experience mold contamination, including new building codes and home transfer incentives (Wu et al., 2007). Such building codes would be modified to require the use of antifungal building materials in locations that have a high risk for mold contamination. Public education could include a curriculum with a Water Intrusion Management Plan

(Silicato et al., 2006) involving regular checks for leaks and water intrusion events by either the homeowner or a certified plumber. These strategies, in combination with surface disinfection, can help to reduce mold and allergen levels.

The CDC recommends that porous surfaces with mold contamination be removed and that hard non-porous surfaces be treated with a diluted bleach solution (1 cup bleach to a gallon of water). After washing with soap and water, the surface should be scrubbed using a brush and treated with the diluted bleach solution before being rinsed with water (Brandt et al., 2006). Even though the CDC recommends a bleach solution for hard non-porous surfaces many other state and federal public health guidelines do not, and others consider its use controversial (USEPA, 2001; Bureau of EODE; Committee on EH, 2006).

Most buildings do not have exposed drywall. Instead, the drywall tends to be painted or covered in plaster. This increases the nutrient availability and encourages mold growth (Menetrez et al., 2008). Antimicrobial paints may be used that are proven to limit the growth of mold on these surfaces (Menetrez et al., 2009).

Other strategies proven to minimize mold contamination in the home include weekly cleaning, proper ventilation, and moisture reduction. Ventilation helps to reduce the moisture levels in the home and strategies to increase ventilation include the opening of windows and using mechanical ventilation systems, particularly in the bathroom, the laundry room, and cooking areas (Wu et al., 2007; CDC, 2009a). The relative humidity in the home should be kept between 40-60% (CDC, 2010a).

<u>Influenza A</u>

Health Effects

Influenza A causes a contagious respiratory illness known as influenza or the flu. It can range from mild to severe and causes an estimated 35,000 deaths a year in the United States. Between 1976 and 2006, the number of deaths ranged from 3,000 to 49,000. Flu symptoms include fever, chills, cough, body and headaches, fatigue, chest discomfort, sore throat, and runny nose. Many people develop complications that include bacterial pneumonia, ear infections, sinus infections, and dehydration. Also other chronic medical conditions can worsen (e.g., congestive heart failure, asthma and diabetes). High-risk populations for contracting influenza include children under the age of five years, adults over the age of 65 years, pregnant women, people with respiratory/liver/kidney/heart disorders, the morbidly obese, and people with compromised immune systems. (CDC, 2013)

In addition to seasonal flu symptoms, 25% of patients experience diarrhea and vomiting with an infection caused by H1N1 influenza A (2009). Diarrhea and vomiting does occur in other seasonal influenza cases, but they are rare (CDC, 2013; Hu, 2010). The 2009 H1N1 influenza virus infected mainly young adults and children, compared to other seasonal influenza which infects mainly young children and the elderly. The 2009 H1N1 outbreak was associated with 18, 449 deaths worldwide (WHO, 2010). The low

death rate can be attributed to the population most affected, young adults, whose immune systems are better able to fight off the infection.

Viral shedding can occur up to two days prior to the development of symptoms and can last for 10 days after the onset of illness (Cowling et al., 2010; Lau et al., 2010). In individuals with asymptomatic infections, 14% actively shed the virus throughout the duration of infection (Lau et al., 2010).

There are two types of vaccines available for seasonal influenza. They include the inactivated vaccine (flu shot) and the live attenuated nasal spray vaccine. The inactivated vaccine is approved for people over the age of six months while the live attenuated vaccine is approved for people over the age of two years and younger than 49 years. It is recommended that high-risk populations, as well as caregivers for these high-risk individuals, get the vaccine yearly (CDC, 2010b). The vaccine is reformulated each year and its effectiveness can vary based on the predominant strain that is circulating that year. It generally becomes available in September. Vaccination can help to limit the spread of the seasonal influenza by 24 to 99.8%.

There are four different types of antiviral drugs that are effective against seasonal influenza; these include amantadine, rimantadine, zanamivir and oseltamivir. These drugs come in the form of pills, liquids or in an inhaler. They are used for the treatment as well as the prevention of influenza (CDC, 2010a). The treatment of influenza cases with antiviral drugs is the most effective when administered within 48 hours of the onset of the illness. These treatments generally last for five days except with extreme cases.

Influenza Strains

Seasonal (H3N2)

Influenza A is an enveloped virus belonging to the Orthomyxoviridae family and the *Influenzavirus A* genus (Strauss and Strauss, 2002). The virus consists of eight minus-sense single-stranded RNA segments that encode 11 different proteins including; hemagglutinin (HA), neuraminidase (NA), matrix 1 (M1), matrix 2 (M2), nucleoprotein (NP), non-structural protein 1 (NS1), non-structural protein 2 (NS2), polymerase acidic protein (PA), polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2), and polymerase basic protein 1-F2 (PB1-F2) (Hu, 2010).

Influenza viruses are characterized by the major surface antigens, HA and NA. There are 15 different subtypes of HA and nine different subtypes of NA. Both HA and NA subtypes are labeled numerically. Only three HA and two NA subtypes have been isolated from epidemic strains (Strauss and Strauss, 2002). All other subtypes have been found in the major reservoirs, including wild ducks and other waterfowl. In migratory birds, different viruses evolve through the re-assortment of genes with other strains of influenza.

Each year, new influenza subtypes and strains evolve throughout the world. Historical outbreaks of influenza show that the emergence of new subtypes and strains can have a dramatic effect on the virus infectivity and case fatality rates. A new seasonal vaccine must be developed each year due to this evolution. Table 1 lists the different

subtypes, mortality peaks, and the vaccine effectiveness for influenza A for the seasons between 2006 and 2012.

The lifecycle of influenza A consists of 7 steps: 1) entry into the host cell, 2) entry of viral ribonucleoproteins into the host nucleus, 3) transcription, 4) replication, 5) export of viral ribonucleoproteins from the nucleus, 6) virus assembly, and 7) budding at the host cell membrane. This is different from most RNA viruses, which tend to replicate in the host cytoplasm instead of the nucleus (Strauss and Strauss, 2002).

Novel H1N1

In 2009, two novel strains of influenza A circulated in U.S. and global populations, including the epidemic and pandemic influenza A (H1N1), both commonly known as H1N1. The H1N1 virus was first discovered in Mexico City, Mexico in April, from where it spread to other parts of the country and the world within weeks. The death rates in Mexican populations (1.23%) were higher than the rest of the world. The European Union had the lowest mortality rate (0.2%) (Echevarria-Zuno et al., 2009). By September 27, 2009, a total of 4,100 deaths were attributed to the H1N1 influenza virus worldwide, with 146 deaths in Mexico. Higher mortality rates were attributed to the delayed healthcare of those infected, particularly in Mexico as it was the first country with intensive virus circulation (Echevarria-Zuno et al., 2009). In addition, a majority of the deaths in Mexico were in highly populated areas with increased incidence. The mortality rate in Mexico eventually stabilized and was comparable to the rest of the world. High-risk populations for contracting H1N1 influenza include; children under the

age of five years, and adults under the age of 50 years, pregnant women, people with respiratory/liver/kidney/heart disorders, the morbidly obese and people with compromised immune systems.

The 2009 H1N1 influenza virus is similar to other influenza A viruses, yet has some key differences. Through genetic analysis, the virus was found to contain a combination of four different strains of influenza virus; there had been a quadruple reassortment of proteins including those from avian (PB2 and PA), human H3N2 (PB1), classical swine (HA, NP and NS), and Eurasian avian-like swine H1N1 (NA and M) (Hu, 2010). Typical seasonal H3N2 influenzas contain a triple reassortment of proteins (Ravi, 2009). The 2009 H1N1 is similar to the H1N1 influenza pandemic of 1918 (discussed in detail below), with a few differences. The HA protein is responsible for binding to human α 2-3 sialylated glycan receptors. In the 1918 strain, the amino acid responsible for this attachment was residue 225, aspartic acid. In the 2009 epidemic H1N1 strain, both residue 225 and 190 (also aspartic acid) were responsible for this attachment, allowing for increased transmission of the virus (Table 2). In addition to both of these regions being prominent, it is also possible that other nearby residues are able to bind to avian α 2-3 sialylated glycan receptors, which could lead to further exchange of genetic material with other avian influenza strains (Hu, 2010).

The 2009 epidemic and pandemic H1N1 influenza viruses also have different susceptibilities to the available antiviral drugs that are commonly used against seasonal H3N2 influenza A. The epidemic H1N1 strain carries a mutation on the NA gene that confers a resistance to oseltamivir. This mutation is not found in the pandemic strain yet

with both strains circulating at the same time, the exchange of genetic material is possible, favoring this mutation (CDC, 2010a; Hu, 2010).

H1N1 influenza a (2009) caused a panic in populations across the world. This novel virus had not been previously seen and appeared to be highly transmissible, though the molecular markers of pathogenicity were missing (Wang and Palese, 2009). Though symptoms were mild in humans there was fear that mutations would occur that would result in a more dangerous virus and subsequent increases in incidences (Shen et al., 2009).

Historical Strains

The largest outbreak of influenza occurred in 1918 due to an influenza A (H1N1) strain known as the Spanish Flu, which infected 1/3 of the world's population (~500 million people). Death counts are estimated between 50 and 100 million people. This virus had an extremely high case fatality rate of >2.5% and as high as 10%. Typical seasonal influenza infections have a case-fatality rate of <0.1% (Taubenberger and Morens, 2006; Dominguez-Cherit et al., 2009). In late August of 2009, the H1N1 outbreak had an estimated case-fatality rate of 1%; however, as H1N1 is now accepted as the dominant influenza virus and testing to determine the HA and NA subtypes in infected patients is no longer required (Dominguez-Cherit et al., 2009) and thus the mortality rate could be overestimated.

The 1918 Influenza pandemic is unique in that most influenza viruses originally spread from Asia. This influenza spread simultaneously from Asia, Europe, and North America, with three different significant increases of incidence. The first increased incidence was seen in March of 1918, with the second appearing from September to November. The third increase occurred in the beginning of 1919. The second increased incidence was associated with the highest mortality rate (Taubenberger and Morens, 2006).

The Spanish Flu virus was also unique in that it persisted in the summer months, whereas most seasonal influenza viruses tend to wane in the late spring and return in the fall due to temperature, humidity levels and herd immunity. This virus also had a high attack rate within the young adult population, compared to seasonal influenzas, which affect the elderly and young children. Its persistence in the summer and high attack rates are similar to those of the novel H1N1 pandemic virus of 2009.

Genetically, the Spanish Flu virus contained HA and NA proteins derived from avian-like influenza viruses. The 1918 influenza virus is also considered to be the "mother of all pandemics", as it is genetically the ancestor of all H1N1, H3N2, and the "extinct" H2N2 influenza viruses (Taubenberger and Morens, 2006). Through antigenic shift, the 1918 H1N1 influenza strain evolved into the H2N2 influenza strain (no longer found in wild types), which in turn evolved into the H3N2 influenza strain (Scholtissek et al., 1978).

Outbreaks commonly do not affect a certain population, in the first wave of and epidemic/pandemic, only to have a higher attack rate in that population in the following

waves. In 1957, an influenza virus arose from Southeast Asia, termed Asian Flu, and was an H2N2 influenza A virus that lasted for a total of three flu seasons. Its highest mortality rate was in the elderly population, with the exception of the first season (Brasseur, 2007). The Asian Flu virus killed an estimated 1-3 million people worldwide with 70,000 deaths in the United States (Brasseur, 2007; Ghendon, 1994).

The next major flu pandemic occurred in 1968 and was termed the Hong Kong Flu. Like the Asian Flu, the Hong Kong Flu also originated from Southeast Asia. The Hong Kong flu pandemic was caused by an H3N2 influenza A virus. It appeared in the U.S. in late 1968. An estimated 1-3 million deaths occurred worldwide, with 39,000 deaths in the U.S. alone. The 1968 pandemic has been the mildest influenza pandemic to date (the only exception being the 2009 H1N1 pandemic), with a case-fatality rate of <0.1% (Dominguez-Cherit et al., 2009; Brasseur, 2007; Ghendon, 1994). The low case-fatality rate seen during this pandemic is partly due to the low attack rate of the elderly. Many of the elderly had been exposed to similar viruses in the past, and thus had some cross-reactivity with the virus and antibodies present in their systems, resulting in a lower attack rate.

Pandemic H1N1 (2009) has been shown to be the mildest influenza outbreak compared to past pandemics. It had been reported in over 214 countries by August 1, 2010 and had been associated with more than 18,449 deaths (WHO, 2010). The death toll was extremely low when compared to historical pandemics or even to the seasonal influenza strains that had been previously circulating. This virus is now considered to be one of the commonly circulating strains and fear still exists that a viral mutation could

produce a more dangerous strain in the future since this strain has a greater rate of

infectivity than most influenza strains (Shen et al., 2009).

Influenza Control

There are four different modes of transmission for influenza viruses. They include

direct contact, fomites, air droplets ($\geq$5µm), and air nuclei (<5µm), with the latter two

being very similar to one another (Shaman and Kohn, 2009; Brankston et al., 2007).

Multiple modes of transmission require a multistep approach for control.

Recommendations vary on the use of specific facemasks to prevent the spread and

acquisition of influenza viruses. A study completed by Johnson et al. (2009) found that

surgical masks and N95 respirators are both effective at preventing the dissemination of

the influenza virus from infected individuals for a short period of time. N95 respirators

are considered a better choice for preventing the acquisition of influenza virus (Johnson,

2009). The filter's barrier/removal efficiency is 95% of particles ranging from 0.1 to 0.3

pm in size, compared to a surgical mask which has a 71% barrier efficiency for particles

with the same size range (Qian et al., 1998). The infectious dose 50% (ID_{50}) of H3N2

influenza virus is 0.6-3.0 Tissue Culture Infectious Dose ($TCID_{50}$) (Weber and

Stilianakis, 2008). Even with such a low infectious dose for influenza A, both types of

face masks are effective at reducing the amount of disease dissemination and acquisition.

Additional public health interventions have been effective at slowing influenza

virus transmission including the closing of schools, quarantining infected individuals, and

implementing travel restrictions. The closing of schools can reduce the number of cases by 13-17% and household quarantines could have a higher rate of reduced infections if the populace is compliant (Coburn et al., 2009). Travel restrictions, such as banning infected persons from flights, are not as effective due to the difficulties in determining who is actively shedding the virus, and thus are not used as frequently as other public health interventions (Han et al., 2009). Travel restrictions are thought to only slow the spread of an influenza virus by a matter of weeks (Coburn et al., 2009).

One of the most effective public health interventions is vaccination. Widespread vaccination has been shown to reduce the amount of clinical illness in healthy adults by 10% (Bridges et al., 2000). The vaccination of high-risk groups, such as the elderly, has been shown to reduce the level of clinical illness by up to 33%, and reduced mortality by 74% (Gross and Hermogenes, 1995). In one study, the introduction of immunization programs, at K-12 schools reduced the prevalence of households with flu-like illnesses in children and adults by 9% and 4%, respectively, with an incremental cost savings of $171.96 per household (Schmier et al., 2008). Widespread vaccination would increase the herd immunity, also resulting in a decreased incidence of influenza cases. Countries with limited supplies and accessibility often require help from other countries to be able to vaccinate their citizens (Coburn et al., 2009).

The development of a vaccine takes several months. Another issue is the difficulty in getting enough people to get the vaccine. Vaccination rates range from 30.2% to 76.6% for seasonal influenza A (Abramson and Levi, 2008; Horney et al., 2010). The compliance with seasonal vaccination programs varies due to the potential

side effects of the vaccine and the perception that influenza is only a minor disease (Balicer et al., 2007). Such noncompliance with vaccine programs results in viral persistence and in increased transmission.

Human behaviors in private and in public have an effect on the surface transmission of influenza viruses. In public, people tend to worry more about people seeing their actions and thus they are more reserved. The rate of nose picking and eye rubbing when not being observed is 0.4 hr^{-1} compared to a 10-fold decrease when people are facing each other (Chang et al., 2009). Thus, people are more likely to infect themselves from fomite transfer when in private, emphasizing the need for good surface hygiene in households.

Conclusion

Fomites serve as effective vectors for pathogen transmission, resulting in an importance for disinfection of surfaces. The control of nosocomial pathogens, such as MRSA and *Clostridium difficile* on surfaces has resulted in substantial decreases in morbidity and mortality rates, as well as the costs associated with related illnesses. The dose response relationship between mold exposures and human health risks is poorly understood. Surface disinfection can minimize the exposure to molds, which will in turn reduce the risk of adverse health effects. In addition, pathogens are continuously emerging and evolving, as is seen with influenza A. Public health measures such as vaccination and the use of respirators can mitigate the impact posed to human health by

emerging and evolving pathogens. Disinfection is also an important strategy that should

be utilized to control for pathogens on surfaces.

Dissertation Format:

This dissertation contains 3 separate appendices. Appendix A is a manuscript titled "Reduction in the Microbial Load on High-Touch Surfaces in Hospital Rooms by Treatment with a Portable, Saturated Steam Vapor Disinfection System." This manuscript evaluates the use of steam vapor as a potential disinfectant for the hospital environment and is published in the American Journal of Infection Control. Appendix B contains a manuscript titled "Indoor Mold Control on Porous Surfaces Using Household Bleach." This manuscript reports of the efficacy of household bleach to reduce mold contamination on gypsum wall-board and is planned for submission to the Journal of Occupational and Environmental Hygiene. Appendix C contains a manuscript titled "Implications of Influenza Strain Differences on Human Health." This manuscript examines contributing factors associated with the risk posed to human health from strain differences of influenza A and is planned for submission to Risk Analysis. The manuscripts in both Appendices B and C are ready for submission to a peer reviewed journal.

The dissertation author was responsible for data analysis and writing of the manuscript for the study in Appendix A and all research for the studies presented in Appendices B and C.

CHAPTER 2: PRESENT STUDY

The objective of the present study was to determine the occurrence of pathogens on surfaces in a variety of environments, evaluate alternative disinfection methods and examine factors that contribute to human health risks from differences in pathogen strain. Hospitals, daycares and elementary schools were the environments that were evaluated. Disinfection methods included household bleach for porous surfaces and steam vapor. The methods, results and conclusions of this study are presented in the papers appended to this dissertation. The following is a summary of the most important findings.

The manuscript entitled "Reduction in the Microbial Load on High-Touch Surfaces in Hospital Rooms by Treatment with a Portable, Saturated Steam Vapor Disinfection System" can be found in Appendix A. This study evaluates an alternative method to traditional chemical based disinfectants. Surfaces, in a long-term care ward of a hospital, were analyzed for heterotrophic plate count bacteria (HPC), total coliforms, Methicillin-intermediate and –resistant *Staphylococcus aureus* (MISA and MRSA) and *Clostridium difficile*, before and after surface treatment. The steam vapor system reduced the microbial load on surfaces by >90%. This device provides an effective way of reducing the prevalence of microorganisms on surfaces without the use of chemicals, in a hospital environment.

The manuscript entitled, "Indoor Mold Control on Porous Surfaces Using Household Bleach" can be found in Appendix B. This study evaluates the effectiveness of using a household bleach solution to treat porous gypsum-wall board contaminated with mold spores and mycelium. Organisms evaluated were *Penicillium brevi-*

compactum and *Alternaria alternata.* Solutions, prepared following manufacturer's instruction, were effective at reducing mold contamination by up to 4 $\log_{10}$ over a 7 day period. Reduction was greatest against mold spores and with repeated application. This study shows that household bleach can be a useful component to an overall strategy to reduce mold contaminants in an indoor environment.

The manuscript, "Implications of Influenza Strain Differences on Human Health" can be found in Appendix C. This study examines a variety of variables related to host, pathogen and environment factors that contribute to adverse human health outcomes due to influenza A strains. Different strains are associated with different fomite survival, morbidity, and mortality rates. These differences can impact human health outcomes and are important to the development of accurate risk assessment models and intervention strategies aimed at minimizing risk.

Table 1: Vaccine Effectiveness in the United States*

Season	H and N Subtypes	Deaths Atrributed to Pneumonia and Influenza**	Vaccine Effectiveness***
2006-07 (CDC, 2007a-b)	H1, H3N2	0.075-0.077	0.24 (H3N2)
2007-08 (CDC, 2008a-b)	H1N1, H3N2	0.091	0.66-0.77
2008-09 (CDC, 2009a-b)	H1N1, H3N2	0.061-0.076	0.62
2009-10 (CDC, 2010d-e)	Novel H1N1	0.081-0.082	NA
2010-11 (CDC, 2011a-b)	H3N2, Novel H1N1	0.08-0.91	0.968-0.998
2011-12 (CDC, 2012a-b)	H3N2, Novel H1N1	0.079-0.091	0.52

*Reported by 122 Cities Mortality Reporting System

**Death peaks above background levels over the course of 1 week; reported as a rate; Number of deaths attributed to pneumonia and influenza/total deaths

***Reported as a rate; number of vaccinated people with no illness/total number of vaccinated people

Table 2: Comparison of Residues Responsible for Attachment of Influenza Strains

Influenza Virus Strain	Attachment Residue Number	Amino Acid
1918 H1N1	225	Aspartic Acid
2009 Epidemic H1N1	190	Aspartic Acid
2009 Pandemic H1N1	190 and 225	Aspartic Acid

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APPENDIX A:

REDUCTION IN THE MICROBIAL LOAD ON HIGH-TOUCH SURFACES IN HOSPITAL ROOMS BY TREATMENT WITH A PORTABLE, SATURATED STEAM VAPOR DISINFECTION SYSTEM

Jonathan D. Sexton[1*], M.S., Benjamin D. Tanner[2], Ph.D., Sheri L. Maxwell[1], B.S., Charles P. Gerba[1], Ph.D.

1. University of Arizona, Department of Soil, Water and Environmental Science, 1117 E. Lowell St., Tucson, AZ 85721
2. Antimicrobial Test Laboratories, 3000 Joe Dimaggio Blvd Suite 32, Round Rock, TX 78665

Author for correspondence:
* Jonathan D Sexton
The University of Arizona
Dept. of Soil, Water & Environmental Science
Building 90, Room 408
Tucson, Arizona 85721
Tel: (520) 621-6910
Fax: (520) 621-6910
Email: sexton@email.arizona.edu

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Elaine Larson, RN, PhD
Columbia University
School of Nursing
617 West 168th Street
New York, NY 10032
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Author Signature: _Jonathan Sexton_____ Date Signed: 7-16-10_____
Author Signature: _Benjamin Tanner_____ Date Signed: 7-16-10_____
Author Signature: _Sheri Maxwell _____ Date Signed: _7-16-10_____
Author Signature: __Charles Gerba_____ Date Signed: 7-16-10_____
Author Signature: _____ Date Signed: _____
Author Signature: _____ Date Signed: _____

ABSTRACT

Introduction. Recent scientific literature suggests that portable steam vapor systems are capable of rapid, chemical-free surface disinfection in controlled laboratory studies. This study evaluated the efficacy of a portable steam vapor system in a hospital setting.

Methods. The study was carried out in eight occupied rooms of a long-term care wing of a hospital. Six surfaces per room were swabbed before and after steam treatment and analyzed for heterotrophic plate count (HPC), total coliforms, Methicillin-intermediate and -resistant *Staphylococcus aureus* (MISA and MRSA), and *Clostridium difficile*.

Results. The steam vapor device consistently reduced total microbial and pathogen load on hospital surfaces, to below detection in most instances. Treatment reduced the presence of total coliforms on surfaces from 83% (40/48) to 13% (6/48). Treatment reduced presumptive MISA (12/48) and MRSA (3/48) to below detection after cleaning, except for one post-treatment isolation of MISA (1/48). A single *C. difficile* colony was isolated from a door push panel prior to treatment, but no *C. difficile* was detected after.

Conclusions. The steam vapor system reduced bacterial levels by >90%, and reduced pathogen levels on most surfaces to below the detection limit. The steam vapor system provides a means to reduce the risks of microorganisms on hospital surfaces without the drawbacks associated with chemicals, and may decrease risks of cross-contamination.

INTRODUCTION

Infections acquired in hospitals are a major problem worldwide. In the United States alone, there are an estimated 35 million admissions to acute care facilities each year. Of these, 1.7 million are affected by a secondary infection. Approximately 100 thousand secondary infections results in death[1]. The risk of secondary infection increases with the time spent in the hospital[2].

Many of the pathogens associated with hospital-acquired infections survive well on surfaces, and surfaces are believed to play a substantial role in the transmission of these pathogens from one person to the next[3]. For instance, a review of intensive care unit (ICU) admissions has shown that prior room occupation by patients carrying vancomycin-resistant Enterocccus (VRE) increases the odds that the next room occupant will contract a VRE infection[4]. Thus, routine disinfection of inanimate surfaces in the hospital environment is expected to reduce transmission of these pathogens.

Liquid chemicals are most frequently used to disinfect surfaces in the healthcare environment though most liquid chemical disinfectants have drawbacks, including lengthy requisite contact times (5-10 minutes), potential reactivity with acids and other chemicals that produce toxic fumes, deactivation through contact with organic matter, irritation to skin, eyes, and the respiratory tract[5].

A previous study, carried out in a laboratory setting, identified portable steam vapor disinfection systems as a potential supplement to, or replacement for, liquid chemical disinfectants[5]. In that study, a steam vapor system was shown to reduce

microbial contamination against a broad range of pathogens (Methicillin Resisant

Staphylococcus aureus, Vancomycin Resistant Enterococci, *Pseudomonas aeruginosa*)

from an initial inoculumn of 7-$\log_{10}$ to levels below the detection limit in less than five

seconds. The study concluded that the device may offer special advantages with respect

to surface disinfection in a healthcare setting, mainly with respect to decreased toxicity or

chemical irritation if used around sensitive patient populations.

This study used heterotrophic plate count (HPC) bacteria, *Clostridium difficile*,

Methicillin Intermediate *Staphylococcus aureus* (MISA), Methicillin Resistant

Staphylococcus aureus (MRSA), and total coliform bacteria as indicators of

environmental contamination. Methicillin resistant *Staphylococcus aureus* (MRSA) and

Clostridium difficile cause infections in the hospital environment that result in death

(16,340 and 12,000 respectively), and total coliform bacteria have traditionally been used

as indicators of fecal contamination of surfaces though presence of total coliforms does

not necessarily confirm this type of contamination[6]. MISA was chosen as a target

organism because it was thought to represent a relatively common environmental

contaminant from human sources within the hospital environment.

Clostridium difficile endospores are resistant to environmental stresses

(desiccation and temperature) and chemical disinfection and can survive on surfaces for

up to five months[7]. They have been isolated on hospital surfaces with frequencies as high

as 58%. Surfaces are more contaminated in rooms in which the patient is colonized or

infected. The most commonly contaminated surfaces are bedpans, washbasins, walls and

floors[7]. *Clostridium difficile* infections are normally brought about by antibiotic use[8], but

evidence suggests that particularly virulent strains may spread from one person to the next via contaminated surfaces.

Staphylococcus aureus is a Gram-positive, aerobic coccus that can be isolated on the epidermis and in the nasal passage of 32% (89.4 million people) of the United States population[9]. This opportunistic pathogen is able to cause conditions such as minor skin infections, life-threatening pneumonias, septicemia and death[10]. Some strains of *S. aureus* have developed resistance to β-lactam antibiotics[11] and are termed MRSA. MISA is the term for *S. aureus* bacterium that has a decreased susceptibility to these antibiotics. Like *C. difficile*, MRSA has the ability to survive on surfaces from a few hours up to several months[12, 13, 14]. Survival rate depends on the moisture content and the amount of organic matter present. Many studies have investigated MRSA on surfaces in the hospital settings with detection frequencies ranging from 1-100%[15, 16, 17]. The sites with the highest isolation rates include tables, beds, ambulances, and personal devices that the patients come into contact with (i.e. phones, remotes).

Total coliform bacteria are well-studied indicator microorganisms. Total coliform bacteria are normal inhabitants of the intestines of warm-blooded mammals and are more numerous than pathogenic bacteria in feces[6]. They are very hardy in the environment when compared to other pathogens. They are useful indicators in a healthcare setting because they are not expected to be present on surfaces unless the surface has indirectly or directly become contaminated[18].

The purpose of this study was to evaluate the efficacy of a steam vapor disinfection system (Advanced Vapor Technologies, Seattle, WA) under actual use conditions in active, occupied hospital rooms.

MATERIALS AND METHODS

Description of the Study Site

The study site was a long term care wing of a prominent hospital in Arizona. It was selected on the basis of proximity to the University of Arizona, as well as informed, interested, and cooperative hospital infection control, environmental services, and nursing staff.

Study Chronology and Selection of Rooms for the Study

Four rooms were sampled on Tuesday, August 11, 2009, and four more rooms were sampled on Wednesday, August 12, 2009. Six surfaces were sampled before and after treatment with the device in each room. Rooms were sampled while the patients were away (eating lunch, attending appointments with medical specialists, etc). The last room sampled, "H," was noted by nursing staff to be a VRE containment room. All other rooms were normal long term care rooms.

Description of the Device

The device used for this study was a steam vapor system, from Advanced Vapor Technologies (Seattle, WA). The dimensions of the unit are 50 x 35 x 30 cm, and it weighs 5.5 kg dry. The unit used for the study comes with a variety of attachments and a utility cart. For the study described here, it was outfitted with a hose connected to a triangular 14 x 14 x 14 cm triangular cleaning head and moved about the hospital on the utility cart. The unit was filled with ordinary tap water, activated, and allowed to reach the functional operating boiler pressure of 66 psi. The steam delivery output was set to 12-15 psi for all experiments.

Description of Surface Treatment

A towel, machine-laundered with ordinary detergent, was affixed to the cleaning head per manufacturer instructions prior to entering each patient room. High touch surfaces (Guest Chair Arm, Left Bedrail, Right Bedrail, Table, Sink, and Door Push Panel) were treated in a normal, natural fashion with light pressure with the device set to 12-15 psi for approximately 10-20 seconds per surface.

Description of the Sampling Methodology

Samples for microbiological analysis were collected using sterile 2 x 2 inch 100% cotton fabric swatches machine-washed with simple detergent, dried, cut to 2 x 2 inch squares, then autoclave sterilized in aluminum foil. Immediately upon entering a room, a series of 6 "pre-treatment" samples were collected using a team of four microbiologists. To collect a sample, a scientist donned latex gloves which were then sprayed liberally with 95% ethanol by an assisting scientist and then air dried. A swatch was grasped by the scientist with ethanol-sanitized gloved hands, folded twice lengthwise, dipped into a cool (~4˚C) 50 ml sterile plastic centrifuge tube containing 10 ml 1:10 D/E Neutralizing Broth (BD, Franklin Lakes, NJ) (tube opened by assisting scientist) to wet the swatch, then an approximately 25 square inch (5 x 5 inches or 2.5 x 10 inches) area was rubbed vigorously for approximately 10 seconds. After rubbing the surface, the swatch was placed back into the sterile plastic centrifuge tube, capped tightly, sealed with parafilm, and then immediately placed in a cooler on ice for overnight shipment to a commercial testing facility, Antimicrobial Test Laboratories (Round Rock, TX), where samples were processed. After treatment of the surfaces, a corresponding "post-treatment" sample was collected from the same surfaces as the "pre-treatment sample" but in a different part of the surface. For example, the pre-treatment sink samples were taken on the left-hand side, then the entire sink was treated with the device, then the post-treatment sample was collected from the right-hand side. Additionally, the triangular bottom portion of the

cleaning towel was sectioned immediately after treatment of the surfaces (approximately 13.25 square inches).

Initial Microbiological Sample Processing

50 ml plastic centrifuge tubes containing 10 ml 1:10 D/E broth and the used swatch were received cold by the processing laboratory on the mornings following the two sampling events. Aliquots were plated directly to R2A agar (BD) and incubated for five days at 20-25˚C. After incubation, all colonies except fungi were counted. Aliquots were plated simultaneously to Mannitol-Salt Agar and incubated for two days at 36±1˚C (see further processing information, below). Aliquots were also plated simultaneously to m-ENDO LES agar (BD), which is selective for total coliform bacteria, and incubated for two days at 36±1˚C. The m-Endo agar plates were observed for growth typical of total coliforms (dark, relatively large colonies) with a sheen. The same day, five ml of the sample was centrifuged (Hamilton-Bell, Montvale, NJ) at approximately $7000 \cdot g$ for 10 minutes. The supernatant was poured off, except for approximately 0.30 mL. The tube was then vortexed and plated in its entirety to Oxyrase Anaerobic Schaedler's Blood Agar Plates (Oxyrase, Mansfield, OH) supplemented with trace sodium taurocholate (Fisher Scientific, Pittsburgh, PA) and lysozyme (Fisher Scientific, Pittsburgh, PA) and incubated anaerobically for 48 hours at 36±1˚C. All presumptive *C. difficile* positives were then Gram stained and streaked to anaerobic blood agar and observed for typical characteristics next to a positive American Type Culture Collection (ATCC) 43598

control. Typical characteristics included horse-stable odor, grey color, and large, irregularly shaped colony structure.

Identification of MRSA

Mannitol-Salt agar plates showing colonies characteristic of *S. aureus* (round, symmetrical, raised, and bright yellow, with a surrounding yellow zone) were streaked to tryptic soy agar (TSA) plates. In the event that single plating yielded more than 10 presumptive positives, 10 were streaked on TSA and the percentage of total colonies analyzed was factored into subsequent calculations. These plates were incubated for approximately 24 hours at $36\pm1°$C and then observed for colonies typical of *S. aureus* as grown on TSA. Prospective *S. aureus* colonies were then subjected to a catalase test to rule out Enterococci. Isolates demonstrating strong catalase activity were streaked to TSA plates with BD Oxacillin (1µg), and incubated approximately 24 hours at $36\pm1°$C. Proximity of growth to the antibiotic disc was then measured using a standard ruler. Antibiotic sensitivity was then judged in accordance with CLSI guidelines[19].

RESULTS

The results for this study are summarized in Tables 1 and 2 and Figure 1.

HPC and Total Coliforms

The two sites most contaminated with heterotrophic plate count bacteria before cleaning were the bedrail and the bedside table with $1.59 \times 10^3 \pm 3.19 \times 10^3$ cfu/in^2 (n=16) and $952 \pm 1.79 \times 10^3$ cfu/in^2 (n=8), respectively. After cleaning, the average bacterial load in the rooms was reduced by 1.19 and 1.71 $\log_{10}$, respectively, which is greater than a 90% reduction. The sinks had the lowest average concentration of HPC bacteria before cleaning (125 ± 145 cfu/in^2) (n=8). The bacterial numbers was reduced by 0.78 $\log_{10}$ after cleaning.

The surfaces with the greatest $\log_{10}$ reduction were the door push outside and the bedside table, with $\log_{10}$ reductions of 1.76 and 1.71, respectively. The sink had the lowest $\log_{10}$ reduction of 0.78 $\log_{10}$ for HPC.

The bedrail and the bedside table also had the highest concentration of total coliforms before cleaning, 106 ± 182 cfu/ in^2 (n=16) and 56 ± 66.2 cfu/ in^2 (n=8), respectively. After cleaning, these bacterial numbers were reduced by 1.42 and 1.15 $\log_{10}$, respectively. These sites also represent the greatest $\log_{10}$ reduction for total coliform bacteria. The lowest $\log_{10}$ reduction for total coliforms occurred on the door push outside, 0.47 $\log_{10}$, which had an initial starting concentration of 11.8 ± 21.9 cfu/in^2 (n=8). Before cleaning 81% (29/48) of the surfaces were contaminated with total coliforms. This was reduced to 13% (6/48) after cleaning.

Pathogens

The greatest $\log_{10}$ reduction for the presumptive MISA and MRSA was achieved on the bedside table, 0.24 and 0.35 logs, respectively. The bedside table had 7.0 ± 6.0 cfu/in^2 (n=8) of presumptive MISA and 9.0 ± 11.3 cfu/in^2 (n=8) of presumptive MRSA. The lowest $\log_{10}$ reduction occurred on the bedrail with 0.05 and 0 $\log_{10}$ for presumptive MISA and MRSA, respectively. Presumptive MISA had an initial concentration of 4.5 ± 2.0 cfu/in^2 (n=8) and MRSA was below the detection limit of 4 cfu/in^2 in all samples (n=8). There was no decrease in *C. difficile* at the sites tested. It was recovered in one sample, door push outside, at a concentration of 0.08 cfu/in^2.

Presumptive MISA was isolated from 25% (12/48) of the sites before cleaning. After cleaning, the isolation frequency was reduced to 2% (1/48). Presumptive MRSA and *C. difficile* had isolation frequencies of 6% (3/48) and 2% (1/48), respectively, before cleaning and were not isolated after cleaning. In regards to all pathogens, 27% (13/48) of the surfaces were contaminated before cleaning. This was reduced to 2% (1/48) after cleaning with the steam vapor system.

DISCUSSION

The steam vapor system has been tested in a lab scale study using a modified EPA protocol against a variety of pathogens including MRSA, VRE, *Salmonella enterica*,

Pseudomonas aeruginosa, Escherichia coli, and *C. difficile*[5]. The study design was similar to that of chemical disinfectants. In that study the steam vapor system reduced the concentration of pathogens by 5-7 $\log_{10}$ within five seconds. The testing was done on small flat, rough clay "coupons." The log reductions observed for the present study are lower than those reported in the *in vitro* study. The lower log reductions are likely due to the initial concentrations of bacteria. The bacterial concentrations were fairly low so $\log_{10}$ reductions were limited to the $\log_{10}$ value of the initial population. In most cases, microorganisms of concern were reduced to below the limit of detection after treatment.

The sampling method used, splitting the site in half for a before and after sample, might have an effect on the $\log_{10}$ reductions that were calculated. The level of contamination on both halves of the surfaces may not have been the same. It is not known if there was more bacteria present on the before or the after. The lab scale study shows that regardless of the initial inoculum, all pathogens were reduced to below the detection limit within five seconds. The initial inoculums ranged from 1.0×10^2 cfu/test surface for *C. difficile* to 8.0×10^7 cfu/test surface for VRE. In addition to bacteria, fungi and bacteriophage were also tested[5].

For HPC bacteria, the greatest $\log_{10}$ reductions were achieved on the door push outside (1.76) and the bedside table (1.71). Both of these surfaces are smooth and flat which likely ensured better heat transfer from the device, allowing for constant contact of the steamer head with the surface. In comparison, surfaces that are smaller or that have rounded edges, such as the bedrails and the sink, achieved lower $\log_{10}$ reductions, 1.19 and 0.78, respectively. These rounded surfaces make it more difficult for the steamer

head to remain in contact with the surface and allowed more of the steam to escape. It is possible that the increase in the escape of the steam was responsible for the lower log reductions observed for the bedrails and sink. These comparisons are based on general trends and have not been statistically evaluated.

The surfaces tested for the study were made of different materials with different heat retention properties, which may have been partially responsible for differences in efficacy from surface to surface. For example, the door push panels were made of stainless steel, which has a higher capacity for heat conduction. In contrast, the bedrails were made of plastic, which has relatively low heat retention. Correspondingly, the door push panel demonstrated a greater HPC reduction than was observed for the bedrail. Previous laboratory studies (unpublished data) suggest that disinfection from the steam vapor system takes place quickly and independently of the heat retention properties of the surface, but this study suggests that further research into the matter may be warranted.

The average $\log_{10}$ reductions achieved in this study falls short of the EPA laboratory testing requirements of 5 $\log_{10}$ and 3 $\log_{10}$ reduction for disinfectants and sanitizers, respectively[20], though the lab scale study achieved reductions greater than 5 $\log_{10}$ using modified EPA methods. However, very few studies have evaluated the efficacy of EPA-registered disinfectants under normal use conditions such as was done for this study. Data from a few studies suggests that there is a wide gap between log reductions observable from laboratory testing and log reductions to be expected from actual usage[21]. Likewise, the same was observed for the steam vapor device, where previous *in vitro* studies showed >5.0 $\log_{10}$ reductions, while reductions in actual use

were lower. Thus, the 1.76 log reduction of environmental HPCs achieved on the door push by the steam device may actually be greater than that which would be achieved by a standard EPA registered disinfectant under typical use conditions. More research is clearly needed on this matter to establish a "real world" performance expectation for chemical disinfectants before a fair comparison can be made between the steam device and disinfectants.

The concentration of bacteria on the surface before cleaning limits the log_{10} reduction for this study. The highest concentration before cleaning was $1.59 \times 10^3 \pm 3.19 \times 10^3$ found on the bedrail. This would limit the reduction to less than $3 \log_{10}$ once the limit of detection has been accounted for in the calculation. In addition to the low starting concentration, 69% (33/48) of the HPC counts were at or near the limit of detection for the assay after cleaning. This suggests that the steam vapor device could achieve greater log_{10} reductions if the starting concentration of bacteria were higher.

The reductions could also be improved upon by increasing the contact time. In this study the disinfecting head only remained in contact with a given "high touch" surface for approximately 20 seconds, in an effort to mimic normal, natural use of the product in a hospital. The duration of contact with a given part of each high touch surface was probably closer to 2-4 seconds. Thus, we expect that disinfection efficacy could be increased by simply increasing the period of surface treatment. Even with the increase in time it would still require less contact time than common chemical disinfectants which commonly require a 10 minute contact time. The steamer head could

also be modified so that it is better able to handle surfaces that are round or that have rounded edges. These changes could greatly increase the effectiveness of the device.

MRSA, MISA and *C. difficile* were isolated from 6% (3/48), 25% (12/48) and 2% (1/48) of surfaces, respectively, before cleaning. After cleaning, only MISA was isolated, at a frequency of 2% (1/48). These results suggest that the steam vapor system is able to meaningfully and consistently reduce the presence of pathogens on surfaces. A review of the literature suggests that conventional disinfection fails to reduce pathogens to this low level. In a study completed by French et. al. (2004), it was shown that conventional disinfection failed to completely eliminate the presence of MRSA on 66% (82/114) of the tested surfaces[21]. Similarly, Byers et al. (1998) found that conventional disinfection failed to eliminate VRE contamination in 15.9% (60/376) of sampled sites[22]. These studies present the results as presence/absence and did not quantify the reduction based on colony forming units. Future testing in a more contaminated environment may help to further elucidate the frequency with which the steam vapor system can be expected to reduce pathogen loads in actual hospitals to below the limit of detection.

One limitation of this study was that Gram staining and coagulase testing was not incorporated into the identification process for MISA and MRSA. Coagulase testing has been suggested for both MRSA and MISA[23]. These tests could have been used to complement the Mannitol Salt Agar (MSA) used in the study and thus help to more accurately identify *S. aureus*. Studies have also shown that by including the coagulase and other biochemical steps and using media other than MSA can increase the rate of isolation two fold when compared to using MSA alone[24]. Inclusion of the coagluase test

and Gram stain in this study would have potentially decreased the overall detection of

MRSA/MISA, due to elimination of false positives, while increasing the accuracy of

identification.

Another limitation of this study was the method for total coliform enumeration.

Total coliforms were only plated on m-Endo media. While it is selective for total

coliforms, confirmation tests such as oxidase would help to eliminate any false positive

results. In addition to total coliforms, enumeration of fecal coliforms would have been

beneficial.

C. difficile endospores can be found in low levels in the hospital environment.

The low levels of these endospores can make it difficult to isolate. An enrichment step

could be added to the isolation protocol in future studies, in addition to concentrating the

sample to increase sensitivity for this particular microorganism[25]. The lab scale study

showed that *C. difficile* endospores can be effectively treated with this device. In that

study a 2 $\log_{10}$ reduction was seen with an initial inoculumn of $1.0x10^2$cfu/test surface

after 5 seconds[5].

Chlorine based cleaners are proven to kill *C. difficile* endospores but can be

hazardous to equipment (corrosion and pitting) as well as workers or visitors nearby, as it

is a respiratory irritant[26]. This product utilizes steam, which is non hazardous to hard

surface equipment and people if proper cleaning protocols are followed. Typically,

disinfectants require a pre-cleaning step to remove organic matter, which can interfere

with the effectiveness of disinfection. However, the device tested here depends on heat

for efficacy, so the presence of organic matter would not be expected to interfere with efficacy.

After each room was treated, the cleaning implement (a towel wrapped over the head of the device) was analyzed to determine the potential for cross-contamination. Cross contamination is a real concern with traditional disinfectants, as shown by recent studies[27], due to improper use scenarios. HPC bacteria were found on 3 out of 8 towels, with an average of 533 ± 480 total CFU, after cleaning, though analysis of the towel showed that no microorganisms of concern were present (MRSA, MISA, *C. difficile*). This strongly suggests that the device does not pose a risk of cross-contamination of surfaces, such as may be expected with depleted chemical disinfectants. Due to the small number of towels tested in this study, further testing of the towel would be beneficial to confirm this finding.

The conclusions of this study were based on general trends due to low levels of contamination and a small sample size. The sample size of this study was 48 samples therefore, the statistical significance of the results could not be determined. Future studies could include areas that have higher levels of contamination in addition to a collecting a larger amount of samples.

The current study did not look at the effectiveness of the steam vapor system against viruses and fungi, though the previous lab scale study by Tanner (2008) showed up to 6 $\log_{10}$ inactivation of MS2, *Candida albicans* and *Aspergillus niger* within five seconds[5]. Future field studies could be completed to investigate efficacy against viruses and fungi.

CONCLUSION

The steam vapor system was effectively able to reduce the presence of general bacteria at all sites by at least 1-$\log_{10}$ with the exception of the sink, which achieved a 0.78 $\log_{10}$ reduction. These reductions can be improved upon by increasing contact time and altering the device head to provide for better surface contact. Pathogens were initially found on 27% (13/48) of tested surfaces. This was reduced to 2% (1/48) after cleaning. Due to low levels of initial pathogen contamination, $\log_{10}$ reductions of pathogens ranged from 0.05 to 0.35 $\log_{10}$.

The steam vapor system has been proven to reduce microbial contamination on hard surfaces. This device will reduce microbial loads in less time without the production of dangerous byproducts as in chlorine based disinfectants. Further testing needs to be conducted in a more contaminated environment to evaluate the efficacy limits of the treatment and to provide additional information for a statistical comparison against traditional chemical disinfectants.

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Table 1 Average* Bacterial Numbers for Before and After Cleaning Based on Site

	HPC (cfu/sq. in.)**	Coliforms (cfu/sq. in.)**	MISA (cfu/sq. in.)**	MRSA (cfu/sq. in.)**	C. difficile (cfu/ sq. in.)***
Before					
Sample Site (n)					
Chair Arm (8)	154 .9 $\pm$ 197	16.5 $\pm$ 23.0	5 .0 $\pm$ 1.9	4.5 $\pm$ 1.4	<0.08
Bedrail (16)	1.59 x 10^3 $\pm$ 3.19 x 10^3	105.6 $\pm$ 182.7	4.5 $\pm$ 2.0	<4.0	<0.08
Table (8)	952.0 $\pm$ 1.79 x 10^3	56.0 $\pm$ 66.2	7 .0 $\pm$ 6.0	9.0 $\pm$ 11.3	<0.08
Sink (8)	126.0 $\pm$ 145	19.0 $\pm$ 23.5	6 .0 $\pm$ 4.3	5.5 $\pm$ 4.2	<0.08
Door Push Outside (8)	243.0 $\pm$ 480	11.8 $\pm$ 21.9	6.5 $\pm$ 4.8	5.0 $\pm$ 2.8	$\leq$0.08

	HPC (cfu/sq. in.)**	Coliforms (cfu/sq. in.)**	MISA (cfu/sq. in.)**	MRSA (cfu/sq. in.)**	C. difficile (cfu/ sq. in.)***
After					
Chair Arm (8)	70 $\pm$ 8.5	$\leq$4.0	<4.0	<4.0	<0.08
Bedrail (16)	103.1 $\pm$ 351.5	$\leq$4.0	<4.0	<4.0	<0.08
Table (8)	18.5 $\pm$ 27.6	4	<4.0	<4.0	<0.08
Sink (8)	21 .0 $\pm$ 27.3	4.3 $\pm$ 0.7	$\leq$4.0	<4.0	<0.08
Door Push Outside (8)	4.3 $\pm$ 0.7	<4.0	<4.0	<4.0	<0.08

* Arithmetic average
** Detection limit of 4 cfu/in^2
***Detection limit of 0.08 cfu/in^2

Table 2: Log$_{10}$ Reduction of Bacterial Numbers Based on Site

Sample Site	HPC	Total Coliforms	MISA	MRSA	C. difficile
Chair Arm (8)	1.34	0.62	0.1	0.05	N/A
Bedrail (16)	1.19	1.42	0.05	0	N/A
Table (8)	1.71	1.15	0.24	0.35	N/A
Sink (8)	0.78	0.65	0.18	0.14	N/A
Door Push Outside (8)	1.76	0.47	0.21	0.1	N/A

Figure 1: Bacteria of Concern Before and After Cleaning with the Steam Vapor Disinfecting System

Bacteria of Concern, Before Treatment

Bacteria of Concern, After Treatment

APPENDIX B:

INDOOR MOLD CONTROL ON POROUS SURFACES USING HOUSEHOLD BLEACH

Jonathan D. Sexton[1*], PH.D., Kelly A. Reynolds[2], PH.D.

1. The University of Arizona, Department of Soil, Water and Environmental Science, 1117 E. Lowell St., Tucson, AZ 85721
2. The University of Arizona, Mel and Enid Zuckerman College of Public Health, 1295 N. Martin Ave., Tucson AZ 85724

Author for correspondence:
* Jonathan D Sexton
The University of Arizona
Dept. of Soil, Water & Environmental Science
Building 90, Room 408
Tucson, Arizona 85721
Tel: (520) 621-6910
Fax: (520) 621-6910
Email: sexton@email.arizona.edu

ABSTRACT

Introduction: Although generally recognized as an effective hard surface disinfectant, no studies have adequately assessed bleach efficacy on mold-contaminated porous surfaces. Thus, the efficacy of household bleach for preventing growth of mold on porous gypsum-wall board was evaluated.

Methods: Drywall tiles were inoculated with *Penicillium brevi-compactum* and *Alternaria alternata* spores. The molds were then grown under saturated conditions for one week to encourage mycelium production and the tiles were treated with a household bleach solution (5250 ppm sodium hypochlorite; 6%) with a 10 minute contact time. Treatment with water and no treatment were included as controls. The tile surfaces were sampled for mold on days 0, 2, 5, and 7 following treatment using neutralizing letheen swabs and the eluates were incubated on selective media for 3 days at 24°C. Tiles with observable mycelium growth on day 5 were treated a second time.

Results: Household bleach reduced ≥99% of mold on drywall surfaces. The greatest $\log_{10}$ reduction was 4.1 after two applications of bleach for *P. brevi-compactum*. The lowest $\log_{10}$ reduction was 2.58 against *A. alternata* spores after one application.

Conclusion: Household bleach is effective at removing mold spores on porous surfaces with repeated application. Routine use of bleach on porous surfaces may therefore be useful as part of an overall strategy to reduce mold contaminants in indoor environments.

INTRODUCTION

In the United States, an estimated 50% of buildings are contaminated with visible mold or have moisture problems that can lead to mold growth (Mudarri and Fisk, 2007). Exposure to mold or indoor moisture is known to exacerbate symptoms for the 19% of the general population that is estimated to have respiratory ailments such as asthma, and allergic reactions (CDC, 2009). These reactions include, but are not limited to, hay fever-type symptoms, sneezing, runny nose, red eyes, and dermatitis (CDC, 2009). In addition to allergic reactions, mold can colonize the airways and deep tissue and cause bloodstream and superficial infections and toxicity due to mycotoxins (Hardin et al., 2003). Standardized methods have yet to be developed for the comprehensive monitoring of mold exposures and little information exists quantifying the relationship between mold exposures and human health risks (Douwes and Pearce, 2003).

Molds utilize building materials such as drywall, ceiling tiles, and wood (plywood and support beams) as carbon sources (Silicato and Cannon, 2006). Growth on these surfaces can occur more rapidly under conditions of >60% humidity, or once they have been exposed to water, by allowing the spores to germinate and form mycelium. Mold growth on porous materials is common, particularly with increased humidity, and such materials are a challenge to remediate or treat. Following flooding events, for example, general recommendations are that porous materials and items that cannot be thoroughly dried be removed and/or replaced with new mold-free materials (Brandt et al., 2006); however, effective and less costly alternatives are needed. Therefore more information is

necessary regarding the practical use of commercial fungicides on porous surfaces to reduce the growth and allergenic properties of molds.

Many different fungicidal compounds have been commonly used to treat indoor molds on hard, non-porous surfaces such as bleach, phenolics and quaternary ammonium compounds (Light, 2009). Of the commonly used fungicides, bleach is one of the most effective at eliminating mold growth and altering the allergenic properties of the spores (Light, 2009; Gupta et al., 2002, Reynolds et al., 2012); nevertheless, research is lacking on its efficacy at reducing molds on porous surfaces.

Billions of dollars are spent each year in the U.S. for mold abatement and to treat mold-related illnesses. In 2004, the total cost associated with asthma-related illnesses attributed to dampness and mold, was $3.5 billion, based values for mortality, morbidity, and lost days of school and work (Mudarri and Fisk, 2007). Effective mold abatement typically involves the use of disinfectants on hard surfaces, the removal of contaminated porous materials, and changes in ventilation, resulting in a cost of an additional $3.4 billion a year (Levin, 2005). The Insurance Information Institute reported an average affected household claim of $15,000 to $30,000 for the year 2002 (Hartwig and Wilkinson, 2003). For many, the cost of porous material remediation is prohibitive, suggesting the need for effective, on-site treatment recommendations.

Mold remediation costs can be dependent on the species of mold present (Henning et al., 2001). Many species appear at specific times of the year. The summer months are generally dominated by hyphae producing molds and the winters by the yeast-like molds. *Penicillium* and *Aspergillus* species are hyphae-producing molds that are

mainly found in the summer (Scott et al., 2004). *Penicillium brevi-compactum* and *Alternaria alternata* are commonly present in both the indoor and outdoor environment, even in the absence of visible mold growth. *Penicillium* species have been isolated in 68% to 90% of homes and are one of the most common fungi associated with damp buildings and household dust in North America and Western Europe (Gravesen et al., 1999; Roussel et al., 2008). *Alternaria alternata* is the main cause of allergies and asthma in children aged 6-11 years (Pieckova and Jesenska, 1999). Associations have also been shown between the sensitization to *A. alternata* spores in adults and the development of asthma in both damp buildings and the desert environment, with an odds ratio of 2.34 (Zureik et al., 2002; Halonen et al., 1997). Currently, the U.S. Environmental Protection Agency (USEPA) lacks any regulations or guidelines with regard to the remediation of molds in building materials, though there is evidence to support the ability of chlorine bleach to kill molds and to reduce a child's likeliness of developing asthma and sensitivities to indoor allergens (Menetrez et al., 2009; Martyny et al., 2005; Nickmilder et al., 2007).

The purpose of this study was to quantitate the efficacy of household bleach disinfectant for the reduction of two mold species, *Penicillium brevi-compactum* and *Alternaria alternata*, on contaminated drywall, in a controlled laboratory setting. The results of this study can be used to inform future exposure assessments and to build a database on the growth and inactivation of specific mold species, on common building materials in addition to developing guidelines for risk reduction.

MATERIALS AND METHODS

Prior to the evaluation of household bleach efficacy on porous surfaces, its efficacy was evaluated on hard non-porous surfaces against *A. alternata* and *P. brevi-compactum* using the USEPA test protocol for hard surfaces (USEPA, 2009). In order to evaluate its efficacy against molds (spores and mycelia) on drywall, a modified version of the standard USEPA test protocol for hard surfaces was used (USEPA, 2009). The experimental protocol is summarized in Figure 1. In addition, five different concentrations of household bleach (5250 ppm, 525 ppm, 52.5 ppm, 5.25 ppm, and 0.525 ppm) were evaluated against *Penicillium brevi-compactum* after a ten minute contact time, on drywall in order to determine the optimal concentration bleach to be used in subsequent trials.

Spore Enrichment

A. alternata was grown on modified Sorsen agar slants at 24°C for three weeks under a black light, until the presence of dark brown/black spores were visible. *P. brevi-compactum* was grown on dichloran glyceral agar (DG-18) slants for five days at 24°C, until the presence of green spores was visible. The mold spores were detached and collected by manually scraping the top layer of the hyphae with a cell scraper. The spores were then suspended in 4 ml of a 0.85% saline (NaCl) solution.

Disinfectant Preparation

A 5250 ppm (6%) solution of sodium hypochlorite was prepared immediately before use, following the manufacturer's instructions for hard surface disinfection of 1 cup of bleach added to 1 gallon of water, using laboratory grade deionized water (DI).

Inoculation of Drywall

A piece of ¼" drywall (gypsum wallboard) was purchased from a local hardware supply store (Home Depot, Tucson, AZ) and manually cut into 2" x 2" squares. This drywall type was chosen as it is one of the most commonly used with regard to thickness and composition in household construction. Drywall tiles were inoculated with 100 µl of the fungal spore suspension (with an average concentration of 10^6 spores/ml for *P. brevi-compactum,* and 10^4 spores/ml for *A. alternata*) in the form of ten 10 µl drops per tile. Two additional replicate tiles were included, for a total of three tiles per treatment. The inoculated drywall tiles were allowed to air dry at room temperature for 30 minutes.

In a separate experiment, the drywall tiles were inoculated with the fungal spore suspension following the protocol described above. The tiles were then placed in deionized water (depth of 1/8 inch) and allowed to incubate at 24ºC for seven days, to allow for spore germination and mycelia growth. The tiles were then allowed to air dry

for up to eight days at 24ºC until they were visibly dry in order to mimic a contamination/drying event.

Disinfection

The tiles were treated with disinfectant and control solutions (DI water) using a standard household spray bottle, requiring two to three pumps at a distance of 15 to 20 cm to fully saturate the tiles. The average volume applied was 2.9 ± 0.34 ml/tile. The tiles were treated for 10 minutes with reapplication of the disinfectant and control solutions every one to two minutes. The reapplication times vary based on how quickly the solutions were absorbed into the drywall. The tiles containing mycelia absorbed the solutions quicker than the tiles with spores and thus required more frequent application. Tiles that did not receive any application of a solution were used as an additional control. The tiles containing mycelia were disinfected a second time after five days incubation at room temperature following the same protocol. The tiles were incubated for seven days at room temperature with a relative humidity of ~50%.

The surviving molds were recovered using RediSwabs (Biotrace, Forest City, IA) containing 1ml of neutralizing Letheen broth on days 0, 2, 5 and 7. The swabs were vortexed for 30 seconds and assayed using the standard spread plate technique. *P. brevi-compactum* was assayed on DG-18 agar and *A. alternata* on Sabouraud dextrose agar with chloramphenicol (Hardy Diagnostics, Santa Maria, CA). The plates were allowed to incubate for three days at 24ºC. When no mold was detected, a value of 10 cfu/ml (the

detection limit) was used to calculate the averages and standard deviation. Each experiment was repeated for a total of three experiments per treatment.

RESULTS

Prior to the porous surface study, the efficacy of bleach was measured for the reduction of *P. brevi-compactum* and *A. alternata* on hard non-porous surfaces. A 4.74 $\log_{10}$ reduction for *P. brevi-compactum* and a ≥ 2.73 $\log_{10}$ reduction (below the detection limit of the assay) for *A. alternata* resulted after a 10 minute contact disinfection. Greater reductions with *A. alternata* could not be observed due to difficulties in obtaining high spore titer stocks.

The concentration of 5250 ppm resulted in the highest reduction (4.27 $\log_{10}$) of *P. brevi-compactum* on drywall. Other concentrations did not work as well and thus were not evaluated any further.

The mold type and growth stage (spore versus mycelium) had an effect on the results. All reductions were calculated based on the untreated control plate counts for day 0, while the reductions in the untreated controls at day 0 were based off the initial inocula concentrations. The initial drying of the spore inocula in conjunction with the efficiency of the methods produced reductions of 0.88 and 1.07 $\log_{10}$ for *P. brevi-compactum* and *A. alternata,* respectively. Subsequent $\log_{10}$ reductions were calculated using mold counts after this initial loss from drying and method efficency. A 5250 ppm (6%) sodium hypochlorite solution achieved ≥ 3.88 and ≥ 2.58 $\log_{10}$ reductions for *P. brevi-compactum*

and *A. alternata* spores, respectively in comparison to the untreated control tiles after seven days. This treatment resulted in 58% of the mold counts falling to below the limit of detection for *A. alternata*. The concentration of the *P. brevi-compactum* and *A. alternata* spores fell by 0.56 and 0.12 $\log_{10}$, respectively on the untreated control tiles, while the water reduced the concentrations by 0.80 and 0.42 $\log_{10}$. These results are summarized in Figures 2-3.

The treatment with bleach resulted in a 1.99 $\log_{10}$ reduction for *A. alternata* and a 2.02 $\log_{10}$ reduction for *P. brevi-compactum* mycelia after the initial application to the drywall. A second application of bleach resulted in a 4.10 $\log_{10}$ reduction for *P. brevi-compactum* and a 2.67 $\log_{10}$ reduction for *A. alternata.* Mold levels were reduced on the untreated tiles by 0.51 and 0.10 $\log_{10}$ for *P. brevi-compactum* and *A. alternata,* respectively. A 0.81 $\log_{10}$ reduction was observed for the *P. brevi-compactum* with the application of the water control, while no reduction was observed for *A. alternata*. Figures 4-5 summarize these results. The growth of the mycelia visible on the surface of the tiles was consistent throughout the trials.

The initial application of bleach was more effective by day 2 against the spores in comparison to the mycelia (4.02 $\log_{10}$ versus 2.02 $\log_{10}$ reductions, respectively for *P. brevi*-compactum and 2.61 $\log_{10}$ versus 1.99 $\log_{10}$ reductions for *A. alternate*. Following the second application of bleach, greater mycelia reductions were observed (Figures 4-5). Regrowth was not observed with either mold species throughout the 7-day study period.

DISCUSSION

There are currently no standardized methods for soft surface testing and thus this study utilized the best available methods, borrowing from hard surface protocols. Nonetheless, real-world scenarios would be further complicated by variations in temperature, growth conditions, humidity levels, and the presence of multiple mold species.

Spore Trials

Penicillium brevi-compactum and *A. alternata* had similar rates of inactivation in all of the spore trials. The untreated controls exhibited a trend of approximately a 1-$\log_{10}$ reduction on day 0 with a recovery of less than 0.5-$\log_{10}$ reduction by day 2. An exception to this was the of *A. alternata* mycelia, in which no reductions were observed. This is likely due to the fact that these tiles were not dried. Initial drying of mold spores caused a 0.88 and 1.07 $\log_{10}$ reduction for *P. brevi-compactum* and *A. alternata* respectively, when compared with the starting inocula.

In the spore trials, a reduction with the water control was not observed until after 2 days. This was more apparent with *P. brevi-compactum*, which had no reduction on day 0 and a 0.60 $\log_{10}$ reduction on day 2. Samples for day 0 were collected immediately following the 10 minute contact time, prior to drying, thus the effects of drying were not evident until subsequent samples were collected. The effect of drying was noted in the untreated control for day 0 in comparison to the initial inocula.

Regardless of mold type or the growth conditions and concentrations of mold spores, a $\geq$2-$\log_{10}$ reduction was achieved using 6% household bleach. This data is consistent with Martyny et al (2005), who showed that a dilute (5000 ppm) bleach solution was able to reduce viable concentrations of *Apergillus fumigatus* on building materials. Martyny et al. (2005) also showed a structural change in the conidia of the fungus, which led to a decreased recognition of the mold spores (conidia) in ELISA tests and reduced their allergenic properties. In the current study, bleach appeared to have a greater effect on *P. brevi-compactum* spores than on *A. alternata* spores, with 3.88 and 2.58 $\log_{10}$ reductions, respectively, but the study was limited by difficulties in achieving high titer stocks of *A. alternata*.

A. alternata sporulation is dependent on multiple factors including media, temperature, light conditions, the addition of supplements, and a varying combination of techniques (Masangkay et al., 2000). The inoculum of *P. brevi-compactum* was two logs greater than that of *A. alternata,* (5.07×10^6 and 4.00×10^4, respectively). *P. brevi-compactum* produced visible spores over the entire surface of the colony within three days of growth, while *A. alternata* did not produce visible spores on the colony until day seven, at which point the conidia covered less than half the mycelium. Only after 21 days of incubation did the conidia cover half the mycelium. Even under optimized growth conditions (e.g., black light and ~90% relative humidity), greater sporulation rates could not be obtained. Future studies are needed using a higher inoculum titer to evaluate the quantitative endpoint of bleach efficacy on such fastidious molds.

Mycelium Trials

In homes with water damage, molds are found growing as mycelia (or hyphae) and not just as spores (Genuis, 2007). Molds, at different phases of growth contain varying amounts of allergenic proteins and may react differently to disinfectants (Xu et al., 2007). This study provides a baseline evaluation of the ability of bleach to penetrate porous media to kill fungal mycelia. Reduction of mycelia is used to show the efficacy of bleach in real-world applications in situations where there may be mixed and complex cultures of molds present. This study provides evidence that bleach can be effective against mold at various life cycle stages of molds on porous surfaces.

The water control solution did not have as large of an effect on the reduction of the mycelia in comparison to the spores. This is at least partly due to the fact that the mycelia did not completely dry due to differences in the experimental protocol from the protocol followed for the spore experiments. The spores were reduced by up to 0.70 $\log_{10}$ from day 0 to day 2 compared to only a 0.10 $\log_{10}$ reduction in the mycelia. Regrowth of mold was not observed even after 7 days.

Molds require a moist environment to grow and reproduce. *Stachybotrys* species are known to be indoor contaminants and release mycotoxins that cause adverse health effects in humans (Johanning et al., 1995). Menetrez et al. (2009) found that *Stachybotrys chartarum* could grow at a relative humidity as low as 64%, but required additional moisture. *S. chartarum* could also grow at 100% relative humidity, while requiring no additional surface moisture (Menetrez et al., 2009). Molds secrete enzymes that break down nutrients externally and thus require water, as well as a high relative humidity

(Silicato and Cannon, 2006). Humidity and water growth requirements vary based on the mold species. A relative humidity of ~50% was maintained in the current experiments, a common humidity level in homes (Chan-Yeung et al., 1995). Thus, the low availability of water could explain why no regrowth was seen throughout the 7 days of the experiments. Regrowth could possibly have occurred if a higher humidity (>60%) had been maintained and/or if additional sources of moisture had been available.

Bleach was reapplied on day 5 for the mycelia experiments and resulted in greater reductions overall when compared to spores (4.10 versus 3.88 $\log_{10}$ reductions, respectively) after seven days. Therefore, more information is needed to develop guidelines for improved disinfectant efficacy on porous surfaces using multiple applications. The portion of mycelium that is not killed is able to grow and can offer some protection from the disinfectant, warranting repeated application.

Recommendations for Disinfection of Mold-Contaminated Drywall

P. brevi-compactum and *A. alternata* growth patterns varied on the drywall tiles. *P. brevi-compactum* produced small colonies with little extension, while *A. alternata* covered an area of 1" x 1" within 5 days. Also, the growth of *P. brevi-compactum* was limited to the surface cardboard layer, whereas *A. alternata* hyphae were visible across the cardboard-gypsum interface, yet with no visible signs of penetration further into the internal gypsum layer by 7 days. Such differences in the growth patterns suggest that bleach will have varying degrees of effectiveness for different species of molds. As

molds grow further into the drywall, the disinfectant will have limited effectiveness due to the chlorine demand of the gypsum, whereas molds that only grow near the surface will be easier to disinfect and physically remove.

An important limiting factor for disinfectant efficacy is the chlorine demand of drywall, as well as the ability to absorb the disinfectant, which limits disinfection at its surface. Presumably, as molds grow further into the drywall there will be less available chlorine to disinfect, allowing for the regrowth of the mold after the initial bleach application. Subsequent exposure to water will increase the likelihood of regrowth. Although regrowth was not observed during the 7 days of this study, more data is needed to better model the real world relationship of time, regrowth, and the repeated use of disinfectants.

The porosity of the drywall allows for a disinfectant solution to be absorbed, leaving surface areas dry after two minutes. The drywall exposed to water (mycelium trial) absorbed water and the disinfectant at a higher rate than the drywall that was not exposed to water (spore trial). In the spore experiments, the tiles required disinfectant and control solution reapplication every two minutes compared to every minute in the mycelium trials. The problem with absorption is further complicated by the position of the drywall. In buildings and homes drywall is primarily vertical, allowing disinfectant solutions to move along the vertical profile, and thus reducing their contact with contaminated areas.

In comparison with non-porous surfaces, a 5,250 ppm bleach solution reduced the concentrations of *P. brevi-compactum* by 4.74-$\log_{10}$ with a ten minute contact time

compared to a ≥ 3.88 $\log_{10}$ reduction on porous drywall surfaces. The bleach on non-porous and porous surfaces achieved a ≥ 2.73 and ≥ 2.58 log reduction, respectively, for *A. alternata.* The smaller (4.00×10^4 cfu/ml) initial inoculum did not allow for measureable reductions greater than this level. *P. brevi-compactum* achieved a 4- $\log_{10}$ reduction on non-porous surfaces and a near 4-$\log_{10}$ reduction on porous surfaces, suggesting that bleach is effective at reducing the exposure to this mold. The reductions could be increased by repeated application of bleach solutions.

Recommendations to increasing the efficacy of bleach include: increasing the contact time or the frequency of application. In addition, physical removal by wiping with a towel reduces the levels of mold mycelia and spores on surfaces; however this would not remove the mold that is below the surface. Such wiping could remove organics and other surface contaminants that otherwise increase the chlorine demand and decrease its efficacy.

Following indoor mold contamination, the Center for Disease Control and Prevention (CDC) recommends that porous surfaces be removed, as they have additional properties that allow for mold growth through the surface (Brandt, 2006). The CDC recommends that hard non-porous surfaces be washed with a bleach solution of one cup of bleach per gallon of water (~5,250 ppm). After washing, the surfaces should be scrubbed using a brush and rinsed with water (Brandt et al., 2006). Nevertheless, many other state and federal public health guidelines do not recommend bleach for non-porous surfaces and others consider its use controversial (USEPA, 2001; Committee on Environmental Health, 2006: Bureau of Environmental and Occupational Disease, 2008).

The results of this study demonstrate that a bleach solution can be used to reduce mold contamination on porous surfaces. Repeated application over time is shown to improve reductions observed with bleach and could be an effective method of remediating small areas of mold contamination due to isolated leaks and minor flooding events. This should be further evaluated as a method to reduce the level of human exposure to molds.

In addition to reducing the concentration of molds growing in drywall, bleach solutions are also able to reduce the allergenicity of mold spores (Martyny et al., 2005; Reynolds, 2012). Spores are considered the causative agent of mold-induced allergies, although fragments from the fungal mycelium contain many of the same receptors and biomarkers as spores and are also able to cause adverse health effects (Gorny et al., 2002).

The exact mechanisms of mold allergenicity are not well characterized. Such responses may be caused by immuno-globulin E-inducing allergens, mycotoxins, and glucans produced by molds that are found on the cell wall or are excreted from the organism (Douwes and Pearce, 2003). Fungal proteases (that are related to human proteases) and surface antigens found in the cell wall of the spores and hyphae of fungi may also lead to allergenicity (O'Driscoll et al., 2005).

Other generally recommended strategies to minimize mold contamination in homes include: regular cleaning and disinfecting, ensuring proper ventilation, and reducing moisture. In the home environment, there are no quantitative standards for molds, primarily due to the fact that there is currently a lack of dose-response data necessary for risk assessments. Given the clear association between indoor moisture/mold

contamination and adverse health effects in sensitive individuals, there is a need for improved exposure and risk assessment and risk management approaches. Modified indoor radon and lead reduction strategies have been suggested for mold contaminants, including building codes, home transfer incentives, and the education of homeowners experiencing mold and moisture problems (Wu et al., 2007). Building codes would be modified to require antifungal building materials in locations that have a high risk for mold contamination. Public education could include a Water Intrusion Management Plan (Silicato and Cannon, 2006) involving regular checks for leaks and water intrusion events by either the homeowner or a certified plumber. Such efforts combined with individual participatory action within the home, such as increased ventilation, dehumidification, and the disinfection of surfaces with bleach could provide a practical multi-barrier approach to indoor mold abatement.

Remediation involving the replacement of building materials is often prohibitively costly, averaging \$15,000 to \$30,000 (Hartwig and Wilkinson, 2003). A failure to remediate or treat molds on surfaces could result in an increased contamination of building materials, allowing for greater human exposure. This exposure could in turn exacerbate human health symptoms that are commonly associated with mold. In the absence of complete remediation of contaminated materials, a bleach treatment can be used to achieve a nearly 4-$\log_{10}$ reduction of certain molds (the required reduction for hard non-porous surface disinfectants).

This study has provided new evidence of bleach efficacy on porous surfaces and the need for standardized approaches to evaluate disinfectants as a best practice approach

to mold control in the absence of costly remediation involving the removal of construction materials. Nevertheless, more studies are needed to evaluate mold contamination and regrowth on a greater variety of indoor porous media. For example, most buildings have painted drywall that may act to increase the growth of molds due to more available nutrients (Menetrez et al., 2008). Some brands of paint, however, contain antimicrobials to limit the growth of molds on surfaces (Menetrez et al., 2009). Information on mold disinfection on drywall and other non-porous surfaces needs to be collected. The next steps to consider differential study designs to better mimic real-world conditions such as including a vertical placement of tiles, a mixture of mold contaminants, and varying humidity ranges. Further research also needs to be conducted to see if a bleach solution is able to effectively reduce spores and fungal fragments from other species that may produce adverse health effects in humans.
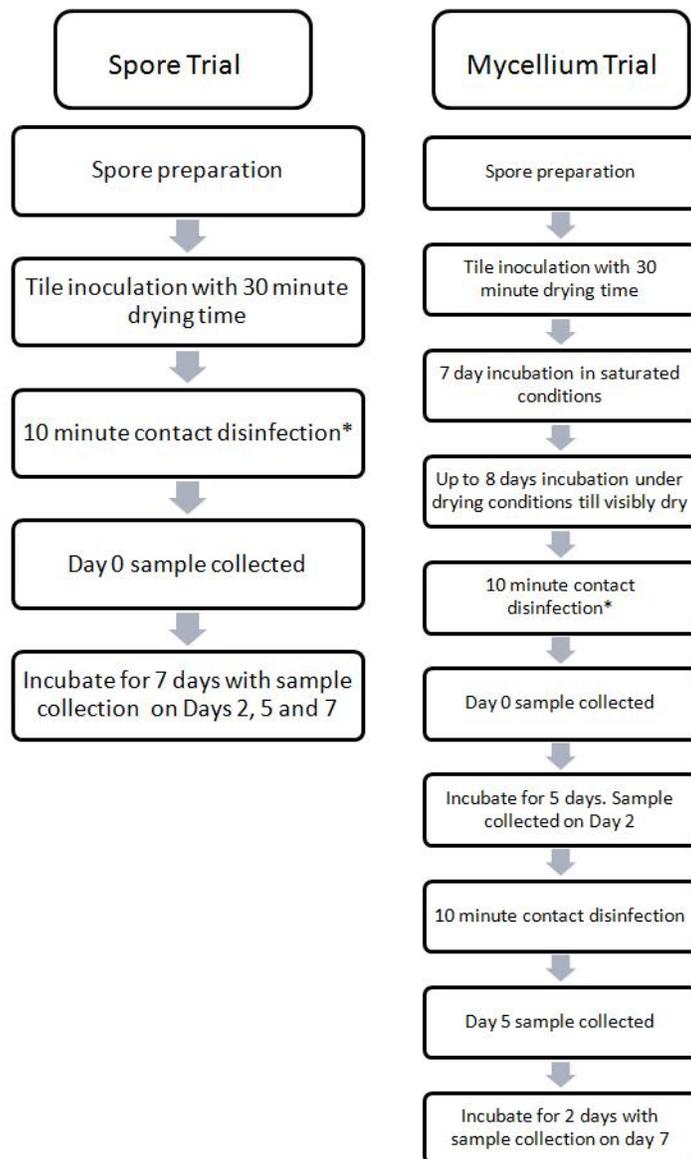
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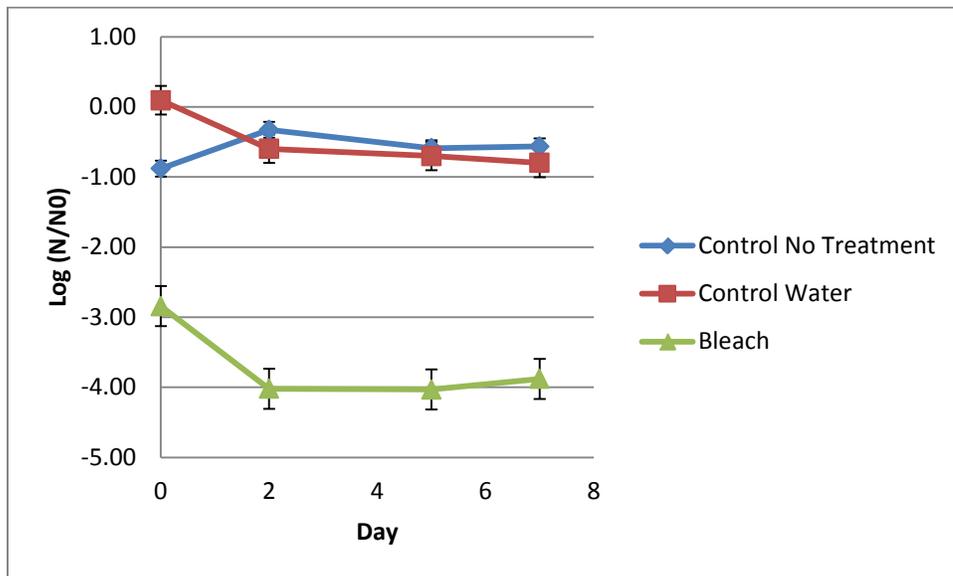Mudarri D, Fisk WJ. Public health and economic impact of dampness and mold. Indoor Air. 2007 Jun;17(3):226-35.

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Figure 1: Experimental Protocol

Spore Trial

- Spore preparation
- Tile inoculation with 30 minute drying time
- 10 minute contact disinfection*
- Day 0 sample collected
- Incubate for 7 days with sample collection on Days 2, 5 and 7

Mycellium Trial

- Spore preparation
- Tile inoculation with 30 minute drying time
- 7 day incubation in saturated conditions
- Up to 8 days incubation under drying conditions till visibly dry
- 10 minute contact disinfection*
- Day 0 sample collected
- Incubate for 5 days. Sample collected on Day 2
- 10 minute contact disinfection
- Day 5 sample collected
- Incubate for 2 days with sample collection on day 7

* Day 0 begins

Figure 2: Reduction of *P. brevi-compactum* Spores on Drywall*

* Results representative of arithmetic mean of 3 replicates.

Figure 3: Reduction of *A. alternata* Spores on Drywall*
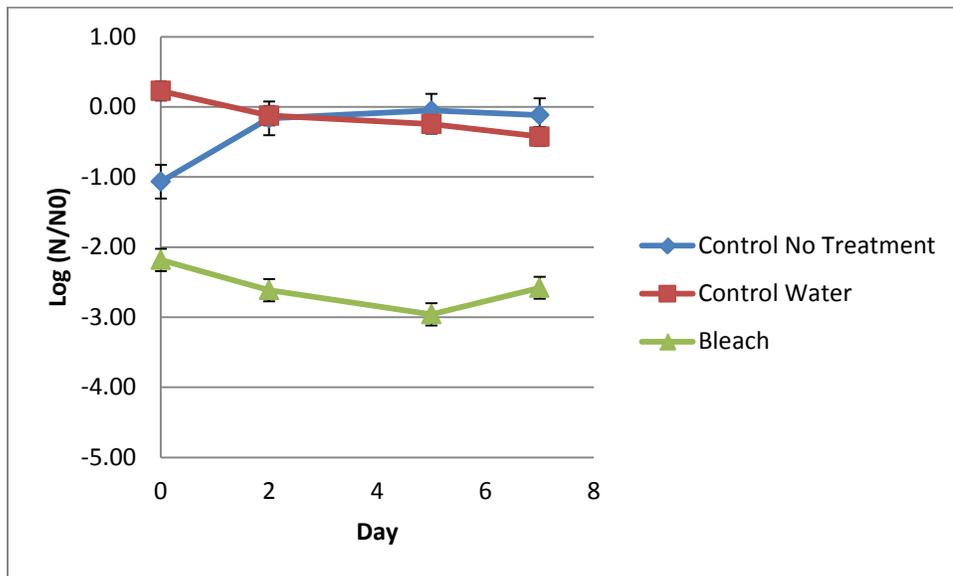

* Results representative of arithmetic mean of 3 replicates.

Figure 4: Reduction of *P. brevi-compactum* Mycelium on Drywall*
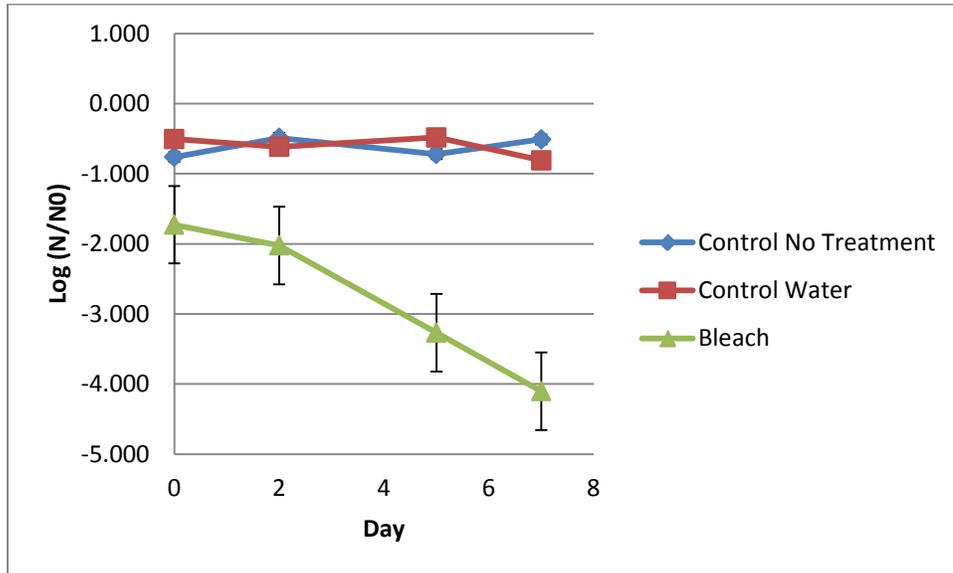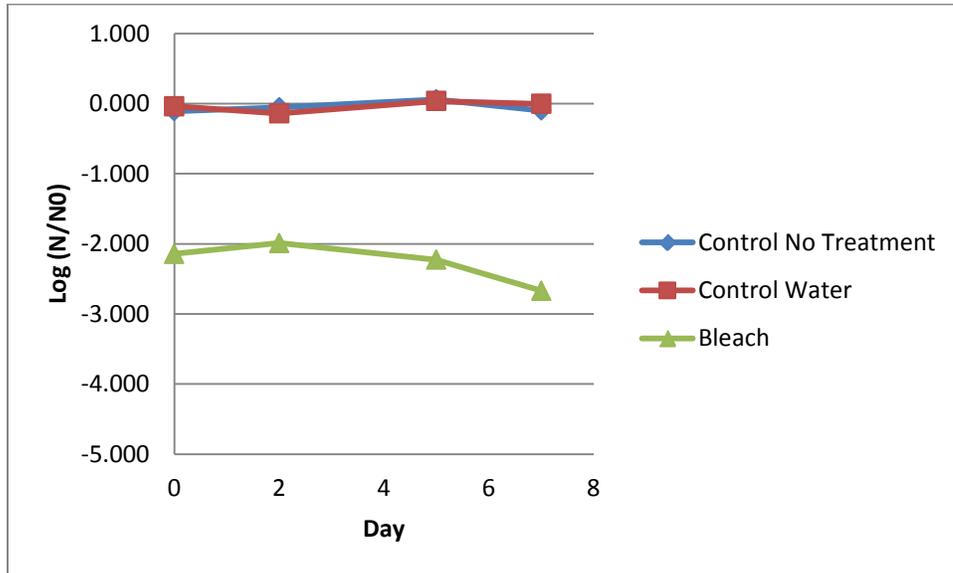

* Results representative of arithmetic mean of 3 replicates.

Figure 5: Reduction of *A. alternata* Mycelium on Drywall*

* Results representative of arithmetic mean of 3 replicates.

APPENDIX C:

EXPOSURE ASSESSMENT VARIABLES RELATED TO INFLUENZA STRAIN DIFFERENCES

Jonathan D. Sexton[1*], Ph.D., Kelly A. Reynolds[2], Ph.D.

1. The University of Arizona, Department of Soil, Water and Environmental Science, 1117 E. Lowell St., Tucson, AZ 85721
2. The University of Arizona, Mel and Enid Zuckerman College of Public Health, 1295 N. Martin Ave., Tucson AZ 85724

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Author for correspondence:
* Jonathan D Sexton
The University of Arizona
Dept. of Soil, Water & Environmental Science
Building 90, Room 408
Tucson, Arizona 85721
Tel: (520) 621-6910
Fax: (520) 621-6910
Email: sexton@email.arizona.edu

ABSTRACT

Risk associated with influenza is based on host, environmental and viral characteristics including strain specific factors, age and immune status of host, relative humidity and composition of contaminated fomites. Differences between strains of influenza can be used to develop more accurate risk models to predict risk of infection from emergence of future strains. Historical outbreaks of influenza show that emergence of new subtypes and strains have a significant effect on the infectivity and mortality rates. The purpose of this review is to compile a database of influenza A exposure and risk factors needed to inform mathematical models aimed at predicting risk of infection and evaluating intervention needs and efficacy. H3N2 and H1N1 (2009) influenza A strains were compared based on strain specific factors, including secondary attack rates, and ability to survive and persist in the environment. Although H3N2 influenza survives longer on fomites compared to H1N1 (2009) influenza A, the latter has a higher infectivity, but lower mortality rate. Comparative risk modeling can be used to quantitatively evaluate which variable characteristics have the most impact on disease outcomes and improve our understanding of current and emerging influenza epidemiology. This information can be used to develop more accurate models to predict exposure and risk of infection associated with different strains of influenza A.

INTRODUCTION

Influenza A causes a contagious respiratory illness known as the flu. The infection causes mild to severe illness and is associated with an estimated 35,000 deaths a year in the United States, with a range of 3,000 to 49,000 (CDC, 2010a). Symptoms include fever/chills, cough, body and headaches, fatigue, chest discomfort, sore throat and runny nose. Influenza infections may also promote bacterial pneumonia, ear infections, sinus infections, dehydration and other chronic medical conditions (congestive heart failure, asthma and diabetes). High-risk populations for contracting influenza and experiencing more adverse outcomes include children under the age of 5 years and adults over the age of 65 years, pregnant woman, people with respiratory/liver/kidney/heart disorders, clinically obese and anyone with compromised immune systems (CDC, 2010a).

Seasonal influenza generally infects 15-20% of the U.S. population. This figure does not differentiate between primary and secondary transmission. Historically influenza outbreaks had higher morbidity and mortality rates when compared to the 2009 outbreak (Mills et al., 2004). The largest recorded outbreak of influenza occurred in 1918 and was caused by an influenza A (H1N1) virus, often referred to as the Spanish Flu. This outbreak infected 1/3 of the world's population (~500 million people), killing between 50 and 100 million people. This virus had an extremely high case fatality rate of >2.5% and as high as 10%. In the last 40 years seasonal influenza infections have a case-fatality rate

of <0.1% (Taubenberger and Morens, 2006; Domingues-Cherit et al., 2009; Wilson and Baker, 2009). Historical outbreaks can be used to validate models for potential adverse health outcomes of new and emerging strains based on infectivity and virulence factors. In addition, the range of outcomes in outbreaks can aid in the development of public health guidelines to minimize impacts by utilizing sensitivity analyses and modeling the effectiveness of previously used interventions such as limiting congregations of people and the closing of schools and businesses.

Influenza viruses are transmitted via four different modes including direct contact with infected persons, fomites, air droplets ($\geq$5µm) and air nuclei (<5µm), with the latter two being very similar to one another due to the route of transmission; air (Shaman and Kohn, 2009; Brankston et al., 2007). The relative significance of each route is currently unknown.

In 2009, two novel strains of influenza circulated globally resulting in a pandemic. These strains were genetically similar, with minor differences such as antiviral susceptibilities and transmission factors, and were collectively known as 2009 H1N1. The 2009 H1N1 virus first emerged in Mexico City, Mexico, in April, where it quickly spread to other parts of the country and the world. This 2009 H1N1 virus is similar to other influenza A viruses but with key differences related to host, environmental and viral factors (Garten et al., 2009; Shiley et al., 2010).

The purpose of this review is to gather and examine contributing factors associated with the risk of exposure and infection of different strains of influenza A. While contributing factors have been examined for influenza A, strain specific factors

have not be reviewed previously. This paper compiles those variables for consistency and ease of evaluating risk models related to unknown strain severities.

DOSE RESPONSE-INFECTIVITY

The dose response model recommended for influenza risk assessment is the Beta-Poisson model (Equation 1) (CAMRA, 2013). The Beta-Poisson model was developed to predict the risk of infection via the intranasal and oral route of infection. It contains an α value of 5.81×10^{-1} which represents host and microbe interactions. There is also an N_{50} value of 9.45×10^{5} which is the dose at which 50% of the population would become infected with the organism. This model was developed from two separate experiments completed by Murphy et al. (1984) and Murphy et al. (1985), using two different influenza strains common in human outbreaks (H1N1 and H3N2). Statistical precision is increased due to the combining of the two experiments into one Beta-Poisson model by narrowing the confidence interval.

Equation 1:

$$P(response) = 1 - \left[1 + dose \frac{\left(2^{\frac{1}{\alpha}} - 1 \right)}{N_{50}} \right]^{-\alpha}$$

(CAMRA, 2013)

Infectious doses may vary based on the route of infection. The infectious dose from surfaces (hand to nose) is 100-1000 Tissue Culture Infectious Dose ($TCID_{50}$) while the airborne infectious dose is 0.6-3.0 $TCID_{50}$ for H3N2 influenza A (Weber and Stilianakis, 2008). Current literature suggests that 2009 H1N1 influenza A is more infectious than H3N2 influenza though no quantitative values have been reported regarding the minimum infectious dose (Munster et al., 2009; Chan et al., 2010).

The morbidity rate for H3N2 influenza ranges from 10-20% with a mortality rate of <0.1% (Koelle et al., 2006; Taubenberger and Morens, 2006; Domingues-Cherit et al., 2009). The morbidity rate is different for H1N1 influenza ranging from 4-43%. H1N1 influenza had an initial mortality rate in Mexico of 0.9% (Echevarria-Zuno et al., 2010). As the pandemic progressed the mortality rate was lowered to 4.0×10^{-4}% (Wu et al, 2010).

EXPOSURE ASSESSMENT

Exposure assessment is a combination of variables related to host (shedding rates, hand to face contacts, vaccine compliance and efficiencies), environmental (relative humidity, vapor pressure, survival on porous and non-porous surfaces) and viral (infectious dose, attack rates, surface contamination and survival based on different strains) factors that contribute to adverse human health outcomes. Different strains of influenza A are associated with variable fomite and air survival, morbidity, and mortality rates. These differences can impact risk of infection and are important to the development

of accurate risk assessment models and evaluating the most important dose and exposure factors relevant to risk.

The risk model input values discussed have a point value or are uniformly distributed. A uniform distribution is assumed to be representative of average values with the mean occurring within the range. Studies have been completed focusing on host, environmental and viral factors but results have not been reported in a way that is conducive to completing a distribution analysis. Additional quantitative results need to be complied to determine a more probalistic distribution analysis.

Host Factors

Table 1 contains a summary of possible host factor input values for the development of a human health risk model.

Epidemiological studies have shown that host age contributes to the severity of illness depending on varying strains of influenza (Simonsen et al., 1998; Olson et al., 2005; Louie et al., 2009). In the U.S., 40% of 2009 H1N1 influenza cases were seen in 10-18 year olds, with 95% under 50 years (Louie et al., 2009). This is different from seasonal cases of H3N2 influenza A. During the 1995-2008 influenza seasons, 39% are under 20 years old, 32% are older than 40 years and 11% are older than 80 years (Khiabanian et al., 2009). The percentage of deaths in people over the age of 65 years is 80-90%, during traditional influenza seasons, compared to 18-20% during the 2009 H1N1 influenza season for people over the age of 50 years (Simonsen et al., 1998; Louie

et al., 2009). Youth social networking likely increased the spread of the 2009 H1N1 influenza virus among the younger populations (Chang et al., 2009). Social networking is likely to increase the transmission rate during traditional influenza seasons, but was more evident during the 2009 season, due to the high rate of infection in people under the age of 18 years.

Children under the age of 5 years are at a greater risk for contracting and spreading the influenza virus, regardless of strain type. Children are commonly associated with day care facilities, which are linked to an increased incidence of respiratory disease (Dales et al., 2004). Increased periods of influenza virus shedding is commonly experienced in children, lasting an average of 11 days compared to 7 days for adults (Viboud et al., 2004; Li et al., 2010). In severe cases of influenza, children may shed the virus for up to 27 days (Li et al., 2010). Viral shedding is greatest during the first two days of illness with an average of 4.13 $\log_{10}$ copies/mL throat swab eluent.

Immune status of the host also contributes to the risk of influenza infection. Younger children (<5 years old) have a less developed immune system and thus are more susceptible to infections (Hanson et al., 2003). In addition, greater than 20% of the United States population is considered immunocompromised (Kendall et al., 2003; Flaherman et al., 2007). Young adults, age 18-24 years, typically have fewer chronic ailments and generally stronger immune systems and thus are more capable of fighting off disease, compared to the elderly, $\geq$65 years. The elderly are considered immuno-compromised, but in some cases advanced age can be a protective factor, given that the elderly have been exposed to many more strains of influenza compared to the younger

population. Previous exposure has been shown to offer cross-protection to new and evolving strains of similar genetic structure (Louie et al., 2009; Chang et al., 2009).

Human activity patterns (rate of hand to face contacts and hand washing) can have an impact on risk estimates. Changes in human behaviors in private and in public have an effect on the transmission of influenza virus. In public, people are more conscious of their actions and tend to be more reserved. The rate of hand-washing increases when one is being observed. In the presence of one additional person the rate of hand-washing increased from 39% to 77% (Munger and Harris, 1989). The rate of facial touching, including nose picking, mouth touching and eye rubbing, when unknowingly observed is 0.4 touch/hr and can be as high as 15 touch/hr, compared to a 10-fold decrease when people are facing each other (Weber and Stilianakis, 2008; Nicas and Rose, 2009). Thus, people are more likely to infect themselves with influenza from fomite transfer when in private.

The infection pathway for influenza on fomites includes factors such as the contamination level on the surface and the rate of transfer from surface to hand and hand to mouth. The transfer efficiency for influenza virus from hand to mouth is not known. PRD-1 has been used as a surrogate for influenza and has a transfer efficiency rate of 0.339 (Rusin et al., 2002; Nicas and Rose, 2009).

The use of personal protective equipment also has an influence on the rate of influenza transmission and is an important public health control strategy to consider when outbreaks occur. Johnson et al. (2009) found that surgical masks and N95 respirators were both effective at preventing the transmission of the influenza virus from infected

individuals, for a short period of time. Participants, positive for influenza virus, coughed 5 times into a 90 mm diameter petri dish containing 1 mL of viral transport media, which was then assayed to determine the concentration of virus present. The mean viral titer quantified was ~2 $\log_{10}$ when no masks were used, compared to no detection when either surgical masks or N95 respirators were worn. When the 2009 H1N1 influenza was first discovered, 36.5% of the Mexican population used facemasks for protection when riding public transportation with a range of 11.5-63.5% (Condon and Sinha, 2010). The use of facemasks in the United States is not as common as in Mexico with only 4-8% of the population utilizing facemasks in public (SteelFisher et al., 2010).

Vaccination has been shown to reduce clinical illness in healthy adults by 10-99.8% (Bridges et al., 2000; CDC, 2011b). Vaccination of high risk groups, such as the elderly, has been shown to reduce clinical illness by 33-72%, and reduced mortality by 67-74% (Gross and Hermogenes, 1995; Voordouw et al., 2003). In addition widespread vaccination programs increase herd immunity resulting in decreased incidence. Vaccination of children in day cares has resulted in an 80% decrease of illness in unvaccinated siblings (Hurwitz et al., 2000). Problems with vaccination include access and supplies, where countries that have limited supplies and accessibility would require help from other countries to be able to vaccinate its population (Coburn et al., 2009). Development of a vaccine takes several months. The 2009 H1N1 influenza vaccine was in limited supply until December 1, 2009, due to slower reproduction of the novel strain and a larger quantity of viral particles were needed to illicit an immune response (Sullivan et al., 2010). Another problem would be getting enough of the population to

take the vaccine. U.S. vaccination compliance rates range from 30.2%-76.6% for seasonal influenza A (Abramson and Levi, 2008; Horney et al., 2010). Compliance with seasonal vaccination programs varies due to potential side effects of the vaccine and the perception that influenza is only a minor disease (Balicer et al., 2007). Noncompliance with vaccine programs results in viral persistence and increased transmission which can be predicted using a quantitative risk model approach. This can be used to help educate the public on the importance of receiving the influenza vaccine.

Environmental Factors

The significance of the each exposure route (direct contact, air droplets, air nuclei and fomites) is currently unknown and thus factors related to each will be examined.

Air

Tables 2 and 3 contain environmental factors related to virus survival and inactivation in air, in regards to varying humidity ranges and vapor pressure that are useful for the development of a risk model to predict influenza exposures. Understanding environmental factors aids in establishing public health strategies aimed at changing the environment and implementing interventions to reduce risk of influenza infection based on macro- and micro-environments.

Two factors that are thought to influence transmission are relative humidity and vapor pressure (absolute humidity). Vapor pressure presents a stronger relationship

compared to relative humidity (P= 0.00027, P=0.059, respectively), though relative humidity has been studied more in depth (Shaman and Kohn, 2009). Relative humidity changes based on the macro- (arid southwest vs. tropical southeast) and micro- (outdoors vs. indoors) environments. This relationship is nonlinear and is affected by temperature. Higher temperatures have been shown to be significant in regards to vapor pressure but not with relative humidity (Shaman and Kohn, 2009).

Humidity affects transmission in two ways. Firstly, air nuclei are more efficiently produced at lower humidity levels and have been shown to contain larger quantities of viral particles. Droplets go through the process of sedimentation and evaporation. The smaller the droplet, the longer it will remain suspended in the air, thus increasing the possibility of airborne exposures. Low vapor pressure leads to evaporation of larger droplets resulting in air nuclei (Shaman and Kohn, 2009). Under conditions of moderate and high humidity, no significant differences are observed between the concentrations detected in the air initially and after one minute following aerosolization. The percentage of initial concentration detected after 1 minute ranged from 3-30% for moderate humidity and 6-34% for high humidity (Brankston et al., 2007). Therefore, low humidity conditions are expected to result in higher detectable virus titers over a longer period of time in air.

Low humidity also increases virus survival and subsequent transmission. Vapor pressure has a greater impact on survival relative to temperature accounting for up to 90% of virus survival variance (Shaman and Kohn, 2009). Lower vapor pressure results in increased survival of influenza over time. A vapor pressure of 10 mb over 1 hour

results in 60% virus survival compared to 20% at a pressure of 20 mb (Shaman and

Kohn, 2009). This can be seen in the winter in the macro-environment of the Northern

Hemisphere when vapor pressure is low and influenza virus survival and transmission are

high (Shaman and Kohn, 2009). Under conditions of low humidity viruses have remained

viable in the air for up to 24 hours after artificial aerosolization compared to 60 minutes

with high humidity (Brankston et al., 2007). There is uncertainty in which environmental

conditions contribute most to influenza survival and transmission. Influenza often peaks

during the rainy season thus the fomite route may have a larger influence on transmission

than the airborne route, though this conclusion is not widely supported (Weber and

Stilianakis, 2008).

Another factor that influences the airborne survival of influenza includes ultra

violet radiation (UV). UV has a greater effect at higher elevations as total irradiance

increases 8% for every 1000 meter rise in elevation (Blumthaler et al., 1997). Under

conditions of high humidity (85-95%), 95% of *Serratia marcescens* bacteria remained

viable after 8 seconds of UV exposure, suggesting that influenza could potentially

survival as well (Ko et al., 2000). There are also effects from other types of

environmental radiation, ozone and pollutants that are poorly characterized for viruses

(Weber and Stilianakis, 2008). Further studies need to be conducted to determine the

overall effect environmental conditions have on viruses. The outdoor environment is

known to be a more inhospitable medium for the virus to survive compared to indoor

environments. Even though virus survival outdoors is limited, viruses can still remain

viable for a several days outdoors when enveloped in aerosols (Weber and Stilianakis, 2008).

Fomites

Input values that should be considered when developing a risk assessment model based on environmental factors associated with fomite survival and inactivation can be found in Tables 2 and 3. Inactivation rates, discussed below, were calculated using the Chick-Watson Law, a first order kinetics equation (Equation 2) (Masschelein, 2008).

Equation 2:

$$N=N_0e^{-kt}$$ (Masschelein, 2008)

In this equation N is equal to the number of surviving organisms, N_0 is the initial concentration of organisms, t is time and k is an empirical constant that describes microorganism and inactivation factors (unit of time^{-1}).

Influenza survives longer on non-porous compared to porous surfaces. Influenza A (H1N1, non 2009 strain) isolated from a patient and from surfaces was utilized in survival studies (Bean et al., 1982; Weber and Stilianakis., 2008). On non-porous surfaces (stainless steel and plastic), influenza survived for 24-48 hours with an inactivation rate of 2.9 day^{-1}, compared to 8-12 hours on porous surfaces (facial tissues)

with an inactivation rate of 24 day^{-1}. The composition of surfaces also affects influenza survival. Stainless steel, which is known to have antibacterial properties, had little effect on influenza survival (>24 hour survival), while copper surfaces had a measurable effect. On copper surfaces, influenza survived for up to 6 hours with an inactivation rate of 33.2 day^{-1} (Weber and Stilianakis, 2008).

The survival of the virus on the surface to the hand was also determined by Bean et al. (1982) and Weber and Stilianakis (2008) (Table 4). Influenza was transferred from a hard non-porous surface to a hand for up to 24 hours, though the viable concentration significantly decreased after only 5 minutes, to levels near the detection limit. Transfer from a porous surface to hand was limited to 15 minutes, with a similar decay rate over five minutes. Recovery from porous surfaces resulted in a loss of 0.7 log$_{10}$ TCID$_{50}$ influenza virus compared to non-porous surfaces. In the case porous and non-porous surface transfer to hands, virus survival on the skin exhibited a high decay, with inactivation rates ranging from 1300 to 2100 day^{-1}. High inactivation rates decrease the plausibility of the fomite route of infection in favor of direct contact and air-droplet transmission; though high touch fomites can be contaminated with high enough concentrations of influenza to infect an individual through the fomite route of transmission.

The variability of survival on surfaces is important to risk modeling. These differences can be used to forecast not just adverse health outcomes and increased exposure but also be used to determine the relative importance of each contributing factor.

Viral Factors

Table 4 contains values for influenza A exposure input factors, infectious dose, and infection ratios that can be used in risk assessment models to predict adverse health outcomes based on non-strain specific influenza characteristics. Pathogen factors can be analyzed further based on specific strain characteristics and used to assess models developed for current and future outbreaks (Table 5).

The infectious dose of influenza varies depending on the route of infection and strain, though data is currently lacking on strain specific infectious dose. A Tissue Culture Infectious Dose ($TCID_{50}$) of 100-1000 initiates an infection from fomites, while only 0.6-3.0 $TCID_{50}$ initiates infection through the airborne route (Weber and Stilianakis, 2008). The $TCID_{50}$ distribution can be used to calculate a range of possible N_{50} values and can be further divided into strain specific distributions.

The 2009 H1N1 influenza virus is similar to other influenza A viruses but with several key differences. Through genetic analysis the virus was determined to be a combination of four different strains of influenza virus based on proteins (quadruple reassortment), compared to seasonal virus (triple reassortment) (Ravi, 2009). These include avian (PB2 and PA), human H3N2 (PB1), classical swine (HA, NP and NS) and Eurasian avian-like swine H1N1 (NA and M) (Hu, 2010). These differences in genetic assortment could lead to a new trend of further mutation affecting infectivity and morbidity rates. Table 6 shows the differences in seasonal strains, percentage of deaths

attributed to pneumonia and influenza and influenza vaccine effectiveness, as reported by 122 Cities Mortality Reporting System. Evolution and emergence of new strains influences the effectiveness of vaccines, as well as can result in increased/decreased number of deaths.

The 2009 H1N1 has genetic similarities to the H1N1 influenza pandemic of 1918, with a few differences. The HA protein is responsible for binding to human α 2-3 sialylated glycan receptors. In the 1918 strain, the amino acid responsible for this attachment was residue 225, aspartic acid. In one the 2009 H1N1 strains, the residue responsible for attachment was 190 (aspartic acid). The second strain favored both residues 190 and 225, which potentially allowed for increased transmission due to the availability of multiple attachment receptor sites. In addition to both these regions being favored, it is also possible that other nearby residues are able to bind to avian α 2-3 sialylated glycan receptors, which could lead to further exchange of genetic material with other avian influenza strains (Hu, 2010). These multiple attachment sites can result in more efficient binding to host receptors (higher infectivity).

Different strains are also able to cause different symptoms in the host. The 2009 H1N1 influenza caused traditional symptoms associated with influenza infections and in 25% of patients, diarrhea and vomiting was also present, which is rare in seasonal influenza cases (CDC, 2010a; Hu, 2010).

There are also differences in influenza strain susceptibility to antiviral drugs. H3N2 influenza can be treated with oseltamivir, in the early stage of the disease. The 2009 strains of H1N1 influenza viruses also have different susceptibilities to this antiviral

drug with one strain carrying a mutation on the NA gene that confers a resistance to oseltamivir of greater than 80 nM (Dharan et al., 2009; CDC, 2010b; Hu, 2010).

The strain of the virus also has an effect on the survival in the air. Four different strains including human, avian, swine and equine influenza viruses were studied and their survival was compared. Avian influenza viruses remained viable the longest ranging from 24-36 hours, with equine influenza being second at 21-30 hours. Swine influenza was able to survive in the air for 16 hours and human influenza viruses ranged from 6-16 hours (Brankston et al., 2007).

The secondary attack rate can also vary between strains. H3N2 influenza has a secondary attack rate ranging from 10-40% (Cauchemez et al., 2009). The attack rate for the 2009 H1N1 influenza ranged from 4-56% (Han et al., 2009; Cauchemez et al., 2009). H1N1 influenza attacks rates were dependent on the number of individuals in a household, age, sex and the proximity to an infected individual. Secondary attack rates ranged from 23-28% in households with 2 members and 4-9% in homes with 6 members (Cauchemez et al., 2009). In a tour group in China it was found that people aged 18-39 years had a secondary attack rate of 41% compared to 21% for people over the age of 40 years. Women had a higher attack rate than men, 50% compared to 13%. Tour group members who were within two meters of the index patient for more than two minutes had an attack rate of 56%. While people were all on the tour bus, members who did not have contact with the patient were not infected, suggesting an aerosol route of exposure (Han et al., 2009). These attack rates have a large impact on the spread of influenza through the population (Meltzer et al., 1999).

<u>Strain Specific Factors</u>

Strain specific influenza factors are important to the development of an accurate risk model. These factors can help to predict the behaviors of influenza strains that have yet to emerge, by providing a range of possible model inputs. Absentee rates can be utilized in early stages of an outbreak and used to assess the validity of the model, so as to minimize adverse health outcomes associated with a new strain of influenza A.

H3N2 Occurrence and Survival

Boone et al. (2005) found that 53% of surfaces in Arizona day care centers were contaminated with influenza A viruses in the spring compared with 23% in the fall. Though humidity data was not collected, this study supports the conclusion that low humidity has a positive effect on influenza survival given fact that Arizona has a lower humidity in the spring when compared to the fall (September-November). Additionally, 59% of surfaces sampled in homes with ill children were positive in the spring (March). The most commonly contaminated surfaces were dishcloths and diaper areas, with the bathroom presenting the least. Diaper areas can be highly contaminated due to viral shedding of influenza A in the stool of children (Pinsky et al., 2010), while dishcloths are commonly used to wipe surfaces.

Different strains of H3N2 influenza have variable survival rates on surfaces. In a study looking at contamination of banknotes, Thomas et al. (2008) found that two

different strains of H3N2 influenza, influenza A/Moscow/10/99 and influenza

A/Wisconsin/67/2005, survived for 1 and 3 days respectively. In the presence of mucus,

influenza A/Moscow/10/99 was able to survive for up to 17 days. To assess these results

under real world conditions, nasal secretions from children with an influenza-like illness

were inoculated onto banknotes. It was found that the viruses were only able to survive

for 48 hours.

H1N1 Occurrence and Survival

Simmerman et al. (2010) found that out of 90 households, with children positive

for H1N1 influenza A, only 16 households had surfaces that were positive for the virus.

Samples were tested using rRT-PCR and positives were confirmed through cell culture

assays. The average number of surfaces that were positive was 1.1 out of 6 in each

household. In addition, it was noted that H1N1 influenza A is shed at lower rates when

compared to H3N2 influenza A. Shedding was determined to last for 3-6 days (Killingley

et al., 2010).

Fomites in homes with children diagnosed with H1N1 influenza A had an

influenza occurrence of only 0.5%, by RT-PCR and immunofluorescence. In addition,

hospital patient rooms, with occupants diagnosed with influenza, resulted in no

occurrence on surfaces (Killingley et al., 2010).

A total of 370 samples were collected from 14 different rooms in four different

day care facilities and elementary schools in Arizona, resulting in no detectable levels of

2009 H1N1 influenza. Samples were assayed using RT-PCR for the presence of 2009 H1N1 influenza A, during the peak of the 2009 pandemic. All fomites sampled were negative for H1N1 influenza A (Sexton and Reynolds, unpublished).

Influenza A strain AHO4/2009 (pandemic H1N1) survived for less than 24 hours on hard non-porous surfaces, when artificially contaminated. Survival was less than 9 hours in 33% of samples. In addition survival on porous surfaces was determined to be less than 4 hours (Greatorex et al., 2011).

Conclusion

Risk of infection, due to different strains of influenza A, is dependent upon a variety of variables related to host, pathogen and environmental factors. Host factors, including age, immune status and behaviors, are important to strain specific risk models as emergence and evolution of influenza strains have different high risk populations and probable routes of infection. Strain differences in regards to pathogen factors include genetic assortment, infectivity, susceptibility to antiviral drugs, and specific traits related to ability to survive in the environment are important to the development of a strain specific exposure model. Environmental factors such as relative humidity, vapor pressure, UV radiation and surface composition are dependent on the strain of influenza. Differences in the strains, in relation to these factors, can have a large impact on human health outcomes. Understanding strain differences will help to develop risk models and implement relevant strategies to minimize the impact of future epidemics and pandemics.

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Table 1: Risk Input Values Based on Host Factors

Host Factors	Distribution	Average	Minimum	Maximum	Units	Description	Source
Shedding rates							
Children (<13 years)	uniform	11	0	27	days		Li, 2010; Viboud, 2004
	uniform		0	4.130	$\log_{10}$ copies/mL		Li, 2010
Adults (>18 years)	uniform	7	5	9	days		Li, 2010; Viboud, 2004
Immune-compromised	point value		0.2*		NA	immuno-compromised population/U.S. population	Kendall, 2003; Flaherman, 2007
Rate of hand to face contact							
Public	point value	0.040			touches/hour		Weber, 2008
Private	uniform		0.400	15	touches/hour		Weber, 2008; Nicas, 2009
Hand to mouth viral transfer efficiency	point value	0.35*			NA	concentration in mouth/initial concentration on hand	Nicas, 2009
Face mask/repirator transmission reduction	point value	2			$\log_{10}$		Johnson, 2009
Vaccine Efficiency							
Adults (>18 years) reduced morbidity	uniform		0.1*	0.998*	NA	reduced morbidity/vaccinated population	Bridges, 2000
Elderly (>65 years) reduced morbidity	point value	0.33*			NA	reduced morbidity/vaccinated population	Gross, 1995
Elderly (>65 years) reduced mortalityy	point value	0.74*			NA	reduced mortality/vaccinated population	Gross, 1995
Vaccination Compliance	uniform		0.302*	0.766*	NA	vaccinated population/U.S. population	Abramsin, 2008; Horney, 2010

*Value reported as a rate

Table 2: Risk Input Values Related to Virus Survival

Virus Survival	Distribution	Average	Minimum	Maximum	Units	Source
Air (Relative Humidity)						
Low Humidity (30-40%)	uniform		1.5	24	hours	Loosli, 1943; Hemmes, 1962
Moderate Humidity (60-70%)	point value	0.5			hours	Hemmes, 1962
High Humidity (<80%)	point value	1			hours	Loosli, 1943
Air (Strains)						
Human	uniform		6	16	hours	Mitchell, 1968; Mitchell, 1972
Avian	uniform		24	36	hours	Mitchell, 1968; Mitchell, 1972
Swine	point value	16			hours	Mitchell, 1972
Equine	uniform		21	30	hours	Mitchell, 1972
Surfaces (Porosity)						
Non-porous	uniform		24	48	hours	Bean, 1982; Weber, 2008
Porous	uniform		8	12	hours	Bean, 1982; Weber, 2008
Surfaces (Composition)						
Stainless steel	point value	24			hours	Weber, 2008
Copper	point value	6			hours	Weber, 2008
Surfaces (Strains)						
H3N2	uniform		1	17	hours	Thomas, 2008
H1N1 (2009)	uniform		4	24	hours	Greatorex, 2011

Table 3: Risk Input values Related Virus Deactivation Rates and Ratios

Virus Survival	Distribution	Average	Minimum	Maximum	Units	Source
Air (Relative Humidity)						
Moderate Humidity for 1 min (50% RH)	uniform		0.03*	0.30*	NA	Schaffer, 1976
High Humidity for 1 min (70% RH)	uniform		0.06*	0.34*	NA	Schaffer, 1976
Air (Vapor Pressure)						
Vapor Pressure 10mb for 1 hour	point value	0.60**			NA	Shaman, 2009
Surfaces (Composition)						
Non-porous	point value	2.9			day^{-1}	Bean, 1982; Weber, 2008
Porous	point value	24			day^{-1}	Bean, 1982; Weber, 2008
Stainless steel	point value	1.4			day^{-1}	Weber, 2008
Copper	point value	33.2			day^{-1}	Weber, 2008
Hand	uniform		1300	2100	day^{-1}	Bean, 1982; Weber, 2008

*Reported as a ratio; 1 minute concentration/initial concentration
**Reported as a ratio; 1 hour concentration/initial concentration

Table 4: Risk Input Values Related to Viral Specific Factors

Viral Factors	Distribution	Average	Minimum	Maximum	Units	Source
Infectious Dose						
Air	uniform		0.6	3	$TCID_{50}$	Weber, 2008
Fomite	uniform		100	1000	$TCID_{50}$	Weber, 2008
Infection Ratio	uniform		0.15*	0.20*	NA	Mills, 2004
Virus Transfer						
Non-porous surface to hand	uniform		0.08	24	hours	Bean, 1982; Weber, 2008
Porous surface to hand	uniform		0.08	0.25	hours	Bean, 1982; Weber, 2008

*Values reported as ratio; number of infected individuals/U.S. population

Table 5: Risk Input Values Related to Strain Specific Factors

Strain Specific Factors		Distribution	Average	Minimum	Maximum	Source
Contaminated Surfaces	**Strain**					
Home Environment	H3N2	point value	0.59*			Boone, 2005
	H1N1 (2009)	point value	0.005*			Killingley, 2010
Day care Environment	H3N2	uniform		0.23*	0.53*	Boone, 2005
	H1N1 (2009)	point value		0*		Sexton, unpublished
Secondary Attack Rates						
	H3N2	uniform		0.10**	0.40**	Cauchemez, 2009
20-39 years	H3N2	uniform		0.12**	0.44**	Frank, 1985; Glezen, 1996
≥ 40 years	H3N2	uniform		0.10**	0.50**	Frank, 1985; Glezen, 1996
	H1N1 (2009)	uniform		0.04**	0.56**	Cauchemez, 2009; Han, 2009
2 member homes	H1N1 (2009)	uniform		0.23**	0.28**	Han, 2009
6 member homes	H1N1 (2009)	uniform		0.04**	0.09**	Han, 2009
18-39 years	H1N1 (2009)	point value	0.41**			Han, 2009
≥ 40 years	H1N1 (2009)	point value	0.21**			Han, 2009
Men	H1N1 (2009)	point value	0.13**			Han, 2009
Women	H1N1 (2009)	point value	0.50**			Han, 2009

*Values reported as a rate; number of contaminated fomites/total fomites

**Values reported as a rate; infected population/total population

Table 6: Vaccine Effectiveness in the United States*

Season	H and N Subtypes	Deaths Atrributed to Pneumonia and Influenza**	Vaccine Effectiveness***
2006-07	H1, H3N2	0.075-0.077	0.24 (H3N2)
2007-08	H1N1, H3N2	0.091	0.66-0.77
2008-09	H1N1, H3N2	0.061-0.076	0.62
2009-10	Novel H1N1	0.081-0.082	NA
2010-11	H3N2, Novel H1N1	0.08-0.91	0.968-0.998
2011-12	H3N2, Novel H1N1	0.079-0.091	0.52

*Reported by 122 Cities Mortality Reporting System

**Death peaks above background levels over the course of 1 week; reported as a rate;

Number of deaths attributed to pneumonia and influenza/total deaths

***Reported as a rate; number of vaccinated people with no illness/total number of vaccinated people