Methicillin-Resistant *Staphylococcus aureus* (MRSA) Exposure Assessment in Hospital Environment

by

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For Damkerng Plipat and in memory of Anchalee Plipat

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ABSTRACT

Methicillin-Resistant *Staphylococcus aureus* (MRSA) Exposure Assessment in Hospital Environment

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of healthcare-associated infections. Contaminated hands of healthcare workers (HCWs) are vectors of transmission, but the contribution of the contaminated environment is not well characterized. The goal of this dissertation is to provide insights into the role of the hospital environment in MRSA exposure to patients.

First, a 20-month prospective study was conducted using nasal swab surveillance data in an intensive care unit (ICU) to examine MRSA acquisition risk associated with having MRSA-positive patients in the ICU during the ICU stay. The study showed that the more recent exposure to MRSA-positive patients in the ICU and the greater number of MRSA-positive patients in the ICU led to a greater hazard of MRSA acquisition among MRSA-negative patients.

Second, we developed an MRSA fate and transport model for two hypothetical hospital rooms based on the Environmental Infection Transmission System (EITS)

framework. We demonstrated a significant role of environmental surfaces in contaminating and re-contaminating HCWs. The model revealed the effect of *S. aureus* continuous shedding from the colonized patient onto room surfaces. The surfaces are quickly re-contaminated with MRSA even after the most efficacious decontamination. Our findings highlight the importance of decontamination frequency in addition to decontamination efficacy.

Third, we constructed a stochastic agent based model using the same structure as the previous model, but with more realistic features. We demonstrated that HCW's compliance is essential in determining the effectiveness of hand hygiene, although the time when it is performed and its efficacy are also important. The model emphasizes the significance of the hand hygiene opportunity before and after touching a patient's surrounding environment, in addition to at the entry and exit of a patient's room. Despite 100% compliance at the entry and exit of a patient's room, we show that contaminated environmental surfaces are the dominant contamination sources to HCWs' hands. Additionally, this model shows the value of hand hygiene efficacy. With 100% compliance and 70% efficacy, HCWs' hands remain contaminated enough to subsequently contaminate the uncolonized patient's environment, which later become another exposure route to the patient.

CHAPTER I

Background and Significance

Staphylococcus aureus, a coagulase-positive, gram-positive bacterium, is among the most successful human pathogens. Both methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) can cause mild to fatal diseases, spread locally and globally, colonize numerous human body parts, and persist in various environments outside of hosts. The objective of this introductory chapter is to provide background knowledge and the significance of MRSA healthcare-associated infections. This chapter includes the history of MRSA, the current classifications, the burden of MRSA diseases, the prevalence of colonization and risk factors for MRSA infections. It also includes a section on community-associated MRSA and its impact on healthcare-associated infections (HAI). Routes of transmission in healthcare settings are discussed based on possible MRSA exposure pathways through healthcare workers' hands and/or the hospital environment. Lastly, the chapter ends with current infection control measures.

1.1 History of methicillin-resistant *Staphylococcus aureus* (MRSA)

Staphyloccoccus aureus was first discovered in 1880 by a surgeon, Alexander Ogston, who described staphylococcal disease and its role in sepsis and abscesses [1].

Over 100 years later, *S. aureus* remains a dangerous threat to human health and has become one of the leading causes of hospital-acquired infection worldwide [1-3].

In the early 1940s, *S. aureus* infection was a fatal disease with the a mortality rate for bacteremia of about 80% [4]. Naturally, *S. aureus* is a susceptible pathogen to any antimicrobial that has ever been developed [5]. This exquisite susceptibility of *S. aureus* led to Alexander Fleming's discovery of penicillin, which was at the time a miracle drug that transformed fatal diseases to curable diseases. A few years after its introduction in the mid-1940s, however, penicillin-resistant *Staphylococcus aureus* was encountered in clinical practice.

In the 1950s a virulent penicillin-resistant clone of *S. aureus* was first reported in Australia and later termed the 80/81 strain according to its bacteriophage susceptibility pattern [3, 6]. The 80/81 strain was responsible for hospital outbreaks in many parts of the world. By the mid-1950s, penicillin-resistant *S. aureus* increased to an extent that penicillin no longer remained useful therapy for staphylococcal infections [7]. Penicillin-resistant *S.aureus* was pandemic throughout the late 1950s to early 1960s [8]. The 80/81 strains began to decline in the 1960s following the introduction of methicillin (formerly named as celbenine), the first semisynthetic derivative of penicillin which was chemically modified to withstand the degradative action of penicillinase [7].

Methicillin-resistant *Staphyloccoccus aureus* (MRSA) was first reported in 1961, within a year of methicillin introduction [9, 10]. Since then, MRSA strains have spread among hospitals and disseminated worldwide. The National Nosocomial Infections Surveillance System (NNISS) reported an increase of MRSA in large U.S. hospitals from

4% in the 1980s to 50% in the late 1990s. In some hospitals, methicillin-resistant strains represented up to 80% of all *S. aureus* strains [7, 11].

Even though MRSA has been recognized as prominent nosocomial pathogen, in the past few decades MRSA has emerged outside of healthcare settings, spreading in the community [12, 13]. These community-associated MRSA strains have also been shown to be the cause of healthcare-associated infections [14]. Further information on community-associated MRSA and its impact on healthcare-associated infections is in section 1.5.

1.1.1 Origin of methicillin resistance

While the first report of methicillin resistance was in 1961, the specific gene responsible for methicillin resistance was not identified until over 20 years later [5]. The structural gene for methicillin resistance, *mec*A, encodes a novel penicillin-binding protein (PBP)-2a (or PBP2`), which has reduced affinity for β-lactam antibiotics. This gene is carried on a mobile genetic element, *Staphylococcal* Chromosomal Cassette (SCC*mec*) [15]. The original donor of *mec*A to staphylococci is unknown, as the element has not yet been identified outside this genus. The origin of the cassette SCC*mec* could be from staphylococci other that *S. aureus* [16]. It has been suggested that *Staphylococcus sciuri* harbored the ancestor of PBP2a, because the PBP found in *S. sciuri* showed 87.8% amino-acid sequence identity with PBP2a [17]. As of 2009, there are eight SCC*mec* types and numerous subtypes described by the International Working Group on the Classification of *Staphylococcal* Cassette Chromosome elements (IWG-SCC)[18].

1.2 Classification

Originally, the infection classification scheme was based on the body site of infection, such as lung, blood stream, urinary tract, etc. and the location of the patient where the pathogen was acquired [19]. This latter criterion has led to the classification of community-acquired and nosocomial (hospital-acquired) infections. The purpose of this classification is to aid clinicians in identifying patients at-risk for antibiotic resistant organisms, which were primarily in hospital settings. Implicit in the management of nosocomial infections is that patients will receive initial therapy with broad-spectrum antibiotics for coverage of potentially resistant organisms. However, using these simple classifications for patient management is no longer adequate [19]. Antibiotic resistance can be found both in hospital and community settings, although resistant profiles may differ between community-associated MRSA and healthcare-associated MRSA. To better address these differences, it is necessary to know not only time of positive MRSA detection (which helps indicate location of acquisition), but also a clinical history to help differentiate healthcare and community associations.

Table 1.1 shows the classification of healthcare-associated infections (HAI) with multidrug resistant organisms including methicillin-resistant *Staphylococcus aureus*. This classification was recommended by the Society of Healthcare Epidemiology of America (SHEA) and the Healthcare Infection Control Practices Advisory Committee (HICPAC) in 2008 [20]. According to the recommendation, healthcare-associated infections encompass hospital-onset and community-onset infections. Infections identified in patients after 48-72 hours of hospitalization or within 48-72 hours after hospital discharge are defined as nosocomial in the absence of evidence of active or incubating infection on admission. These nosocomial infections are termed “hospital-onset”, and are only a

subset of all healthcare-associated infections. Infection with disease onset in the community in persons with recent exposures to a healthcare system is called a "community-onset" healthcare-associated infection [20]. While the criteria may be burdensome because it requires a clinical assessment of the disease onset, it is likely more specific with fewer false positive HAIs. One strategy to simplify the criteria is to use 3 calendar-days instead of 48-72 hours rule. That is, the organism is considered hospital-onset if it is isolated after the third calendar day of hospitalization, with the first day being the day of admission with an overnight stay.

While there is a need to make classification practical, the current criteria may not be guaranteed to be accurate and misclassification could occur. Since colonization may last for months or years, patients may be misclassified as having a healthcare-associated infection, when they actually became infected by endogenous strains acquired from the community. Also, patients with a prior history of MRSA infection may likely be labeled as having recurrent healthcare-associated infections, when the infection was acquired in the community [21, 22].

In this dissertation, when referring to published references, exact terminology according to the citations will be used to maintain the original definitions.

Table 1.1: Definitions used for epidemiologic classification of infections with multidrug-resistant organisms (MDROs) including 1) methicillin-resistant *Staphylococcus aureus*, 2) vancomycin-resistant *Enterococcus* species, 3) multidrug-resistant gram-negative bacilli, and 4) vancomycin-resistant *S. aureus*. This table is from the Society for Healthcare Epidemiology of America (SHEA) and the Healthcare Infection Control Practices Advisory Committee (HICPAC) position paper [20].

Classification	Definitions
Temporal Hospital-onset	Specimen was collected from patient after defined time period of hospitalization to best reflect that the pathogens were acquired in the hospital. Recommended definition is based on specimen being

	collected >3 calendar days after patient was admitted to the hospital (first day is date of admission). This is known as the "3 midnights rule." For example, if a patient is admitted to the hospital at any time on a Monday, only MDROs that are isolated after midnight Wednesday would be considered to represent hospital-onset infection (i.e., specimen was collected on day 4 of hospitalization). All hospital-onset infections are considered healthcare-associated.
Community-onset	Specimen was collected before defined time period of hospitalization to best reflect that the pathogens were acquired either in the community (including other institutions or homes) or during a previous hospitalization. Recommended definition is based on specimens being collected ≤ 3 calendar days after the patient was admitted to the hospital. A subset of community-onset infections may be healthcare-associated.
Clinical Healthcare-associated	Categorization requires evaluation of the patient's clinical history, as well as the timing of specimen collection for clinical cultures. Patient has an identified association with recent healthcare delivery, such as current or recent hospitalization, use of an indwelling venous catheter, residence in a long-term care or rehabilitation hospital, recent surgery, and/or receipt of outpatient dialysis. These types of exposures to healthcare settings may vary as a result of study design and availability of data. Therefore, if data are available, community-onset infections (see above) could be categorized as healthcare-associated, to better understand the role played by healthcare facilities in the potential transmission of MDROs.
Nosocomial	Categorization requires evaluation of the patient's clinical history, as well as the timing of specimen collection for clinical cultures. The infection in a patient was likely to have been acquired during the hospital stay, without any evidence that infection was incubating or present on admission.
Community-associated	Categorization requires the evaluation of the patient clinical history, as well as the timing of the specimen collection for clinical cultures. Patient has no documented healthcare-associated risk factors (i.e., community-onset infection (see above) and there is no identified association between patient and recent healthcare delivery).

1.3 Burden of healthcare-associated staphylococcal diseases

From 1975 to 1995 the National Nosocomial Infections Surveillance (NNIS) system at the Centers for Disease Control and Prevention (CDC) collected monthly reports of nosocomial infections from over 270 institutions in the U.S. From this data, nosocomial infections remained remarkably stable - approximately 5-6 hospital acquired infections per 100 admissions [23]. In 2002, the CDC reported that the estimated number of healthcare-associated infections in U.S. hospitals was approximately 1.7 million [24]. The overall annual direct medical costs of healthcare-associated infections (HAI) to U.S. hospitals ranges from $35.7 billion to $45 billion for inpatient hospital services [25].

A retrospective analysis of the 2000 and 2001 editions of the Agency of Healthcare Research and Quality's Nationwide Inpatient Sample (NIS) database revealed that staphylococcal infections accounted for 0.8% of all hospital inpatients stays, or 295,045 stays per year [26]. These inpatients with *S. aureus* infection had on average 3 times the length of hospital stay, 3 times the total hospital cost, and 5 times the risk of in-hospital deaths [26]. Another analysis from 1998 to 2003 NIS showed substantial increases in inpatient *S. aureus* infections and the economic burden from 1998 to 2003, whereas the in-hospital mortality rate decreased [27].

In view of the differences in the economic impact of methicillin-sensitive (MSSA) and methicillin-resistant *S. aureus* (MRSA) infections, a study using the New York State 1995 Statewide Planning and Research Cooperative System (SPARC) database showed similar direct medical costs between methicillin-sensitive and methicillin-resistant strains, but the resistant infections leads to more deaths [28]. The CDC estimated the nationwide burden of invasive MRSA diseases using population-

based, active case finding, to be over 94,000 life-threatening MRSA infections and nearly 19,000 deaths in 2005 [29].

While the prevalence of MRSA infections is recognized to increase, its impact on the overall incidence of *Staphylococcus aureus* infection is unclear. A systematic review of 45 studies indicates that the emergence of healthcare-associated MRSA and community-associated MRSA had led to an increase in the overall incidence of *S. aureus* infection, with MRSA principally adding to, rather than replacing, methicillin-sensitive *S. aureus* [30].

1.4 Clinical manifestations

S. aureus is among the most common human pathogens, capable of causing infections of any body parts in mild to fatal forms both in community and hospital settings. In a surveillance study conducted by the National Nosocomial Surveillance System (NNIS) from 1990 to 1999, *S. aureus* was the most common cause of nosocomial infections overall [31, 32]. Other studies have shown that *S. aureus* is the leading cause of nosocomial bloodstream infections [31, 33, 34]. A British study of two large hospitals including 216,644 inpatients from April 1997 to March 2004 noted that the overall incidence of *S. aureus* bloodstream infections had significantly increased, primarily driven by the increase of MRSA bacteremia [35]. This finding is magnified by the worse outcome of MRSA bacteremia and infective endocarditis, which is the most severe complication of bacteremia, when compared with MSSA [36].

Staphylococcal pneumonia was once uncommon, accounting for 1-5 % of all community-acquired pneumonia and occurring mostly in association with influenza [37-39]. In healthcare settings, *S. aureus* pneumonia was considered an important, but

infrequent cause of nosocomial pneumonia. However, in this past 2 decades *S. aureus* pulmonary infections have increased [37]. A retrospective cohort study of 59 U.S. hospital inpatient databases showed that *S. aureus* was a major pathogen of all pneumonia including healthcare-associated pneumonia, community-acquired pneumonia, hospital-acquired pneumonia and ventilator-associated pneumonia [38]. In this cohort, *S. aureus* was identified as the only pathogen independently associated with pneumonia mortality. Currently, MRSA accounts for 20-40% of all hospital-acquired pneumonia and ventilator-associated pneumonia. This is likely due to the overall increase of methicillin resistance in *S. aureus,* and the frequent and prolonged use of ventilator support in aging and vulnerable patients [37].

Other clinical manifestations of MRSA infections may include infections in skin and soft tissue, bone and joint, urinary tract, and central nervous system. The rise of *S. aureus* skin and soft tissue infections (SSTI) is largely related to community-associated MRSA.

1.5 Community-associated methicillin-resistant *Staphylococcus aureus* and its impact on healthcare-associated infection

Since its emergence in 1961, MRSA has historically affected individuals with healthcare exposures almost exclusively [10, 40]. MRSA outside of healthcare settings was first reported in Detroit, Michigan in 1982. These MRSA strains were called "community-acquired MRSA", and were noted to be a source of nosocomial outbreaks, accounting for 30% of all nosocomial staphylococcal infections in January 1981 [41, 42]. The associated risk factors for these patients were drug use, serious underlying illness, previous hospitalization or previous antimicrobial therapy. However, according to the classifications shown in Table 1.1, some of these patients would not fit in the community-

associated category, but in the community-onset healthcare-associated MRSA category [20, 41]. Later in the same decade, there were two additional U.S. reports from two Children's hospitals which also noted community origins of MRSA infections [43, 44].

During this same time in the early 1980s, there was an emergence of community-associated MRSA in the indigenous population of Western Australia and in the Northern Territory. These patients were from rural and remote Aboriginal communities without prior hospital contacts, which fit in the current category of community associated MRSA. In contrast to the U.S., where one strain (USA 300) was responsible for the majority of community-associated infections, in Australia there were several genetically diverse strains, which independently emerged from geographically distinct regions [45]. Since then, MRSA in community settings has been described among young, otherwise previously healthy individuals in many regions of the world [46-51].

The characteristic *S. aureus* infections in the community are skin and soft tissue infections (SSTI) ranging from mild to severe manifestations, such as deep soft tissue abscesses or necrotizing fasciitis [21]. Other types of infections include community-acquired pneumonia, blood stream infections, bone and joint infections, toxic shock syndrome, staphylococcal scalded skin syndrome, or food poisoning [52].

Community-associated MRSA strains possess certain unique, but not exclusive, characteristics. They usually have small *SCC*mec cassettes (type IV or V), and are generally not as resistant to antibiotics as healthcare-associated MRSA strains—community-associated strains being more likely susceptible to non- β-lactam antibiotics [52]. This profile is in contrast to the traditional nosocomial MRSA which has a

multidrug-resistant profile, possibly related to the continuing antibiotic pressures in hospital environments.

Many community-associated strains produce Panton-Valentine Leucocidin (PVL), a cytolytic toxin which targets human neutrophils [53]. In 1999, a French observational study of 172 clinical isolates suggested that PVL was a virulence factor associated with more severe illnesses of SSTI [54]. Nonetheless, the role of PVL in determining severity and outcomes of complicated SSTIs has been controversial. A more recent study in 2009 included 522 clinical MRSA isolates from 17 countries and showed that patients with PVL-positive MRSA isolates were more likely to be young North Americans who presented with large abscesses, than patients whose MRSA isolates were PVL-negative. However, patients with PVL-positive MRSA were more likely to be cured compared to those with PVL-negative MRSA [55]. The finding suggested that the presence of the PVL-encoding gene in an MRSA strain by itself should not be an indicator for a specific clinical treatment. In the U.S., PVL may represent a diagnostic marker for the most abundant CA-MRSA strain, known as USA 300 [56].

Although community-associated MRSA strains originated from the community, they have also been noted to emerge as a cause of healthcare-associated infections in developed countries. A systematic review of 18 outbreaks of community-acquired MRSA strains in healthcare settings between 2003 and 2010 provided interesting outbreak features and supporting evidence that community-associated MRSA strains may be overtaking traditional healthcare-associated MRSA as a common cause of hospital infections [14]. From the review, most of the outbreaks were caused by a single strain. Twelve of 18 outbreaks were in pediatric and obstetrics, specialities where the

healthcare-associated MRSA prevalence is low, and several outbreaks demonstrated transmission within households. Interestingly, healthcare workers were found commonly to be the source of the outbreaks and the target of infections [14].

In addition to the emergence of human community-associated MRSA strains into healthcare settings, in this past decade there have also been reports of animal and livestock MRSA strains which have led to outbreaks in hospitals [57-59].

This phenomenon of community-associated MRSA taking over healthcare settings has several important implications [14].First, mixing of the community and the hospital strains healthcare setting increases the pool of susceptible populations to include not only the elderly and/or chronically ill patients, but also healthcare workers, visitors and their community contacts.

Second, having community-associated MRSA strains in hospital settings also means exposing these relatively more susceptible strains to more antibiotic pressure in the hospital environment, which may influence their future resistance profile.
Third, this mixing also exposes PVL-producing community-associated MRSA strains to hospitalized patients, which may increase the morbidity in nosocomial MRSA infections. Nonetheless, a study from Detroit, Michigan showed that community-associated strains when inside hospitals behave more like healthcare-associated infections, causing invasive infections rather than complicated SSTIs like in community settings [60]. Finally, while efforts to control MRSA have primarily focused in the healthcare system, the expanding community reservoir and the dynamic environment within healthcare settings may pose a new dimension of infection control strategies to more actively include control within the community as well [14].

1.6 Colonization

S. aureus is a common commensal organism on human skin and mucosa. The anterior nares of the nose are the main ecological niche, while numerous other body sites may also harbor *S. aureus* including skin, pharynx, perineum and the gastrointestinal tract [61-68].

Measures of colonization status may vary depending on the studied population, the type of study (cross-sectional or longitudinal), the sampling quality, the sampling site(s) and the detection methods [22]. Globally, it is noted that the *S. aureus* colonization prevalence may be lower in tropical countries [61]. A nasal swab survey from July 2001 to May 2002 in two university hospitals in Indonesia included 3,995 inpatients, outpatients and relatives of patients and found 362 (9.1%) individuals to be *S.aureus* nasal carriers [69]. In Pakistan, 1,660 nasal swabs were collected from healthy individuals who accompanied patients to a community laboratory from January 2002 to December 2003. A total of 246 (14.8%) individuals were identified as nasal carriers for *S.aureus* [70]. In Malaysia, nasal swabs of 346 health adults found 81 (23.4%) individuals to be *S.aureus* nasal carriers [71].

In view of age, a British study of 100 infant-mother pairs showed colonization status varies substantially from being the most prevalent (45%) during the first 8 weeks of life to 21% by 6 months. The usual sources of infant strains were their mothers [72].

In the general population, the prevalence of nasal colonization varies greatly. About 12-30% are persistent carriers and 16-70% may be intermittent carriers [61, 73, 74]. In 1997 a review reported a mean nasal carriage of 37.2% among the general population [75]. However, a more recent review in 2005, which included studies since 2000, reported a *S. aureus* nasal carriage of 27% among healthy adults. The proposed

explanations of the decline were improved personal hygiene, changes in socioeconomic class, and smaller family sizes [61, 76, 77]. In the U.S. the National Health and Nutrition Examination Survey showed an overall decrease of *S. aureus* nasal colonization from 32.4% in 2001-2002 to 28.6% in 2003-2004, but the prevalence of MRSA nasal colonization has increased from 0.8% to 1.5%, respectively [78].

In longitudinal studies, populations could be defined as persistent, intermittent and non-carriers. That said, there is no general consensus on how many cultures should be taken and how many cultures should be positive to define persistence [61]. One study that used quantitative and qualitative nasal culture data to differentiate persistent and intermittent or non-carriers proposed a "culture rule" [79]. This study suggested that two consecutive weekly positive cultures could predict the persistent carriage state with a reliability of 93.6%.

Determinant factors of colonization may include the host, the environment, and the nasal microbial ecology [22, 80]. In view of host factors, persistent carriers were shown to preferentially reselect their autologous strains from artificial inoculation with mixed strains [81]. Patients with certain diseases were more likely to be colonized with *S. aureus*. These diseases include diabetes mellitus, chronic renal disease, chronic skin diseases (e.g. psoriasis and atopic dermatitis), and nasal anatomical abnormalities [22]. Nose picking was also associated with increased *S.aureus* carriage [82].

In view of environmental factors, crowding in households and hospitals, and the level of hygiene are associated with risk of carriage. Conditions in prisons, public housing projects, military barracks, and daycare centers are also known as associated risk factors for acquisition [22].

For the microbial ecological factors, bacterial interference has been postulated to be a major determinant of carrier and non-carrier states [80, 83-85]. Using a neonatal rat model and culture-based detection method, one study showed that multiple strains of common commensal organisms such as *Streptococcus pneumoniae* or *Haemophilus influenza* can coexist, but *S. aureus* strains require a host to have no other *S. aureus* present to colonize [83]. Studies using culture-independent analysis of 16S rRNA also supported previous findings [84, 85]. A study of human nasal microbiota among 26 inpatients found *S. aureus* nasal colonization to be negatively correlated with the abundances of other commensal organisms including *S. epidermidis* and several actinobacterial groups [84]. A study examining bacterial microbiota of the nostril and oropharynx in seven healthy adults showed an inverse correlation between the prevalence of *Firmicutes* and other phyla; *Proteobacteria* and *Actinobacteria* at both sites. In the nose, this inverse correlation existed between the *Firmicutes* family *Staphylococcaceae* and *Actinobacteria* families, suggesting potential antagonism between these groups [85].

1.7 Risk factors of acquiring healthcare-associated infection

Hosts, pathogen and environmental factors, the three components of the epidemiological triad, all contribute to acquisition risk. Individuals who are colonized with *S. aureus* have an increased risk of subsequent infections with their own strains, i.e. endogenous infections [22, 86-88]. Studies have shown that *S. aureus* carriage is associated with increased risk of staphylococcal diseases in both community and healthcare settings. In the community, carriage has been linked to increased risks of skin and soft tissue infections, osteomyelitis, rhinosinusitis, endocarditis, as well as toxic shock syndrome [22]. In healthcare settings, carriage has been shown to increase risks of

postoperatively acquired surgical site infections and blood stream infections [86, 88, 89]. Furthermore, individuals who are colonized with methicillin-resistant *S. aureus* carry a higher risk of subsequent infections than those with methicillin-sensitive strains [87, 90-92]. A prospective cohort study over a 4-year period in a 24-bed surgical and 19-bed medical ICU performed nasal cultures upon admissions on 9,523 patients [91]. The study found that risk factors for ICU *S. aureus* infections were MRSA nasal colonization upon admission (adjusted hazard ratio, 4.7), and MSSA nasal colonization (adjusted hazard ration, 2.5).

In view of the patient's environment, exposure to healthcare workers who were colonized with *S. aureus* may also be a risk factor for acquisition. In a review of 191 MRSA outbreaks, 26 outbreaks were found where healthcare workers might have been the source. Of these, 11 had strong evidence that healthcare workers were the likely sources [93]. Eight of eleven had indistinguishable strains according to their molecular typing methods when comparing isolates recovered from patients and colonized health care workers. In addition to exposure to healthcare workers who may be carriers, exposure to contaminated rooms can also be a risk factor. A 20-month retrospective cohort study of patients admitted to 8 intensive care units, which performed routine and weekly screening for MRSA, showed that patients admitted to a room that was previously occupied by MRSA-positive patients had increased odds of MRSA acquisition, compared to patients whose prior room occupants were MRSA-negative [94]. Another retrospective cohort study of a 472-bed acute-care teaching hospital showed that roommates of patients with MRSA were at significant risk for becoming colonized. This study followed 198 roommates of patients who had unrecognized MRSA colonization between 1996 and

2004. Subsequently, twenty-five patients (12.6%) acquired MRSA, all with strains indistinuguishable by pulsed-field gel electrophoresis from those of their roommate [95]. These studies showed that MRSA status of the room occupants, either previous room occupant or current roommates, is an important risk factor in MRSA acquisition.

1.8 Routes of transmission

The hands of healthcare workers were recognized as vectors in staphylococcal transmission as early as the 1960's [40, 96]. When infants were housed in the same nursery and near an index infant known to be colonized with a defined strain of *S. aureus*, the exposed infants later became colonized with certain strains from the nurse who cared for them rather than the index infant strain. Exposure to the nurse's hands even during a single session was sufficient for transmission, whereas hours of exposure to the nurse in the same room with no hand touching did not result in transmission. This finding in nurseries suggested a major role of healthcare workers' hands in spreading the pathogens, while the airborne route appeared to be of less importance.

Nevertheless, the circumstances that govern the transfer and acquisitions of *S. aureus* may vary widely in different parts of the hospital or the same part but different settings, so that the effective routes may differ [97]. "Cloud" babies were an example of *S. aureus* transmission via airborne route in nurseries. These babies disseminated an increase amount of *S. aureus* into the air, particularly in association with viral upper respiratory tract infections [98]. An outbreak in a surgical intensive care unit due to a cloud adult, a physician who was a carrier and suffering from a rhinoviral infection, has also been documented [99]. Outbreaks have also occurred where there was no link to healthcare worker as a source, but epidemiological evidence suggested airborne spread

through the air-channel duct into the patient's room [100, 101]. Similarly, outbreaks occurred where the likely sources were contaminated environmental surfaces [102-104]. Collectively, in various hospital units (including general wards, burn units, operating rooms, and intensive care units,) environmental contamination by air or surfaces has been suggested as effective transmission routes [105-112].

Using advanced molecular techniques, several studies estimated that 15-67% of common nosocomial bacterial infections occurred through cross (patient-to-patient) transmission [113-115]. Patient-to-patient transmission is, however, a broad term that does not provide the exact mechanical exposure pathways. Patient-to-patient may be healthcare workers' hand-mediated transmission or environmental-mediated transmission. For this dissertation, the exposure pathways according to the pattern of pathogen flow from the colonized or infected patient to the susceptible or the uncolonized patient are explicitly examined. The possible transmission routes are classified based on the final exposure source to the uncolonized patient.

Figure 1.1 shows the three main exposure pathways: the actual patient-to-patient route, the hand mediated route and the environmental mediated route. Figure 1.1.a depicts the actual patient-to-patient route, where the colonized patient (PTc) makes skin-to-skin contact with the uncolonized patient (PTu) and transmits MRSA to the uncolonized patient. Figure 1.1.b represents the hand-mediated route, where hands of healthcare workers (HCW) can be contaminated by touching the colonized patient or contaminated surfaces. These hands later touch the uncolonized patient and transmit MRSA. This touching event is referred to as direct patient-HCW contact. Figure 1.1.c represents the environment-mediated route, where the environment (Env) may be air or contaminated

surfaces and objects (fomites). MRSA is transmitted when the uncolonized patient touches the contaminated surfaces. The event that the patient touches the surface will be referred to as an indirect contact route.

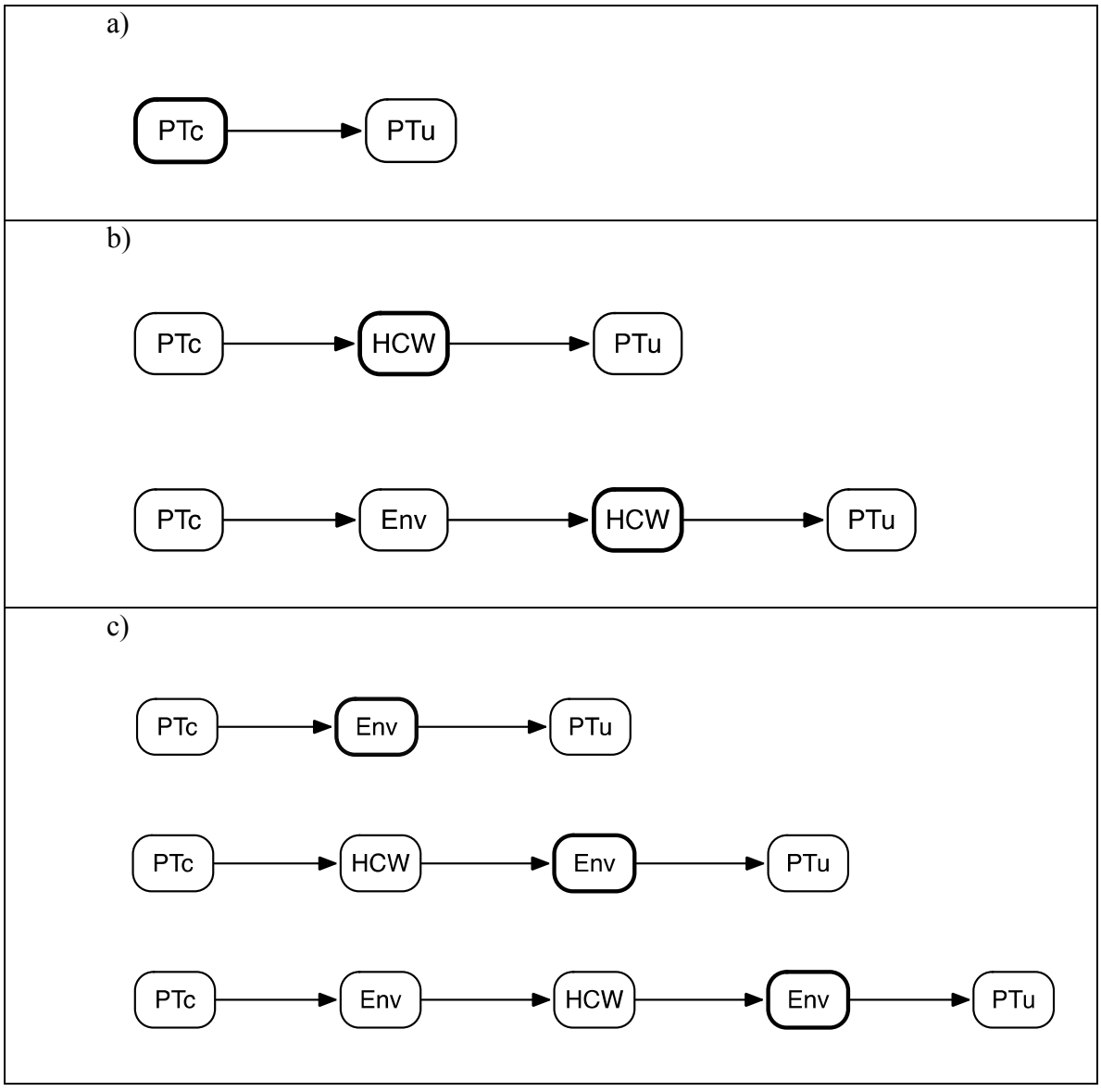

Figure 1.1: Possible routes of MRSA transmission. This figure depicts how MRSA may be transmitted from a colonized patient (PTc) to an uncolonized patient (PTu). The three main categories are based on the source of MRSA that finally transfer to the uncolonized

patient. Figure 1.1a is the actual patient-to-patient route. Figure 1.1b is the hand-mediated route. Figure 1.1c is the environment-mediated route. HCW – healthcare worker. Env – environment.

1.9 Current infection control strategy

Control of antibiotic resistant organisms is a complex problem involving the interplay among pathogens, hosts and their environments. Understanding how resistance develops and how the pathogen spreads between hosts, taking into account their environment, are important in strategizing infection control. Several pathways may be involved in the appearance or spread of resistance in bacteria [116]. Those most relevant to MRSA are: introduction of a few resistant organisms into a population where resistance was previously not present, selection of a small and resistant subpopulation, and dissemination of inherently resistant organisms within the local setting [117].

To suppress resistance development and prevent further spreading, four main strategies have been proposed for endemic MRSA control. These are 1) prevention of selection of methicillin-resistant isolates in a population of *S. aureus* by antibiotic stewardship, 2) identification of carriers by screening and isolation, 3) elimination of the reservoirs by patient decolonization, and 4) prevention of patient-to-patient transmission by hand hygiene, contact precautions, and environmental decontamination [117, 118].

1.9.1 Prevention of selection of methicillin-resistant isolates by antibiotic stewardship.

A review showed supporting evidence that antibiotic usage is directly associated with MRSA infections [117, 119]. This evidence included consistent associations between heavy antibiotic use and high MRSA prevalence in the patient, hospital or hospital unit levels. Patients who are colonized or infected by antibiotic resistant

organisms are more likely to have received prior antibiotic treatment. The proportion of methicillin resistance is higher among *S. aureus* isolates from hospitals, where the antibiotic pressure is higher, compared to *S. aureus* isolates from the communities. Within the same hospitals, the proportion of MRSA in intensive care units, where more antibiotics are used, is higher than in other inpatient units [119, 120]. The dose-response relationship of the antibiotic usage and the proportion of antibiotic resistant organisms were also shown to be linear. Additionally, there were temporal concomitant changes such that as antibiotic use increased, antibiotic resistant increased [121]. Thus, an antibiotic stewardship program is generally recommended to monitor and direct appropriate antimicrobial use at healthcare institutions with the purpose to prevent selective pressure for resistant strains to emerge.

1.9.2 Identification of carriers by screening and isolation

Identification of carriers and isolation are integral components of the search-and-destroy strategy, which has been successful in some regions with low MRSA prevalence such as in the Netherlands [118, 122]. This strategy includes the use of active surveillance of persons at risk, the preemptive isolation of patients at risk, the strict isolation of known carriers, and eradication of MRSA carriage with intranasal mupirocin [123]. In the U.S., an active surveillance and isolation program has been legislatively mandated in some states such as Illinois, New Jersey, Pennsylvania Minnesota, and Maine to screen certain patients for MRSA upon admission [124]. However, two recently-published large scale studies assessing the effectiveness of active surveillance programs reported conflicting results. One of these studies was a Veteran Affairs (VA) system-wide quality-improvement initiative program called ‘MRSA bundle’, which

included nearly 2-million admissions, transfers, or discharges in 150 hospitals with 196 intensive care units (ICU) and 428 non-ICUs. The study concluded that implementation of the MRSA bundle, which consisted of universal nasal surveillance for MRSA, contact precautions, hand hygiene improvement, and institutional culture changes, was associated with a decrease in healthcare-associated transmission and infections with MRSA [125]. The other study was an unmasked, cluster-randomized, controlled trial involving more than 9000 patients admitted to 18 ICUs, which participated in the Strategies to Reduce Transmission of Antimicrobial Resistant Bacteria in intensive care units (STAR-ICUs) trial. In the intervention ICUs, interventions included nasal surveillance cultures and the expanded use of barrier precautions. Once contact precautions were initiated, they were continued for the entire ICU stay. Laboratory results were reported through a web-based system. Patients were placed in contact precautions if they had a history of being MRSA positive in the past year or if clinical or surveillance cultures became positive. In the control ICUs, nasal surveillance was performed, but the ICU staff did not have access to the results. The study concluded that the surveillance was not effective in reducing transmission of MRSA [126]. These two studies differed in several important aspects, including the study designs, the study populations, and the concomitant interventions [127]. Nonetheless, the most influential factor was likely related to their choices of laboratory techniques, which determined the laboratory reporting time. For the VA study this turnaround time was less than a day, since more than 90% of VA hospitals used real-time polymerase-chain-reaction, but for the STAR-ICU trial study, this time was 5.2 days due to the centralized cultured-based method. This turnaround time only allowed 41%

captured isolation patient-days while in the ICUs, much less than the suggested level of over 80% for successful endemic MRSA control [128-130].

1.9.3 Elimination of the reservoirs by patient decolonization

Colonization is an important risk factor for subsequent infection [61]. Individuals who are either colonized or infected are the major sources of spread to others [22]. Thus, decolonization has two main purposes; 1) prevent subsequent infections in individuals who are already colonized, and 2) prevent transmission from colonized individuals to others by eradicating the *S. aureus* reservoir. The approach for eradication has been intranasal application of topical antibiotics, i.e. mupirocin either alone or in combination with antiseptic soaps, i.e. chlorhexidine, or in selected cases, oral systemic antibiotics [131]. A systematic review of 23 clinical trials suggested that short-term intranasal mupirocin is the most effective treatment, with a success rate in eradicating MRSA carriage of 90% in 1 week after treatment and up to 60% after a longer follow-up period [132].

Despite the short-term successful rate in eradicating MRSA, several studies have shown that its impact in suppressing MRSA infections was inconsistent among various study populations [131-133]. Evidence supports that decolonization of *S. aureus* carriers before surgery reduces the risk of postoperative staphylococcal infections, particularly in patients undergoing cardiothoracic procedures. Decolonization might reduce infection rates in patients undergoing haemodialysis or continuous peritoneal dialysis, and could be useful in patients with recurrent staphylococcal skin and soft tissue infections. Routine recommendations for non-surgical carriers are not currently indicated. The role of decolonization in preventing transmission in endemic settings is not conclusive.

Nevertheless, decolonization of colonized healthcare workers or patients as a component of outbreak management may be considered [131, 134].

1.9.4 Prevention of patient-to-patient transmission

1.9.4.1 Hand hygiene

Contaminated hands are considered the main vector of the spread of MRSA. Therefore, hand hygiene has been considered as the cornerstone of transmission prevention. Despite its importance, its simple procedure and the continuing campaigns, compliance remains a constant obstacle. One review reported hand hygiene compliance to be in the range of 20-50% using observational data from various time-periods [135]. An observational study using a 24-hour period in two 28-bed medical wards showed that compliance varied greatly among the 823 hand hygiene opportunities. Compliance before an aseptic task was reported as 100% (3/3); after body fluid exposure 93% (86/93); after patient contact 80% (114/142); before patient contact 68% (196/290); and after contact with surroundings 50% (65/129).

Reported reasons for suboptimal compliance were lack of time, skin irritation from the hand hygiene agents, high workload and poorly accessible sinks [136]. An alternative use of alcohol-based hand disinfectant has overcome some of these obstacles and is now widely recommended [135, 137].

Substantial evidence supports that improvement of hand hygiene can reduce the incidence of healthcare-associated infection [135]. Nevertheless, some studies showed no association of improved hand hygiene compliance and reduction of nosocomial infection rates [138-141]. A 2-year prospective, controlled, cross-over trial of alcohol-based hand gel in 2 adult ICUs in a U.S. tertiary-care teaching hospital showed a statistically

significant improvement in compliance after the introduction of hand gel, increasing from 37% to 68% in one ICU and 38% to 69% in the other unit. However, there was no substantial change in the rates of device-associated infection or infections due to multidrug-resistant pathogens [141]. In contrast, a 4-year prospective quasi-experimental study with hospital-wide program emphasis on using an alcohol-based hand rub showed a significant improvement of hand hygiene from 43% to 96%, with a significant reduction in the healthcare-associated infections of most hospital units [142].

While improving and sustaining hand hygiene compliance has been a challenge in many institutions, controversy exists regarding the targeted compliance level and the utility of attempts to further increase compliance in settings where the baseline levels are already high [118]. Mathematical modeling suggests that such interventions to achieve compliance over 50% may not be beneficial in further reducing MRSA transmission.

1.9.4.2 Environmental decontamination

Over 40 years ago, Earle H. Spalding devised a rational approach to disinfection and sterilization of patient-care items and equipment [143]. This approach classifies patient-care items and equipment into three categories according to the degree of infection risk involved in their use. Critical items are considered high-risk for infection if they are contaminated. These are objects that enter sterile body sites and must be sterilized before use. Semi-critical items are those in contact with mucous membranes or nonintact skin and must undergo high-level disinfection. Non-critical items are those in contact with intact skin and should receive low-level disinfection [143].

Hospital environmental surfaces are known to be contaminated with various organisms, including nosocomial pathogens [144, 145]. Because environmental surfaces

are frequently in contact with intact skin, and are generally viewed as an uncommon source of infection, they have been classified as noncritical and require cleaning and disinfection on a regularly scheduled basis.

Nevertheless, there is growing evidence that contaminated surfaces may be an important transmission route. A review assessing the efficacy of environmental decontamination stated that the effect of surface disinfection is only transient, and microbial contamination can reach its former level within hours [146]. In addition, outbreaks that are linked to contamination in patients' environment have been documented [102-104]. Studies in endemic settings that included clinical and environmental surveillance showed evidence to support environmental contamination as the source for subsequent infections using pulse field gel electrophoresis for *S. aureus* typing to demonstrate molecular identity between environmental and patient isolates [109, 110, 147]. These findings suggest that contaminated environment may also play an important role in MRSA transmission. More detail in supporting evidence of environmental mediation is in Chapter III.

1.10 Summary

MRSA has continued to be a public health threat since it was first discovered. Currently, MRSA is not only a prominent healthcare-associated pathogen, but also an important cause of community-associated infections. Populations at-risk for MRSA have expanded to include young, healthy individuals, in addition to vulnerable patients in healthcare facilities. The pathogen itself has evolved and showed its ability to resist all classes of antibiotics currently used. An effective infection control strategy is a combination of multiple interventions with a multidisciplinary approach requiring

participation from all levels, ranging from patients themselves to legislators. While the burden to the public health at large remains, successful stories of control of MRSA infections in institutions have been reported. Moreover, many advances in study methodology, including mathematical modeling and computer simulations, have been made which may allow us to better understand transmission systems and to better plan for infection control measures. Additionally, the available and feasible molecular tools used in this decade provide great potential to improve our insight in the interactions between hosts, pathogens and environments in the transmission process. To this end, an application of these advances to study MRSA transmission in the healthcare settings can be promising. Review of selected mathematical modeling and molecular techniques is provided in Chapter II.

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CHAPTER II

Selected Transmission Modeling Studies and *S. aureus* Molecular Typing Techniques

This chapter provides a review of selected transmission modeling studies in healthcare settings. The focus is on studies that have incorporated healthcare workers' and patients' environments in some capacity. The latter part of the chapter includes a review of selected *S. aureus* molecular typing techniques that are potential tools to be incorporated in the future transmission study, which will be discussed later in chapter VII.

2.1 Previous modeling studies in healthcare setting

An infection transmission system is a dynamic complex system that includes hosts, pathogens and their environments. To overcome some of the complexities we require simplifying assumptions in modeling, so that we can gain insights into the system. Therefore, inferences from any models depend heavily on the model assumptions. Traditional statistical analysis models in epidemiological studies include a stratified comparison of risks, logistic regression and proportional hazard models, estimate parameters that relate exposure to disease in individuals. They assume that the outcome of one individual is independent of the outcomes of the others [1]. These assumptions may be appropriate in settings where there is no dependency among

individuals. However, they are inappropriate in a study of person-to-person infection transmission such as with the case of MRSA.

On the contrary, the transmission model allows individuals to relate to one another by using parameters that express contact rates and transmission probability [1]. Understanding the contact patterns that lead to transmission is important for infection control planning. In this past few decades there has been an increasing use of mathematical modeling and computer simulations in the study of transmission. These tools allow us to form theoretical concepts, generate and test hypotheses, design studies and gain insights into the transmission system [2, 3].

Many modeling studies in healthcare settings were adapted from the Ross-Macdonald model [4-7]. The Ross-Macdonald was originally used to describe transmission of Malaria, where *Anopheles* mosquitoes were vectors that carried the parasites transiently [8]. This model was later applied to healthcare settings, where hands of healthcare workers were contaminated with nosocomial pathogens and transferred these pathogens to patients. Figure 2.1 shows an example schema of an applied Ross-Macdonald model.

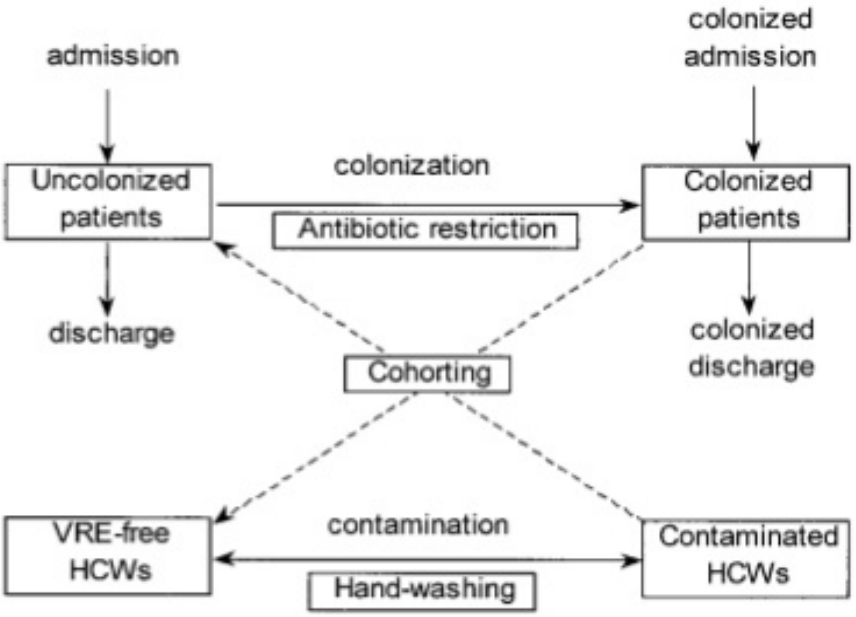

Figure 2.1: An applied Ross-Macdonald model of indirect patient-healthcare worker-patient vancomycin resistant enterococci (VRE) transmission in an ICU showing the possible effect of infection control measures. Once patients become colonized they are assumed to remain colonized for the duration of their stay in the ICU. Dashed lines represent contacts between healthcare workers (HCW) and patients [6].

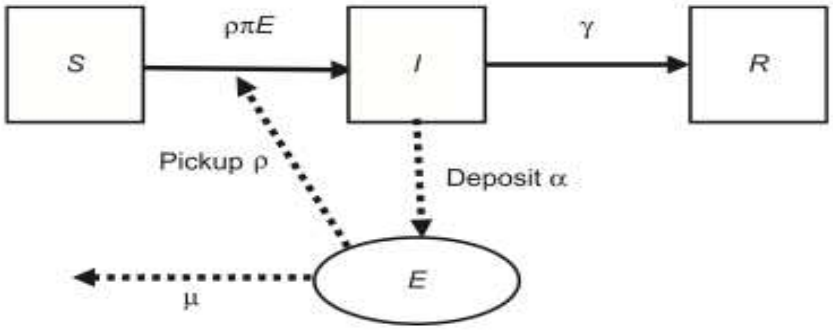

Figure 2.2: A schematic representation of flow of individuals (solid lines) among states and flow of pathogens (dotted lines) in the environment (E) for the environmental infection transmission system (EITS) model [9]. The three states of individuals are susceptible (S), infected (I), and removed or immune (R). The model parameters are pick-up rate (ρ, pathogen/person/day), the probability that a susceptible individual becomes infectious per pathogen picked up (π), recovery rate (γ, 1/day), pathogen deposit rate (α, pathogen/infected/day), and elimination rate (μ, 1/day).

Author, year	MDRO	Model	Assumptions	Interventions	Findings	Sources of parameter estimate
D'Agata, 2005 [10]	VRE	Deterministic differential equation based model, 6 compartments: colonized and uncolonized patients divided into those receiving and not receiving antibiotics, and contaminated and uncontaminated HCW	The loss of VRE colonization requires an absence of antibiotic pressure. Antibiotic pressure is prerequisite for VRE colonization.	Hand hygiene, patient cohorting, HCW/patient ratio, length of stay, antibiotic policy, admission prevalence	1) Reducing antibiotic exposure to uncolonized patients is more effective than reducing antibiotic exposure to colonized patients. 2) Eliminating the influx of VRE results in the eradication of this pathogen from the hospital 3) Decrease length of stay of colonized patient, increased hand hygiene compliance, and lower HCW/patient ratio – all decrease endemic prevalence.	Expert opinion: transmission probability. Database: length of stay, ward size, admission prevalence, staff/patient ratio, antibiotic treatment and cessation rates. Literature sources: hand hygiene compliance

Mcbryde, 2006 [11]	VRE	Extension of D'Agata's model with an additional environmental compartment	The colonized patients and HCW contribute to environmental contamination. Contaminated environment can lead to contamination of HCW, and indirectly leading to patient colonization.	Same as in D'Agata's	1) VRE remains endemic, even if colonized patients are prevented from entering the ward – in contrast to D'Agata's model which had no environment compartment. 2) The magnitude of impact due to the same intervention is decreased compared to D'Agata's model.	Expert opinion: transmission probability
Wolkewitz, 2008 [12]	VRE	Stochastic compartmental model: 6 compartments were patients divided into colonized and uncolonized, HCW and environmental surfaces, divided into contaminated, uncontaminated	HCW and patients can be contaminated when contact with surfaces. Each contaminated HCW can independently create infectious contacts.	Hand hygiene and use of gloves for HCW, increased decontamination of surfaces, patient cohorting, screening and isolation, reduction of antibiotic usage	The improvement of hand hygiene alone has no effect on the endemic prevalence. Only a combination of interventions may control an outbreak.	Expert opinion: transmission probability Literature sources: colonization prevalence upon admission, discharge rate, duration of staff contamination

Li, 2009 [13]	Influenza	Deterministic and stochastic compartmental model: 4 compartments were hosts (susceptible, infected, removed), and pathogens in the environment.	Pathogens in the environment instantaneously and thoroughly mix. Their levels diminish through human pick-up, natural die-off, and decontamination.	Environmental decontamination	Transmission through frequently touched fomites generates frequency-dependent patterns, while transmission through air and infrequently touched fomites generate density-dependent patterns. The model provided a theoretical framework to examine the role of the environment in transmission study.	Expert opinion: recovery rate Literature sources: Transmission probability, deposit rate, pick up rate

Table 2.1: Comparison of transmission studies that incorporated environment in their models. MDRO is multi-drug resistant organisms. MRSA is methicillin-resistant *Staphylococcus aureus*. VRE is vancomycin resistant enterococci. HCW is healthcare workers.

Although many models have incorporated healthcare workers as vectors, few had incorporated the environment as a reservoir (Table 2.1). A mathematical modeling study to quantify the contribution of antibiotic exposure to the dissemination of vancomycin-resistant enterococci (VRE) was performed [10]. The model was an ordinary differential equation based model comprising of 6 main compartments; patients colonized with VRE receiving and not receiving antibiotics, uncolonized patients receiving and not receiving antibiotics, contaminated and uncontaminated healthcare workers. The model predicted that preventing the initiation or enhancing discontinuation of unnecessary antibiotic therapy could have a greater impact if it was targeted on uncolonized patients. Also, the model predicted that eliminating the influx of VRE resulted in the eradication of the pathogens from the hospital [10]. Nevertheless, an extension of this model by adding the environment as an additional compartment provided a new insight regarding the impact of environmental reservoirs on the transmission of VRE [11]. The results from the extended model showed that even if the colonized patient was prevented from entering the ward, VRE remained endemic [10, 11]. This extended model, however, only allowed healthcare workers, not patients, to make contact with the environmental reservoir. Another differential equation model included environment and also allowed both patients and healthcare workers to make contact with the environment. The study concluded that only the combination of interventions (hand hygiene, cohorting, screening and antibiotic reduction) including environmental decontamination could control a VRE outbreak [12].

These previous compartmental models [11, 12] assumed homogenous mixing for the effective contacts that resulted in transmission. A patient's risk of acquisition through healthcare workers and the environment is governed through the probability of

transmission. These studies did not explicitly model contact as a discrete event. Also, they did not include pathogen specific environmental parameters, contact patterns between patients and healthcare workers, nor contact patterns with environmental surfaces. Excluding these elements may impede the capability of these models to analyze the effects of host and environmental based interventions such as hand hygiene and surface decontamination.

Recently, a new framework, Environmental Infection Transmission System (EITS), which incorporates explicit environmental processes, was developed as displayed in Figure 2.2 [9]. The framework incorporates the pathogen fate and transport processes to determine exposure doses to susceptible patients from different exposure routes. Contacts between hosts, pathogens and environments are explicitly incorporated. The exposure dose-response function then determines the acquisition risk. This EITS model has been applied for waterborne, airborne and fomite-mediated transmission [14-16]. In chapters V and VI of this dissertation, the EITS framework has been applied in the assessment of both hand- and environmentally-mediated MRSA exposure in a hypothetical hospital ward.

2.2 *S. aureus* molecular typing methods and their applications

Modern molecular techniques have become powerful tools in epidemiological studies as well as in many other scientific areas. Molecular tools create informative data and sometimes enhance the existing data to more in-depth levels [17]. They also allow nomenclature systems to be developed providing identity for each isolate and diversity for the population. However, each molecular technique has a different discriminatory power. Discriminatory power is the average probability that a typing method will assign

the same strain type to strains randomly sampled from the same group. Determining an appropriate typing technique for a study depends not only on the discriminatory power, but also on the purpose of the investigation and the study time scale, which may affect the evolutionary changes of the pathogen. Thus, the most discriminatory technique may not suit a study, if the resulted groupings are not associated with the outcome of interest [18].

2.2.1 Chromosomal DNA restriction patterns by pulse field gel electrophoresis (PFGE)

PFGE typing is the most widely used typing method, and generates a banding pattern for each isolate that serves as a molecular "fingerprint." This method allows for an evaluation of the entire chromosome, which is the most fundamental component of the cell identity. The chromosomal DNA is first digested by the restriction enzyme *Sma*I. The resulting DNA fragments are then separated by agarose gel electrophoresis in an electric field with an alternating voltage gradient [19, 20]. The banding patterns are then interpreted with certain criteria. Interpreting PFGE banding patterns require knowledge about how random genetic events can alter the patterns [21]. Taking into account these variants due to random genetic events, a guideline proposed by the U.S. Centers for Disease Control and Prevention for an outbreak investigation has frequently been used. It divides isolates into four categories; indistinguishable, closely related, possibly related, or different to the index isolate. According to the guideline, these categories are reliable if the PFGE resolves at least 10 distinct fragments [21].

PFGE is one of the most discriminating typing methods. Depending on the number of bands observed, its discriminatory power can be defined as moderate to high [18]. PFGE typing has been used at local, regional and international levels. It is

applicable in short time scale outbreak investigations, where genetic variation is likely minimal and the investigation requires a method with high discrimination between the index isolate and the other non-index isolates. However, differentiation of short-term outbreaks and endemic infections may be difficult when the outbreak strains also belong to the local endemic strains [21].

One study used PFGE and other methods to evaluate 325 unique patients' bloodstream MRSA isolates from a worldwide collection. The results showed that PFGE was superior in discriminating isolates into their original geographic regions, with four instances of indistinguishable PFGE patterns from more than one continent [22].

An important limitation of PFGE is related to its inter-laboratory reproducibility. Given the nature of the band-based method, strict adherence to standardized protocols is needed for a common nomenclature. However, there has been limited success in harmonizing the PFGE protocols on an international scale [19].

2.2.2 Multilocus sequence typing (MLST)

MLST is a molecular typing method based on the sequence analysis of internal 400 to 500-bp regions of seven *S. aureus* house-keeping genes [19, 23]. For each gene fragment, genetic polymorphisms in sequences are considered distinct alleles. Each strain is defined by the alleles at each of the sequenced housekeeping loci, which together comprise the allelic profile or sequence type [20]. These allelic profiles were then compared based upon the relatedness of lineages using the BURST algorithm (Based Upon Related Sequence Types) [24]. Strains with identical sequences at all seven genetic loci are assigned unique 'sequence type' (ST), and clusters of closely related STs are

called ‘clonal complexes’ (CC). For *S. aureus*, when five of the seven housekeeping genes are identical, strains are then clustered into a single CC [24].

The nomenclature of MRSA is currently based on the ST and the type of *Staphylococal* Chromosomal Cassette (SCC*mec*) element, which carries the structural gene, *mec*A, for methicillin resistance [24]. According to the 2009 guideline for the classification of SCC*mec*, there are 8 SCC*mec* types [25]. For example, strains of ST5 may be ST5-MSSA, ST5-MRSA-I, ST5-MRSA-II or ST5-GISA-II, where GISA is glycopeptide intermediate resistant *Staphylococcus aureus*. Thus, ST5-MRSA-I is the methicillin-resistant *Staphylococcus aureus* sequence type 5, which carries *SCC*mec I resistant gene [25]. Sequences from MLST can be submitted to a central database (available at http://saureus/mlst.net/), which enables online inter-laboratory communication and identification of alleles and STs. As of October 18, 2011 this database contained 2124 STs based on 4226 isolates.

MLST is a useful method for the study of population structure and molecular evolution of *S. aureus*. When used in conjunction with *SCCmec* characterization, it can reveal evolution of major MRSA clones [26]. Application of the recently estimated rate of (short-term) evolution for the MRSA core genome predicts that contemporary STs on average are many years old [26-28]. Thus, newly emerging and spreading strains will rarely be associated with novel STs [26]. So far, MLST excels in its use to identify broad population-based interrelationships. In local clinical settings, however, it is of limited use to trace the spread of individual *S. aureus* clones, due to insufficient discriminatory power. Another limitation of MLST is its high expense, labor and time requirements [26].

2.2.3 Single-locus sequence typing (SLST)

The SLST approach with most promise is the analysis of the polymorphic X region of the staphyloccal protein A (*spa*) gene, which is present in all strains of *S. aureus*. The polymorphism is due to 24-bp repeat sequences that may vary in both the number of repeats and the overall sequences in the polymorphic X or short sequence repeat region [20]. This variation is attributed to point mutation, as well as deletions and duplications of the repeats [19].

Spa typing has a higher discriminative power than that of MLST, but lower than that of PFGE [19, 29]. Since it involves only a single locus sequence, it is also less expensive, less laborious and less time consuming than MLST. The *spa* sequences can be stored in a central database (available at http://spaserver.ridom.de/), which is likely the largest *S. aureus* typing database [19]. As of Oct 21, 2011, the database contained 9,469 *spa* types from 188,276 isolates. The *spa* cluster analysis (*spa* clonal complex) is available based on the repeated pattern (BURP).

Spa typing has become increasingly popular and has been used to study both the molecular evolution as well as hospital outbreaks of MRSA [30-32]. However, it also has its own limitations. The high mutation rates may result in evolutionary convergence. When *spa* typing was compared to a phylogenetic tree that was based on core genome SNPs, several *spa* sequences were found scattered in two or more distinct phylogenetic lineages [28, 33]. This finding could misleadingly suggest the geographical spread of individual clones. On the same note regarding the mutation rate, there appears to be differences between methicillin-sensitive (MSSA) and methicillin-resistant *S. aureus* (MRSA). While MSSA display relatively greater *spa* variability, there is a concern that *spa* typing may provide too little discriminatory power for MRSA despite the high

mutation rate at the *spa* locus. This limitation could be due to the unknown proportion of emerging MRSA strains that are associated with unique *spa* sequences and hence may be recognized by the basis of *spa* typing [26].

A Belgian study comparing PFGE and *spa* typing to MLST based on a collection of 217 *S. aureus* strains during 13 years revealed that PFGE classification rarely violated the MLST assignment of CCs and STs, while the violation was more frequent for the *spa* classification [32]. The study suggested that *spa* typing should preferably be used in conjunction with other markers such as SCCmec typing, or resistance or virulent gene detection. Another study compared PFGE, spa typing and MLST based on a collection of 198 *S. aureus* strains over 15 years from 19 countries [31] and supported the Belgian finding that the combination of *spa* typing and PFGE was better than *spa* alone. When the results of PFGE and *spa* typing were conjugated, if two strains were classified together in the same PFGE-*spa* type, there was a 99.5% probability of also sharing the same MLST clonal complexes [31].

2.2.4 Mapping genome-wide single nucleotide polymorphism (SNP)

A recent investigation analyzed a whole genome of 63 *S.aureus* ST239 isolates from a global collection obtained over 21 years by mapping SNPs, insertions, and deletions to a reference sequence [28]. The study estimated the core genome divergence rate of 1 SNP every ~6 weeks. It analyzed the phylogenetic tree based on core genome SNPs and was able to identify intercontinental transmission events and expansion of subclonal variants which became dominant in the new geographical region [28].

This study highlighted the potential use of the ancestry-based tracking approach to identify recent from distance transmission events, and thereby improving contact

tracing in endemic and outbreak settings [26]. When provided with meaningful epidemiological surveillance samples including isolates from healthcare workers, patients' environmental samples, and patients' own colonized samples, this typing approach could be of great potential to unravel preferential routes of *S. aureus* transmission.

2.3 Summary

The environmental infection transmission system (EITS) framework allows the study of a complex system among hosts, pathogens, environments and their relationships. It is beneficial for the study of MRSA patient-to-patient transmission, given that possible routes are closely linked to the interactions among healthcare workers, patients and the environments. It is also proper for an evaluation of hand-based and environmental-based interventions.

Advanced molecular techniques can greatly enhance and facilitate the understanding of the transmission system. Integrating the use of modeling and molecular tools in a well-designed epidemiological study that includes clinical and environmental MRSA surveillance, as well as host contact patterns can be greatly informative and improve our insight in the MRSA transmission.

CHAPTER II REFERENCES

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CHAPTER III

Supporting Evidence for Environmental Mediated Transmission and Model Parameterization

Since *S. aureus* was first discovered, a considerable body of data has emerged regarding its spreading through the environment. To perform exposure assessment of the environmental mediation process in chapter V and VI, we will derive an environmental infection transmission system (EITS) framework, which allows incorporation of pathogen, environment, patients and healthcare workers in one system. In this chapter we perform a literature review to provide supporting evidence for environmental mediation processes based on the EITS concept. These processes include 1) shedding of pathogen into the environment, 2) pathogen survivability in the environment and on hands, 3) pathogen being transferred to hands of healthcare workers and/or to other patients, and 4) exposure dose-response or acquisition risk. Materials in this chapter will serve as basis for model parameterization in chapter V and VI.

3.1 *S. aureus* is shed to the environment continuously and sometimes profusely.

The ecological niche for *Staphylococcus aureus* are at the anterior nares, in the throat, in the gastrointestinal tract, and on the skin at numerous body parts including perineum, axilla, and wound [1]. With the exception of a less common direct person-to-person transmission, the majority of pathogen transfer occurs through more indirect routes. Some of these routes may include 1) expulsion via respiratory droplets from the

nose, 2) contamination of nasal discharge onto hands, 3) release of pathogen from the skin into air, or 4) excretion in the feces [1, 2].

Studies in 1956 and 1958 reported a series of investigations of these possible routes [2, 3]. To examine the number of *S. aureus* emitted from the nose, culture plates were held below the nose of eleven volunteers (6 nasal carriers and 5 non-carriers) during different types of activities including mouth breathing, nose breathing, coughing, counting, sneezing and snorting. The results showed that *S. aureus* was generally not expelled with these activities. Only as a result of snorting did large number of *S. aureus* emerge [2]. This study also examined 10 nasal carriers and 6 non-carriers for contamination on skin and clothing. The results showed that *S. aureus* was present on the skin and clothing of nasal carriers, but was found infrequently among non-carriers. The same study also quantified the release of *S. aureus* into free air. The experiment was carried out in a cubicle with culture plates held horizontally in each corners of the cubicle. The total number of colonies and those consisting of *S. aureus* on the exposed plates were counted assuming that each colony developed from one organism. Study results were expressed as cfu per 1 ft^2 (930 cm^2) per one minute. *S. aureus* count was the highest when volunteers were fully clothed and exercised in the cubicle, compared to when sitting still or when barely clothed and exercised or when agitating the volunteers' clothes. The range of *S. aureus* deposited on the plates were 0.14 to 47.4 cfu/ft^2/min [2]. Another experiment including 3 nasal carriers and 2 non-carriers washing and scrubbing hands with soap and water for 5 minutes showed a significant increase of *S. aureus* liberated into free air and isolated on culture plates standing in the four corners of the cubicle. *S. aureus* counts from the hand washing were in the range of 0.41 among non-

carriers to 300.5 cfu/ft^2/min among nasal carriers [2]. These original series of investigations in the 1956 study suggested that *S. aureus* likely transfer to others by indirect route involving i) egress in nasal secretions, ii) contamination of the skin, clothing, or bedding, iii) release of the organisms by friction, movement, or washing, and iv) transportation to others by air currents [2].

In 1958, further investigation on the role of skin and clothing contamination was undertaken. Seventy-six technicians, surgical dressers, and final-year medical students had nasal swabs; 30 (39.4%) were found to be nasal carriers. With the use of the cubicle as in Figure 3.1, quantification of *S. aureus* dispersal from skin and their clothing was investigated in 19 nasal carriers and 12 non-carriers. Measure for dispersal was reported in cfu/ft^2/min. The range of MRSA dispersal among the nasal carriers was 0 – 27.8 cfu/ft^2/min. It was a surprising finding that the number of *S. aureus* in the nose gives little indication of the extent of skin and clothing contamination, or the ability to disperse. In particular, there was one individual who had the primary source on the perineum rather than in the nose [3].

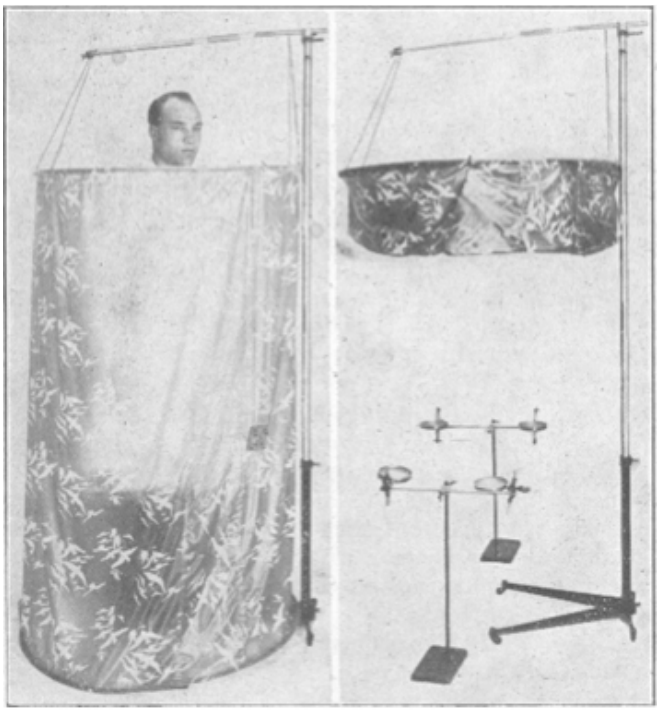


Figure 3.1: Cubicle employed in the dispersal experiments. The position of the four culture plates exposed in each experiment is shown on right. The figure is from Hare and Ridley 1958 [3].

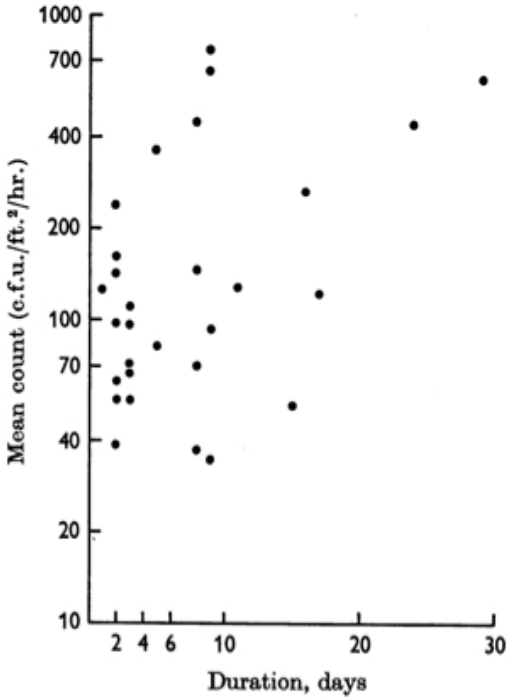
By early 1960s much attention was on the ability of *S. aureus* to disperse into air, but the underlying mechanism was unclear. There were questions of *S. aureus* floating freely in the air or attaching with textile fibers [4]. Skin scales were found in the air as early as in the 1855, and the possibility that they could carry organisms was suggested in 1905. But it was not until 1962 that it was found that these desquamated skin scales are the vehicles that carry most of the bacteria dispersed into the air in hospitals [4, 5]. The average human skin area is 1.75 m^2. This surface area comprise of approximately $2x10^9$ skin scales. A complete layer of cells can be lost and replaced on average every 24 hours. Hence, at least 10^7 skin particles may be shed every day [6, 7]. It was estimated that each airborne skin particle could carry four viable cocci of *S. aureus* [8].

To investigate whether there were differences in dispersal ability between patients and healthy individuals, one study evaluated 127 laboratory staff, students, patients with and without skin diseases for dispersal, as well as collected swabs of the nose and multiple body sites [6]. A dispersal test was performed while subjects were undressing in a cubicle similar to previous studies. But instead of using settling plates, it had two air slit samplers. The results showed that the ability to disperse was largely dependent on the degree of skin contamination. Patients with skin diseases dispersed more than those without. The range of dispersal was 0.25 to 100 cfu/ft^3 in 2 minutes [6].

To investigate the effect of clothing on dispersal and its extent in relation to various colonized body parts and gender, an experiment was conducted among 615 laboratory technicians, doctors and nurses. Nasal swabs were collected and air samplings were performed using a special test chamber [9]. This was a rigid enclosure of about $30m^3$ capacity, which volunteers entered. Air was drawn from the chamber through a tube to slit-samplers outside [10]. The results showed that 28% of women and 27% of men were nasal carriers, while 1% of women and 13% of men shed *S. aureus* into air. Of these dispersers, two men agreed for further experiments on clothing and body sites of shedding. Each man was tested wearing own clothes, then unclothed, then wearing clean or worn operating suits. Then, each man would wear polyethylene materials to cover different parts of the body. The results showed that wearing clothing increased *S. aureus* dispersal, particularly when wearing previously worn operating suits which released the highest quantity of *S. aureus* into air. The main site of shedding was the skin of the perineal area yielding 28-84 cfu/100 cu.ft. This finding supported the previous 1958 study and others that perineal carriers were likely heavy dispersers [3, 11, 12].

Given that these earlier studies were based on experiments in a confined space of a cubicle or a chamber, quantitative interpretation may not be generalized to the hospital air. A study to quantify *S. aureus* air count was conducted for 20 months in a 14-bed surgical ward divided into 4 rooms, and a 22-bed open ward using settling plates [13]. These plates were placed in each room in the surgical ward, and in 4 corners of the open ward. All 307 patients had nasal swabs collected upon entering the ward and weekly thereafter. Nurses and staff had hand swabs collected weekly and nasal swabs for the first two months of the study. The study showed that *S. aureus* dispersal in ward air did not spread from one room to another in a great extent when patients were found to be the source in the surgical ward. However, when the sources were the staff, *S. aureus* of the relevant types was found in all rooms and in the ward office. In the open ward, there were less differences between the counts on the four corners plates. There appeared to be a gradient of counts according to the distance from the highest count plate, i.e. the count on the plate 20 ft. distant averaged 26%, and the count on the plate 70 ft. distant averaged 11% of the high-count plate. The study also showed that 62% (53/87) of nasal carriers dispersed *S. aureus* into the air. As shown in Figure 3.1a, the longer duration of the dispersal event the higher mean air count. Figure 3.1b shows that about 10% of the patients generated air counts that averaged more than 50 cfu/ft^2/24 hrs. with the highest count up to 1000 cfu/ft^2/24 hrs.

a.



b.

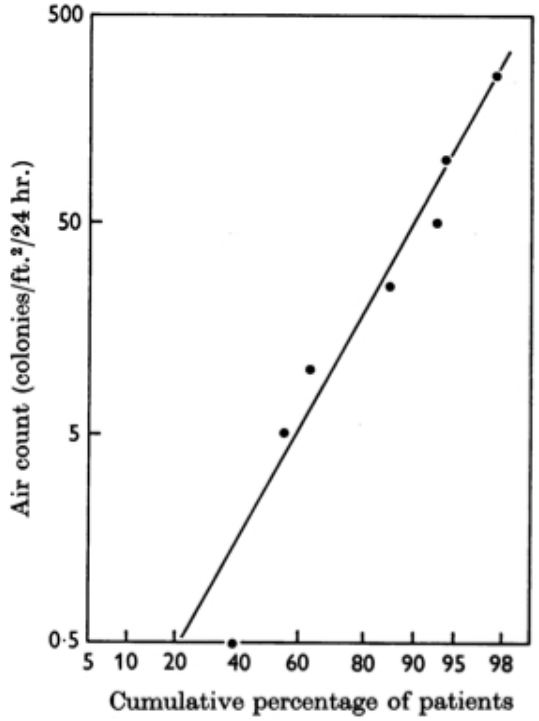


Figure 3.2a: Relation of staphylococcal air count during broadcast to duration of broadcast. Broadcast is the air dispersal event, which could have considerable day-to-day variation. Y-axis is the mean count (cfu/ft^2/hr) of each broadcast. X-axis is the duration of each broadcast. The figure is from William 1967 [13].

Figure 3.2b: Air counts generated by patients admitted as nasal carriers of staphylococci. Y-axis is mean air count (cfu/ft^2/24 hr). X-axis is the cumulative percentage of the nasal carriers. The figure shows that about 10% of the patients generated air counts that averaged more than 50 cfu/ft^2/24 hrs. The figure is from William 1967 [13].

More recently, a study in 2009 performed air sampling to assess methicillin-resistant *S. aureus* (MRSA) dispersal among staphylococcal pneumonia patients (n=20) and cystic fibrosis patients who were colonized with MRSA (n=4) during their hospital stays. The study used 10-minute air samplers, which aspirated air through a perforated plate and the resulting air-stream directly went to the agar surface [14]. MRSA was isolated from 21 out of 24 rooms. The range of air count was 1-78 cfu/m^3, which was lower than the earlier reports. There was no significant difference in MRSA counts

between sampling locations at 0.5, 1 and 2-3 m. from the patient. Several reasons may explain the lower counts in this study compared to previous others. Firstly, air sampling in this study was performed when there was no movement in the room, while patients were in their beds. Secondly, almost all of these patients had received antibiotic therapy. Thirdly, this study was performed in 2009 when the room air-exchange and the ventilation system were likely different from those four decades ago.

Several factors could influence the shedding and dispersal heterogeneity. Men were found as heavy disperser more commonly than women. Although when comparing the numbers of staphylococci dispersed, there was no difference between men and women [15]. Clothing can also affect dispersal by a few ways. First, it may increase dispersal by increasing friction and rubbing on the skin. Second, clothes may be reservoirs where contaminated scales accumulated before they are dispersed by overflow or movement. Third, if they are thick and have small textile pores, they may act as a shield and reduce dispersal [10]. However, this latter effect does not act well with everyday clothes since the pores are large enough for skin scales to pass through. Other factors that may increase dispersal are movement, skin diseases such as psoriasis, hand washing with soap and water, or showering [15-17]. Moreover, in addition to these external factors and differences between individuals, a large variability of *S. aureus* dispersal could also occur within the same individuals [12, 18].

3.1.1 Parameterization for chapter V and VI models

Possible ranges of *S.aureus* dispersal are summarized in Table 3.1. According to the study of the size distribution of airborne particles carrying microorganisms, these particles are in the range of 4-20 μm with the median equivalent diameter of 14 (13-17)

μm [19]. For such particle size, the settling rate is such that the number contained in 1 ft^3 of air is approximately equal to the number that settle on 1 ft^2 in 1 min [13]. Therefore in Table 3.1, the reported *S. aureus* air count is converted to the cfu/cm^2/min for use in chapter 5 and 6, assuming all *S. aureus* in the air completely settle on the horizontal surfaces.

Table 3.1: Selected literature review of *S. aureus* dispersal

First author, year	Study design	Subjects	Setting	Methods	*S.aureus* count in the original reported unit and its conversion to cfu/cm^2/min[a]
Hare, 1956 [2]	Experimental study. This study consisted of 9 different experiments. Results presented in this table are from the 5th experiment to quantify release of organisms into free air.	3 *S.aureus* nasal carriers and 2 non-carriers	Subjects were fully clothed and exercised for 15 minutes.	Setting plates in a cubicle	0.14 – 47.4 cfu/ft^2/min i.e. 1.5x10^{-4} – 5x10^{-2} cfu/cm^2/min
Hare, 1958 [3]	Experimental study	19 nasal carriers and 7 non-carriers	Subjects wore everyday clothing and exercised in the cubicle by marking time and waving their arms.	Setting plates in a cubicle	0 - 27.8 cfu/ft^2/min i.e. 0 – 3x10^{-2} cfu/cm^2/min
Noble, 1965 [6]	Experimental study	127 subjects including laboratory staff, students, hospital in-patients, patients with skin diseases	Subjects undressed in a cubicle during a 2-minutes period	Air sampling through slit samplers of a cubicle	0.25 – 100 cfu/ft^3/2min i.e. 1.3x10^{-4} – 5x10^{-2} cfu/cm^2/min
Noble, 1962 [20]	4-year environmental surveillance study in 3 male surgical wards	3,675 patients were admitted during the study period. 1488	Varied ward activities including disturbance of bedside curtains, wound	Air sampling through slit	0 – 3 cfu/ft^3/min i.e. 0 – 3.2x10^{-3} cfu/cm^2/min

		(40%) were *S.aureus* nasal carriers on admission.	dressing, bed making, patient dressing and undressing.	samplers for 2 hours in the wards	
Williams, 1967 [13]	20-month environmental surveillance study in a 14-bed surgical ward divided into 4 rooms, and a shorter study in a 22-bed open ward	307 patients were admitted.	Varied ward activities	Setting plates in the 4 rooms and in 4 corners of the open ward	0 – 700 cfu/ft^2/hr i.e. 0 – $1.3x10^{-2}$ cfu/cm^2/min
Hill, 1974 [9]	Experimental study	615 laboratory technicians, doctors and nurses: 238 males & 377 females.	Subjects moved arms and legs in a defined manner at a constant rate.	Air sampling in a special test chamber for 2 minutes	0-2800 cfu/100ft^3/2min i.e. 0 – $1.5x10^{-2}$ cfu/cm^2/min
Gehanno, 2009 [14]	Environmental sampling study in hospital wards	20 patients with staphylococcal pneumonia and 4 cystic fibrosis patients who were colonized with MRSA. Overall, 8 had received appropriate antibiotics and 24 had received no, or ineffective antibiotics before the samplings.	Patients were in their beds with no movement.	Air sampling for 10 mins, which represents 1 m^3	1-78 cfu/m^3/10min i.e. $1x10^{-5}$ – $7.8x10^{-4}$ cfu/cm^2/min

a) The settling rate for the airborne particles carrying microorganisms is such that the number of microorganisms contained in 1 ft3 of air is approximately equal to the number that settle on 1 ft2 in 1 minute [13]. One foot (ft) is 30.5 centimetres (cm). One ft^2 is 930.25 cm^2.

3.2 *S.aureus* survives and remains viable on surfaces and hands for a long period of time

The potential of airborne particles to remain in the air or to settle on surfaces and floors is largely determined by the size or the diameter of the particles [21]. With a size of 13-17 μm skin desquamated cells, approximately all particles ultimately settle down to surfaces and floors [13, 21]. As seen in hospital environment, *S.aureus* can be found ubiquitously in various surfaces, including floors, carpets, bed linens, bed frames, over-bed tables, blood pressure cuffs, nurse call buttons, as well as on nurse stations and furniture in public areas [22, 23]. In general, surfaces are frequently referred to as one of the following two categories: porous and nonporous or textile and non-textile. We will use the former category when referring to surfaces. Despite a wide range of gross characteristics, porous material is referred to as material with pores or deep recesses where organisms may reside. Nonporous material is frequently hard with a smooth surface that does not offer crevices in which microorganism may hide.

S. aureus is known to survive in a variety of environmental niches by virtue of its adaptability and resistance to environmental stress [10, 11]. Studies showed that strains causing epidemics had more prolonged survival than non-epidemic strains [12, 13]. Some staphylococci epidemic strains may persist on surfaces for months [24-26]. An outbreak in a dermatology ward lasted for 14 months. With extensive surveillance among patients, healthcare workers and the environment, it was found that a blood pressure cuff and the patient's communal shower were positive for *S. aureus* isolates identical to the patients' isolates [25]. Initiation of infection control and housekeeping policies while ensuring negative environmental surveillance controlled the spread of the outbreak. These

initiations included changes of blood pressure cuffs between individual patients and daily cleaning of all communal areas on the ward and the shower areas. An outbreak in a male surgical ward lasted for 21 months despite emphasis on hand hygiene, isolation of affected patients and staggered closure and cleaning of ward bays. The outbreak came under control following an intervention, which included increasing the domestic cleaning time with emphasis on removal of dust and thorough cleaning of shared medical equipment [26]. The study showed indistinguishable strains between patients and the ward environment.

The prolonged survivability of *S. aureus* in the environment not only contributes to its ability to disseminate but also makes decontamination in the hospital environment both more difficult and more important. A study to investigate *S. aureus* contamination of environmental surfaces in a dermatology ward revealed a significant difference of porous and nonporous surfaces. This study showed contamination of bathtub, stretcher and chair for the shower to be as high as $100\text{-}10^5$ cfu in 900 cm^2. But following disinfection, *S. aureus* continued to be detected on porous surfaces up to 2-1600 cfu depending on disinfectant types, while none was detected on nonporous surfaces [27].

3.2.1 Parameterization for chapter V and VI models

Many studies have been performed to investigate the duration of survivability of various nosocomial pathogens in hospital, household or in experimental settings [28-34]. However, there were many differences in study designs and study conditions, and the outcome measures of these studies were not all consistent. These measures were death rate per unit time [28-31], changes of concentration or % recovery over time [32-34], or duration of days of survival [35]. For model parameters in chapter V and VI, we have

selected references with quantitative measures that allow calculation of the die-off or the inactivation rate (μ) based on the reference initial and final concentrations over time as in equation 3.1 [31].

$$\mu = \frac{log_{10}(M_0) - log_{10}(M_t)}{T_{survival}}$$

, where M_t is $M_o * 10^{-\mu t}$ (3.1)

3.2.2 Survival on porous surfaces

A study was performed to evaluate *S. aureus* survival on contaminated standardized sterile fabrics, commonly used in dental clinics. The result suggested that *S. aureus* could survive 3-7 days on surfaces including cotton/polyester fabric and paper. From our calculations, the die-off rate is 0.000632 log cfu/min for cotton/polyester fabric [14]. Another study using household soiled and clean cloths showed a die-off rate of 0.000612 cfu/min [15]. In chapter V and VI, we use a former result given it is more relevant to hospital settings.

3.2.3 Survival on nonporous surfaces

Laboratory experiments on decay rate of six different nonporous surfaces using culture and PCR methods found a much higher level of inactivation using a culture method in comparison to a quantitative PCR. From this study, we used the decay rate by culture method on plastic, which was 0.012 (log cfu/hr). We assume first order decay in a small time step of one minute; this decay rate on plastic was equated to 0.0002 log

cfu/min [16]. Another study using soiled and clean laminate surfaces showed decay rates of 0.00054 cfu/min and 0.000637 cfu/min on average, respectively [15].

3.2.4 Survival on hands and skin

A study that artificially applied nosocomial pathogens including *S. aureus* on four volunteers' fingertips found that among five different pathogens, *S. aureus* was minimally second to *Klebsiella pneumoniae* in its survivability on fingertips. The greatest loss happened in the first five minutes and was due to desiccation. Thereafter, the decline was less pronounced. Here, we use data from this second phase assuming a first order decay. The die off rate on fingertips was 0.00353 log cfu/min [17].

Despite being a commensal organism on skin, *S. aureus* survives shorter on hands compared to on surfaces. This characteristic is not unique to *S. aureus*; other nosocomial pathogens such as *Candida* species, enterococci, or *Klebsiella* also have shorter survival on hands than on surfaces [33, 36, 37].

3.3 *S. aureus* can be transferred between contacting surfaces. These include both direct contacts (hand-to-hand or hand-to-skin) and indirect contacts (hands-to-surfaces).

Direct contact refers to contact between patients and healthcare workers. Indirect contact refers to contacts between healthcare workers or patients and environmental surfaces. In chapter V and VI, there is another contact when patients or healthcare workers touch their noses with their fingertips. This contact can leads to more contamination to the hands or self-inoculation in the nose of the patients or healthcare workers. After each contact microorganisms can be transferred between the two contacting surfaces [38]. Several factors can influence the microbial transfer between surfaces. These include the nature of the environmental surfaces, moisture of surfaces,

temperature and relative humidity in the air, whether contact was with or without pressure, the amount of bacteria on both contact surfaces as well as the bacterial species [38, 39].

While many factors may determine microbial transfer between surfaces for each contact, the frequency of the contact is also important in determining how much cumulated exposure dose an individual received from each route. A prospective trained-observer study in a 12-bed adult intensive care unit was conducted to determine the contact rates between healthcare workers and patients and used these to estimate the time needed for hand hygiene [40]. Direct contact was defined as healthcare workers' contacts with intact skin, wound, body fluids and intravascular device. Indirect contacts were contacts with immediate patient's environments such as contact with medical equipment, handling patient case notes, or touching equipment within bed space. The study showed that healthcare workers who cared for more than one patient during their shifts made, on average, 22 direct and 107 indirect contacts without adequate hand hygiene per patient per day [40]. Each patient was contacted directly 159 times and indirectly 191 times by many healthcare providers. Observed post-contact hand hygiene rates were 43% for direct contacts and 12% for indirect contacts [40]. As seen in this study contacts with surfaces were more frequent than direct person-to-person contacts, which is likely similar to everyday living. Nevertheless, this more frequent indirect exposure was less likely to be followed with hand hygiene. This finding raises a question if touching these contaminated surfaces leads to higher hands contamination among healthcare workers.

3.3.1 Parameterization for chapter V and VI models

To compare the potential impact of these different contacts, a measure of the fraction of the organisms on one surface that is transferred to another contacting surface is used. This fraction is called transfer efficiency, which varied greatly depending on the tested surfaces and materials such as dishcloths, sponges, ground beef, carrot, stainless steel, and phone receivers [38, 39]. For chapter V and VI, there are transfer efficiencies for surfaces (porous and nonporous surfaces), hands and noses.

3.3.2 Transfer from hands to surfaces and from surfaces to hands

An earlier study in 1990 investigated the extent of which survival of organisms on cloths and laminate surfaces may be associated with cross-contamination of the hands. The study included 5 different organisms including *S. aureus* and the transfer was tested at time 0, 1, 2, and 24 hours after the contamination of surfaces. The results showed that organisms were transferred more efficiently from laminate surfaces than from cloths. The transfer efficiency from contaminated laminate surfaces to hands was the highest (43.5%) at one hour after contamination and decreased subsequently. The transfer efficiency from contaminated clothes to hands was also the highest (5.1%) at one hour after contamination, although there appeared to be regrowth of *S. aureus* at 24 hour. Regrowth of the residual survival led to increase in transfer efficiency. While this study was informative, the reported measures were not all quantitative in nature.

A more recent study in 2001 proposed to develop a quantitative protocol for assessing the transfer of bacteria [38]. The study evaluated transfer of *S. aureus* from two types of fabric (100% cotton and 50-50% cotton-polyester) to fingerpads under three conditions (dry, moist, re-moist) when transfer was tested with or without friction. The results showed the higher levels of transfer between moist donors and/or recipients

surfaces as well as higher transfer when friction was applied [38]. While this study allows quantitative assessment and comparison of various transfer conditions, certain basic differences make direct comparison of the transfer efficiency levels difficult [34, 38]. For example, in the 1990 study, the contact time was 30 seconds, which is three times longer than in this study, and contact surface area was 2 fingertips, compared to 0.5 cm^2 in this study. These differences may partly contribute to higher transfer efficiency in the earlier study.

To determine the transfer efficiency of microorganisms from surfaces to hands and from fingertips to lower lip, a 2002 study was conducted using a different protocol than previously described [39]. The study found a significant difference in transfer efficiency between porous and nonporous surfaces. Efficiency for nonporous surfaces was in the range of 28% to 66%, while for porous it was <1%.

3.3.3 Transfer from fingertip to nose and from nose to fingertip

To our knowledge, there have been no studies on transfer efficiency from hand to nose as of now. However, bacterial transfer efficiency from fingertip to lower lip was studied and was in the range of 34% to 41% [39]. In the models in chapters V and VI, we assume transfer efficiency from hand to nose to be less than transfer efficiency from fingertip to lip due to less direct contact of the fingertip to the anterior nares, where *S.aureus* resides. We assume a lower efficiency of 20%.

3.3.4 Transfer from hand to hand

There is no study on transfer efficiency from hand to hand. We assume the efficiency from hand to hand to be similar to from fingertip to lip [39].

3.4 *S. aureus* in the environment can lead to infection.

S. aureus in the environment has been linked as the source of infections in epidemic, sporadic and endemic hospital settings. Several MRSA outbreaks were shown to be associated with environmental contamination. These outbreaks required extensive additional decontamination interventions [41-44]. A systematic review of 1,022 outbreaks from 1966 to 2002 showed that *S. aureus* was among the most common causes representing 15% of all nosocomial outbreaks, compared to 13 other nosocomial pathogens, which each represented from 2 to 9% of the outbreaks [45]. Of all the outbreaks, the sources were the patients (25.7%), medical equipment or devices (11.9%), the environment (11.6%), the staff (10.9%), and contaminated drugs or food or care equipment (2.9 %). In 37% of the outbreaks, the authors were not able to identify the sources.

In endemic settings, the risk associated with environmental sources has been examined indirectly by assessing the MRSA status of the prior room occupants or roommates. A 20-month retrospective cohort study of patients admitted to 8 intensive care units, which performed routine and weekly screening for MRSA, showed that patients admitted to a room that was previously occupied by MRSA patients had increased odds of MRSA acquisition, compared to patients whose prior room occupants were MRSA negative [46]. A retrospective cohort study of a 472-bed acute-care teaching hospital showed that roommates of patients with MRSA were at significant risk for becoming colonized. This study followed 198 roommates of patients who had unrecognized MRSA colonization between 1996 and 2004. Subsequently, twenty-five patients (12.6%) acquired MRSA, all with strains indistinuguishable by pulsed-field gel electrophoresis from those of their roommates [47]. While these data were suggestive of

risks due to environmental factors, they did not have laboratory confirmation of environmental sources.

A 1-year prospective study was conducted to assess the effect of additional cleaner in a surgical ward and the environmental contamination and clinical outcome of MRSA infections [48]. The study assigned an additional cleaner into two matched wards with each ward receiving enhanced cleaning for six months in a crossover design. Clinical and environmental surveillances of hand-touch sites were monitored. The study showed that enhanced cleaning was associated with a 32.5% reduction in contamination levels and 26.6% reduction in new MRSA infections when wards received enhanced cleaning. Using pulse field gel electrophoresis, the study was able to identify indistinguishable MRSA strains first isolated in the environment, which later caused infections in patients, as well as isolated from the patients that later found in the environment [48].

In general, determining sources of sporadic cases can be rather challenging. A unique report in 1980 of a 6-year surveillance of a single individual revealed how much impact one disperser could cause in both epidemic and sporadic settings [49]. A staphylococcal disperser employed as an operating room technician was found to be the source of 11 cases of wound sepsis over a three-year period. Using a phage-typing technique, the staphylococcal strains from technician's nasal swabs, his aerial dispersal test, infected patients, and the operating room air samples were indistinguishable. Several attempts of various intranasal and systemic antibiotic regimens were given to control his skin dispersal. Subsequently, the dispersal was controlled by daily washing with chlorhexidine detergent. During the following 2 years when he remained on duty and

continued his chlorhexidine baths, there was only one case of wound sepsis attributed to the technician. In retrospect, however, it was later realized that the technician may have been the source of sporadic cases of wound infections over another 3 years. During this time he stopped the baths and ceased working in the operating room to take up another job within the hospital. In addition, his weekly skin dispersal surveillance was stopped [49].

Collectively, these studies revealed that exposure through the environment can pose a risk of *S. aureus* acquisition. To explicitly quantify and understand this risk due to environmental exposures, based on the EITS framework we would first need to perform a quantitative assessment of the exposure dose, and a qualitative assessment of the exposure patterns of the susceptible individuals. Then we can incorporate the dose-response relationship to analyze the risk based on the environmental exposure dose and route. Thus far, there have been several experimental dose-response studies in animals such as rabbit and mice models, in newborns, as well as in adult volunteers where the outcome measures were either infection or colonization [50-56].

3.5 Summary

There is substantial evidence supporting environmental mediation of *S.aureus* transmission. *S. aureus* is a greatly adaptable commensal organism that can live in the nose, on the skin and at numerous other parts of the body. Colonized or infected individuals can shed *S.aureus* continuously and sometimes profusely into air via contaminated skin scales. These aerially dispersed skin scales later deposit on surfaces, floors, or on patients. The contaminated surfaces may serve as a contamination sources to healthcare workers and patients, when they touch these surfaces. Contaminated hands of

healthcare workers may also subsequently transfer *S. aureus* to susceptible patients. These literature reviews apply to both methicillin-sensitive (MSSA) and methicillin-resistant *S. aureus* (MRSA).

3.6 Dissertation goal and outline

The overall goal of this dissertation is to provide further insight of the role of the contaminated environment in transmission of methicillin-resistant *S. aureus* in hospital settings. To do so, three studies were conducted as presented in chapters IV,V, and VI in this dissertation. The objectives and brief introductions for each chapter are as follows.

3.6.1 Chapter IV

The objectives of this chapter are i) to examine the MRSA acquisition risk associated with the presence of MRSA positive patients in the intensive care unit (ICU) among susceptible patients admitted to the same ICU, and ii) to examine the MRSA acquisition risk associated with MRSA status of the previous room occupants and the room vacant time prior to patient's admission. We use Cox proportional hazard regression model analysis. The dataset came from the 20-bed surgical intensive care unit (SICU) at the University of Michigan Health System, a 930-bed tertiary care university hospital. This was part of a hospital targeted active surveillance program from October 1, 2006 to June 15, 2008. This program included nasal swab cultures of all patients within 2 days of admission, weekly and at discharge.

3.6.2 Chapter V

The objectives of this chapter are to determine the effect of MRSA continual shedding on i) the direct and indirect exposure patterns of nurses and the uncolonized

patient, and ii) the surface contamination levels following the decontamination interventions. The two interventions are daily surface decontamination and decontamination by wiping after each nurse touches the nonporous surfaces. We construct and analyze an ordinary differential equation based model representing two hypothetical hospital rooms. The model describes MRSA fate and transport between (1) two patients, a colonized patient and an uncolonized patient, who are in two separate hospital rooms, (2) porous and nonporous environmental surfaces in each room, and (3) nurses.

3.6.3 Chapter VI

The objectives of this chapter are to examine the effects of hand hygiene compliance at the entry and exit of a patient's room to the exposure to the uncolonized patient, 2) to examine the impact of the contaminated environmental levels to hand hygiene compliance effect. For this chapter we construct and analyze a stochastic agent based model with the same structure of two hypothetical hospital rooms as in chapter V.

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CHAPTER IV

Colonization Pressure as a Risk Factor for Methicillin-Resistant *Staphylococcus aureus* Acquisition in a Surgical Intensive Care Unit

4.1 Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to be a leading cause of healthcare-associated infections [1-3] . The route of transmission is generally accepted as patient-to-patient from contaminated healthcare workers' hands [4] . While hands are transmission vectors, the major reservoir for contamination is from colonized or infected individuals [5]. These patients can transfer the pathogen directly to healthcare workers following skin-to-skin contact [4], as well as shed the pathogens on their desquamated skin cells onto the environment, resulting in environmental contamination [6, 7]. Clean hands then become contaminated by touching the contaminated surfaces [8, 9].

The presence of colonized or infected patients is known to affect acquisition risks of other patients [10, 11]. The measure used to quantify the proportion of patients who are MRSA–positive who share the same general ward or intensive care unit with others in a given time is called "colonization pressure". This measure was first described in 1994 and has since been recognized as a risk factor for nosocomial infections [10, 12].

Colonization pressure is a measure of both exposure magnitude as well as exposure time. A systematic review of studies using colonization pressure showed that there have been various definitions of colonization pressure calculated over various lengths of time (one day, three days, one week, and one month) [10]. The inconsistent definitions impaired the ability to assess the actual exposure time or when the MRSA-positive patient affects other individuals at risk. Given the continual shedding process of MRSA-positive patients and the rigorous daily surface decontamination regime in hospitals, we will examine whether a more recent exposure, such as a day before acquisition, may be more relevant and a better predictor than a longer exposure time.

In addition to sharing wards or ICUs with MRSA-positive patients, sharing rooms with MRSA-positive patients also increased acquisition risk. A 20-month retrospective cohort study of patients admitted to 8 intensive care units, which performed routine and weekly screening for MRSA showed that patients admitted to a room that was previously occupied by MRSA patients had increased odds of MRSA acquisition, compared to patients whose prior room occupants were MRSA-negative [13]. Another retrospective cohort study of a 472-bed acute-care teaching hospital showed that roommates of patients with MRSA were at significant risk for becoming colonized. This study followed 198 roommates of patients who had unrecognized MRSA colonization between 1996 and 2004. Subsequently, twenty-five patients (12.6%) acquired MRSA, all with strains indistinuguishable by pulsed-field gel electrophoresis from those of their roommates [14]. These studies showed that the MRSA status of the room occupants' either previous room occupant or current roommates is an important risk factor in MRSA acquisition.

This study aims to examine the relationship of MRSA acquisition risk and the environmental exposure to patients. These environmental exposures include the daily preacquisition colonization pressure in an intensive care unit, the prior room occupant MRSA status, and the vacant room time between patient admissions.

4.2 Methods

4.2.1 Patients and settings

We conducted a prospective cohort study of patients admitted to a 20-bed surgical intensive care unit (SICU). The SICU was an adult critical care unit where patients came from major general surgery, trauma, respiratory or multiple organ failure. The study was a part of the MRSA active surveillance program at a 930-bed tertiary care university hospital from October 1, 2006 to June 15, 2008. The program included nasal swab cultures of all patients within 2 days of admission, weekly and at discharge. Nasal swab specimens were obtained by SICU nurses. These specimens were then inoculated on selective chromogenic agar (MRSASelect: Bio-Rad Laboratories Inc., Hercules, CA) and incubated at 35°C in room air for 24 hours. Results were reported at the end of the 24-hour incubation period. MRSA-positive patients were placed under contact precautions, where healthcare workers had mandatory gowns and gloves and instructions for strict hand hygiene. All rooms were cleaned daily and upon discharge.

4.2.2 Data

The nasal swab culture data was collected as part of the surveillance program. Admission cultures were taken within the first two days of SICU arrival. Then, weekly cultures and cultures at the time of discharge were taken. All clinical specimens from these patients that were positive for MRSA during the study period were also recorded.

The following data were extracted from the hospital data warehouse: age, gender, history of hospitalization in the previous year, diagnosis, date and time of admissions and discharges, and patient room number at any given day during SICU admissions. Acute Physiology and Chronic Health Evaluation (APACHE) III scores were collected from the clinical information and decision support service. This study was approved by the Institutional Review Board of the University of Michigan Health System.

4.2.3 Statistical analysis

The primary outcome was MRSA acquisition among at-risk patients who had more than one culture and whose first culture was negative. MRSA acquisition is defined when subsequent nasal swab or clinical specimen became MRSA positive. We compared environmental exposures and patient characteristics between those who acquired and those who did not acquire MRSA using χ^2 and student's *t*-test for categorical and continuous variables, respectively. The environmental exposures are grouped into i) SICU variables, which represent the contextual exposure during the SICU stay, and ii) room variables, which represent the factors related to patients' rooms.

The SICU variables included daily colonization pressure, bed occupancy, number of admissions and discharges of the previous day and previous week of acquisition, and nurse to patient ratio. Colonization pressure measures both 1) the exposure magnitude, which is the number of MRSA-positive patients, and 2) the exposure time. We assumed that the acquisition day is the same day as the detection of positive culture. Colonization pressure (CP_d) was defined daily as the fraction of all patients in the ICU who were MRSA colonized and/or infected, expressed as

$$CP_d = \frac{P_d}{T_d} \qquad \text{,where } d \in [0,7]$$

With the day $d = 0$ as the first day of positive culture for MRSA-positive patients or the last day of swabs for the MRSA-negative patients, P_d is the number of MRSA-positive patients who were present on day d prior to day 0. T_d is the total number of patients in the SICU on the d day prior to day 0. For example, CP_1 is the colonization pressure on the day prior to the acquisition day. Additionally, we categorized colonization pressure on the day prior to the acquisition day into 4 groups according to the number of positive patients present on that day. CP_1 categories 1, 2, 3, and 4 referred to 0, 1, 2-3 and 4-5 MRSA-positive patients, respectively.

The room variables were prior room occupant status and duration of vacant room time between admissions. Analysis of Variance (ANOVA) was used to compare duration of prior vacant room time among at-risk patients who remained negative, patients who acquired early and had positive second swabs, and patients who acquired later and had positive subsequent swabs.

The patient's information were age, gender, APACHE score, history of previous year hospitalization, length of stay in the hospital before SICU admission (preICU LOS), length of stay in SICU (ICU-LOS) and length of stay in the hospital after SICU discharge (post ICU-LOS). Correlation analysis among host factors was examined. The prediction of exposure by host factors was checked using linear regression analysis with the number of MRSA-positive patients (i.e. the exposure) as the dependent variable. A comparison of host factors between patients with a history of previous hospitalization and those without was assessed using student's *t-test*. Equality of variances was checked. For variables whose variances were unequal, the Satterthwaite method of *t*-test was used.

We used Cox proportional hazards regression models in which the number of days until acquisition of MRSA or the number of days until the last negative culture was the dependent variable. In the univariate analysis, we included the independent variables as follows: the 4 categories of SICU colonization pressure on the day prior to the acquisition, the prior room occupant status, the prior vacant room time, and host factors including previous hospitalization, APACHE score, pre-ICU, ICU and post-ICU length of stay.

According to our diagram of the environmental and healthcare worker's hands mediated acquisition (Figure 4.1), two potential sources of confounders are the room environment contamination factors and host factors. The diagram is described in more detail in Appendix B. We performed a multivariate analysis to assess the adjusted acquisition hazard due to colonization pressure by controlling for the room factor in model 1, for the host factor in model 2, and for the combined room and host factors in model 3. To avoid over-fitting the model since the number of outcomes is small, we selected only a room factor and a host factor (see Appendix B). Interactions between covariates, and between covariates and time were assessed and retained if significant at the 5% level. A proportional hazard assumption was checked by Kaplan-Meier curves and by including the interactions of predictor covariates and the time. A finding of parallel K-M curves and insignificant time-dependent covariates would support proportionality assumption. All analyses were performed using SAS software, version 9.1 (SAS Institute, Cary NC).

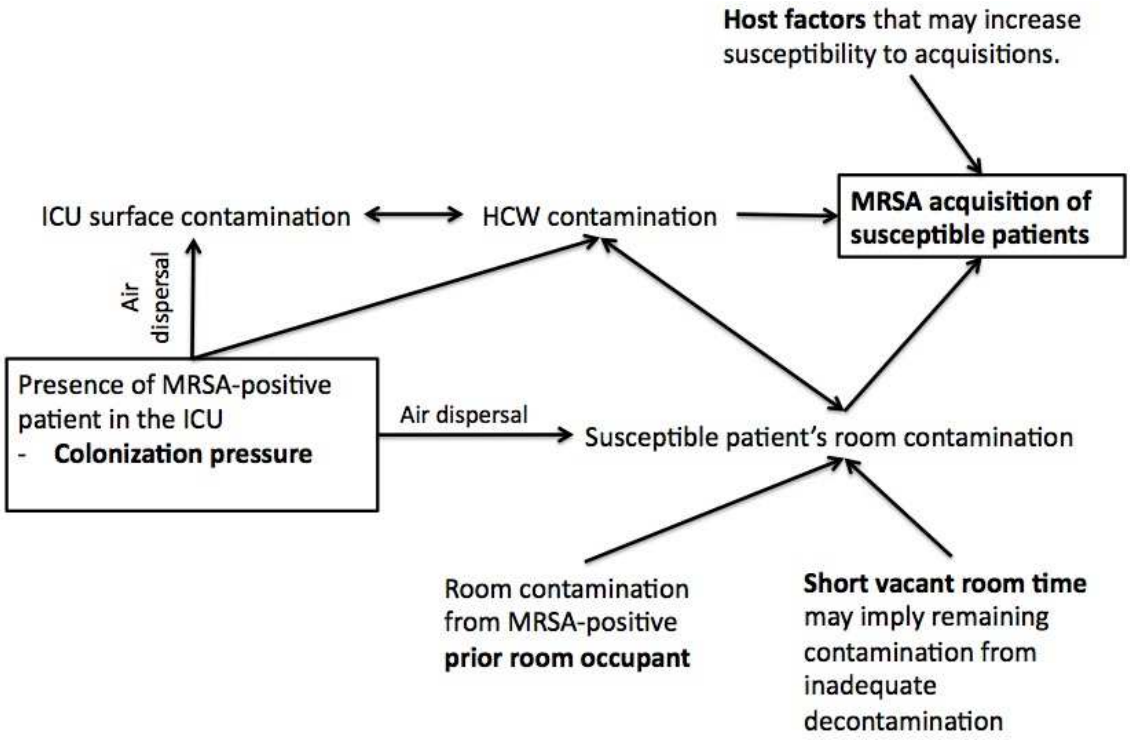

Figure 4.1: Environmental and hand-mediated acquisition diagram. This diagram provides the relationships of the variables in the study. The arrows represent a direct effect of the tail variable on the head variable. The double arrow indicates bidirectional effects. The exposure of interest in this study is the presence of MRSA-positive patient in the surgical intensive care unit (SICU). The outcome is MRSA acquisition during the SICU stay.

4.3 Results

Of the total 2,038 SICU admissions, 1,817 (89%) participated in the surveillance program. Among these participants, 1,779 had their first culture performed within the first 2 days of SICU admission, and 120 (6.7%) were identified to have positive MRSA.

Of 1,817 admissions, a total of 524 admissions had more than one culture taken. The other 1,293 (71%) had only one culture taken during their SICU stay. Of 524, there were 471 patients who had negative first cultures. These compose our prospectively followed cohort referred to earlier.

Table 4.1 compares characteristics of the 24 (5.1%) patients who acquired MRSA after admission to the characteristics of the 447 patients who did not. Patients who acquired MRSA were exposed to higher colonization pressure compared to those who did not acquire MRSA, particularly on the day before the acquisition (7.94 % versus 5.49 %, p=0.15). However, this difference decreased and disappeared on day 4-7 before the acquisition.

In view of the exposure in patients' rooms, patients who acquired MRSA were admitted to rooms that were vacant for a significantly shorter duration than the rooms of those who did not acquire MRSA (0.56 versus 0.94 day, p= 0.01). Even though the difference is less than half a day, the duration distribution barely overlapped. We further examined the relationship between 16 patients who were found positive from their second swabs, 8 patients who were positive in their subsequent swabs and the remaining negative patients. We found that patients with positive second swabs had the shortest vacant room time. Their vacant room time was significantly shorter than patients who remained negative (0.53 vs 0.94, p=0.007).

Regarding host factors, we found no differences in age, gender and APACHE score between patients who acquired and who did not acquire MRSA. Patients who acquired MRSA were more likely to have history of hospitalization in the previous year, have longer pre-ICU, ICU and post-ICU length of stay, although only post-ICU length of stay demonstrated a statistically significant difference. Patients with a history of previous hospitalizations were more likely to have higher colonization pressures and higher APACHE scores, compared to those without. Since patients with a history of previous year hospitalization tended to have higher colonization pressures and also had higher

hazards of MRSA acquisition, there was likely a confounder in the relationship between colonization pressure and the acquisition hazard. We included the previous hospitalization history in the subsequent multivariate analysis.

Table 4.1: Comparison of variables related to patients who acquired MRSA and those who did not acquire MRSA. These 471 patients were patients admitted to 20-bed Surgical Intensive Care Unit between October 1, 2006 and June 15, 2008, and participated in the MRSA Nasal Colonization Active Surveillance Program. They were patients who had more than one culture taken and their first cultures were negative. Of 471 patients, 24 were later found to have positive MRSA.

Variables Mean (95%CI)[a]	**Patients who did not acquire MRSA (n = 447)**	**Patients who acquired MRSA (n=24)**	**P value**
Age	57.14 (55.60 – 58.69)	54.21 (45.61 – 62.81)	0.40
Gender (% male)[b]	55	62.50	0.47
History of hospitalization in previous year [b]	32.70	50	0.08
APACHE score [c]	61.50 (59.10-63.80)	65.50 (56.30 - 74.60)	0.44
Pre-ICU length of stay (days)	7.41 (6.27 – 8.54)	11.50 (5.03 – 17.97)	0.11
ICU length of stay (days)	9.86 (9.00 – 10.72)	14.87 (9.56 – 20.19)	0.06
Post-ICU length of stay (days)	8.96 (7.71 – 10.21)	21.17 (9.62 – 32.71)	0.04
Length of hospitalization (days)	24.22 (22.03 – 26.41)	45.54 (29.38 – 61.70)	0.01
Prior room occupant status (% positive MRSA status)[b]	7.75	9.09	0.82
Duration of prior vacant room time (days)	0.94 (0.76 - 1.12)	0.56 (0.31 – 0.82)	0.02
Bed occupancy (%)	93.35 (92.34 – 94.37)	90 (84.87 – 95.13)	0.14
Daily colonization pressure [d]			
CP_1	5.49 (4.96 – 6.01)	7.94 (4.56 – 11.31)	0.15

CP_2	5.39 (4.86 – 5.91)	6.22 (3.61 – 8.82)	0.48
CP_3	5.64 (5.11 – 6.16)	6.29 (3.68 – 8.89)	0.59
CP_4	5.93 (5.35 – 6.51)	6.21 (3.95 – 8.46)	0.83
CP_5	5.75 (5.18 – 6.33)	5.09 (2.51 – 7.68)	0.61
CP_6	5.37 (4.8 – 5.91)	4.52 (1.98 – 7.06)	0.47
CP_7	5.50 (4.96 – 6.04)	4.75 (2.17 – 7.33)	0.54
Number of daily admissions of the day prior to the culture	2.64 (2.48 – 2.80)	2.58 (2.07 - 3.10)	0.82
Average number of daily admissions of the week prior to the culture	3.41 (3.34 – 3.46)	3.38 (3.15 – 3.63)	0.85
Number of daily discharges of the day prior to the culture	3.05 (2.87 – 3.22)	3.04 (2.45 – 3.63)	0.98
Average number of daily discharges of the week prior to the culture	3.39 (3.33 – 3.44)	3.19 (2.99 – 3.39)	0.11
Nurse to patient ratio	0.69 (0.68 – 0.70)	0.72 (0.67 -0.76)	0.12

[a] These are upper and lower 95% confidence limits of the means using *t*-test.
[b] Comparing proportions using χ^2 test.
[c] APACHE score stands for the Acute Physiology and Chronic Health Evaluation scores. It is an estimate of intensive care unit mortality based on a number of laboratory values and patient signs taking both acute and chronic disease into account.
[d] Colonization pressure (CP_d) is a daily fraction of all patients admitted in the SICU who were MRSA colonized and/or infected. CPd means the colonization pressure on the d day prior to the acquisition.

In the Cox proportional hazard univariate analysis presented in Table 4.2., the fraction of patients colonized on the day prior to the culture was a statistically significant predictor for MRSA acquisition. During the study period of 624 days, the mean and median number of MRSA-positive patients per day was one. As the number of MRSA-positive patients increased in the CP_1 categories, the acquisition hazard increased. The

history of hospitalizations in the past year also increased acquisition hazard by 2 fold, although the relationship was statistically insignificant (p=0.06).

In the multivariate analysis shown in Table 4.3., the dose response relationship of colonization pressure and MRSA acquisition remained. The adjusted hazard increased as the number of MRSA-positive patients increased. These adjusted hazards were not drastically different from the unadjusted in Table 4.2, likely due to the minimal confounding effects from the room and host factors.

In view of the room factors shown in Table 4.1., patients who acquired MRSA had 60% shorter vacant room time prior to admission, when compared to those who did not acquire MRSA (0.56 vs 0.94 days). By increasing vacant room time by one day, the acquisition hazard decreased by 26% (HR 0.74 (0.46 – 1.18), p = 0.20). After controlling for SICU colonization pressure and host factor, the hazard given one day of vacant room time decreased further to 32% (HR 0.68 (0.40 – 1.14), p =0.14).

Table 4.2: Cox proportional hazard univariate analysis of MRSA acquisition. Data was from the MRSA Nasal Colonization Active Surveillance Program of a 20-bed Surgical Intensive Care Unit from October 1, 2006 to June 15, 2008, and included 471 patients who had more than one culture taken with the first cultures being negative for MRSA. Of 471 patients, 24 patients later acquired MRSA.

Variables	Hazard Ratio (95% CI)	P value
Age	0.99 (0.97 – 1.01)	0.48
Gender	0.74 (0.32 – 1.69)	0.48
History of hospitalization in the previous year	2.17 (0.97 – 4.84)	0.06

APACHE [a]	0.99 (0.97 – 1.01)	0.27
Prior room occupant status	0.73 (0.32 – 1.68)	0.47
Prior vacant room days	0.74 (0.46 – 1.18)	0.20
Colonization pressure of the day prior to the culture (CP_1) [b,c]	1.06 (1.00 - 1.13)	0.04
Category 1: CP_1 with no positive patients (reference)		
Category 2: CP_1 with 1 positive patients	0.55 (0.17 – 1.78)	0.32
Category 3: CP_1 with 2-3 positive patients	1.11 (0.40 – 3.14)	0.84
Category 4: CP_1 with 4-5 positive patients	5.91 (1.95 – 17.86)	<0.01
Bed occupancy percentage	0.98 (0.95 – 1.02)	0.31
Number of daily admissions of the day prior to the culture	1.01 (0.79 – 1.29)	0.96
Average number of daily admissions of the week prior to the culture	0.94 (0.44 – 2.00)	0.87
Number of daily discharges of the day prior to the culture	0.98 (0.79 – 1.21)	0.85
Average number of daily discharges of the week prior to the culture	0.59 (0.27 – 1.27)	0.18
Nurse/patient ratio	1.03 (0.98 – 1.07)	0.20

[a] APACHE score stands for the Acute Physiology and Chronic Health Evaluation scores. It is an estimate of intensive care unit mortality based on a number of laboratory values and patient signs taking both acute and chronic disease into account.

[b] Colonization pressure is a daily fraction of all patients admitted in the SICU who were MRSA colonized and/or infected.

[c] The hazard ratio presented in this line represented the effect of colonization pressure as a continuous variable. The effect of colonization pressure as categorical variables were shown in the below lines corresponding to CP_1 catergory 1, 2, 3 and 4.

Table 4.3: Cox proportional hazard multivariate analysis of MRSA acquisition. A multivariate analysis was performed to assess the adjusted acquisition hazard due to colonization pressure by controlling for the room factor in model 1, for the host factor in model 2, and for the combined room and host factors in model 3. Data was from the

MRSA Nasal Colonization Active Surveillance Program of a 20-bed Surgical Intensive Care Unit from October 1, 2006 to June 15, 2008, which included 471 patients who had more than one culture taken with the first cultures being negative for MRSA. Of 471 patients, 24 patients later acquired MRSA.

Variables	**Hazard Ratio (95% CI)**	**P value**
Model 1:		
Prior vacant room days	0.68 (0.40 – 1.14)	0.14
Colonization pressure of the day prior to the culture (CP_1) [a,b]		
Category 1: CP_1 with no positive patients (reference)		
Category 2: CP_1 with 1 positive patient	0.56 (0.17 – 1.85)	0.34
Category 3: CP_1 with 2-3 positive patients	1.17 (0.41 – 3.39)	0.76
Category 4: CP_1 with 4-5 positive patients	6.80 (2.19 – 21.11)	<0.01
Model 2:		
History of previous hospitalization	2.10 (0.94 – 4.69)	0.07
Colonization pressure of the day prior to the culture (CP_1) [a,b]		
Category 1: CP_1 with no positive patients (reference)		
Category 2: CP_1 with 1 positive patient	0.52 (0.16 – 1.70)	0.28
Category 3: CP_1 with 2-3 positive patients	1.12 (0.40 – 3.15)	0.83
Category 4: CP_1 with 4-5 positive patients	5.51 (1.81 – 16.80)	<0.01
Model 3:		
Prior vacant room days	0.69 (0.40 – 1.18)	0.18
History of previous hospitalization	2.06 (0.91 – 4.69)	0.08
Colonization pressure of the day prior to the culture (CP_1) [a,b]		

Category 1: CP_1 with no positive patients (reference)		
Category 2: CP_1 with 1 positive patient	0.54 (0.16 – 1.80)	0.32
Category 3: CP_1 with 2-3 positive patients	1.19 (0.41 – 3.44)	0.74
Category 4: CP_1 with 4-5 positive patients	6.36 (2.03 – 19.92)	<0.01

[a] Colonization pressure is a daily fraction of all patients admitted in the SICU who were MRSA colonized and/or infected.
[b] The hazard ratio presented in this line represented the effect of colonization pressure as a continuous variables. The effect of colonization pressure as categorical variables were shown in below lines corresponding to CP_1 catergory 1, 2, 3 and 4.

4.4 Discussion

Our study supported environmental factors as MRSA acquisition risks. We demonstrated the two exposure aspects of SICU colonization pressure: exposure time and exposure magnitude. Firstly, we found that a higher hazard of SICU exposure to MRSA-positive patients was seen with a more recent exposure, which was in the prior day, compared to with a longer exposure. Secondly, we showed that the presence of greater number of MRSA-positive patients in the SICU led to the greater hazard of acquisition among other patients. When there were more than 3 MRSA-positive patients in the SICU, the acquisition hazard significantly increased by 6-8 fold. In addition to the SICU factor, we found that patients who acquired MRSA were more likely to be admitted to rooms that were vacant for a shorter duration between admissions. Increasing the vacant room time between patient admissions by 1 day decreased the acquisition hazard by 20-30%.

While the concept that sharing the same physical space with MRSA positive patients can increase acquisition risk among other patients is widely accepted, the underlying mechanism is not well described. MRSA-positive patients may contaminate their environment as well as individuals who make direct contact with them [7, 15]. This

contamination may likely be the exposure source to other susceptible patients. A recent 12-month prospective study that included both active MRSA screening and environmental sampling in a 23-bed emergency ward and a 7-bed respiratory intensive care unit showed that the weekly colonization pressure adjusted by degree of environmenal contamination was a better indicator for predicting MRSA acquisition than unadjusted colonization pressure [16].

In regard to the exposure time, the duration that MRSA-positive patients affect risk of others is unclear. As seen in a systematic review of measurement of colonization pressure in MRSA, vancomycin resistant enterococci (VRE) and *Clostridium difficile* acquisition, the definition of colonization pressure varied considerably over periods of varying lengths from a day to a month [10]. Table 4.4 shows previous studies of MRSA acquisition that used colonization pressure in their analysis. Some studies used colonization pressure as correlation measures with MRSA acquisition rates [11, 16-18]. Others used preacquisition colonization pressure as predictors of MRSA acquisitions [19-22].

We found colonization pressure on the day prior to the detection of positive MRSA culture to be a more relevant predictor of acquisition than the colonization pressure earlier. This finding provided support that the exposure over a more recent interval conveys greater transmission risk when compared to an earlier interval. When comparing the magnitude of exposure in the day prior to acquisition, we found that a greater number of positive patients led to a higher acquisition hazard. Our findings might explain the discrepancy of results in previous studies, which used a varied period of time and likely had a varied magnitude of colonization pressure.

In our study we did not find an association between colonization pressure taken from the preceding week and the MRSA acquisition (data not shown). This result agreed with a previous retrospective study that also used colonization pressure from the week preceding acquisition, and did not find an association with MRSA acquisition [23]. This retrospective study instead found associations of acquisition with reduced number of trained nurses and hygiene failures of hand-touch site environmental surfaces [23]. Conversely, two other ICU studies using weekly colonization pressure found a statistically significant association with MRSA acquisition [19, 21]. However, we noted that the colonization pressure in these two studies were much larger than in our study. The colonization pressure in the 2005 and 2000 studies were in the range of 5 to 40% and <10 to <40%, respectively, while in our study the preacquisition colonization pressure measured in the preceding week was in the range of 3.7 to 7.8%.

In regards to room environment, we found no significant association between MRSA acquisition and the prior room occupant's MRSA status. Instead, we found that the vacant room time between admissions was 60% shorter in patients who acquired compared to those who did not. These findings differed from a previous 20-month 8-ICU cohort study which found increased odds of MRSA acquisition among patients whose prior room occupants were MRSA-positive, but did not find an association of vacant room time between patient admissions and MRSA acquisition [13]. However, we noted that the bed occupancy of this previous study was very high and likely impacted this lack of association. Their median vacant room day of all patients was zero, while the mean vacant room day for patients who acquired and who did not acquire MRSA were 0.5 and 0.6 day, respectively.

Our findings are consistent with the previous observations that overcrowding and heavy workload, measured as high bed occupancy and turnover rates, correlate with MRSA acquisitions [24]. In the Netherlands, where there is a national policy of search and destroy regarding MRSA, the prevalence of MRSA among clinical *S.aureus* isolates as well as among those without risk factors is well below 1%, which is among the lowest in the world. In contrast in our study where the bed occupancy was 85-95%, the bed occupancy rate in the Netherlands is approximately 65% [25, 26].

While our study supported that the environmental risk for MRSA acquisition existed, the small number of acquisitions limited our inference. Thus, the definitive inference about the recent exposure time relationship with the MRSA acquisition cannot be finalized. Nevertheless, our finding provided support for pursuing further investigation in these exposure time relationships, as well as assessment of environmental contamination with MRSA.

In summary, our study demonstrated that the patient environment is an important risk factor in MRSA acquisition. Recent exposure to SICU where there were MRSA-positive patients increased acquisition hazards among other patients. As numbers of MRSA-positive patients increased, the hazards increased. In regards to SICU room admission, longer vacant room time between admissions was associated with lower acquisition risks. Although our study had several limitations, we hope our observations of these associations will stimulate more careful attention to this issue in other studies.

Table 4.4: Characteristics of previous studies of MRSA acquisitions that included colonization pressure in their analysis. This table was modified from the 2011 systematic review by Ajao et al [10].

First author, year	Country	Study design	Setting	Laboratory sample	Colonization pressure definition	Analysis, finding[a]	Other factors[b]
Merrer 2000 [19]	France	Prospective 26-month cohort	12-bed medical ICU	1) surveillance samples from nares, axilla and perineum on admissions and weekly, and 2) clinical samples	Number of (MRSA imported + MRSA nosocomial) patient-days in the week x100/Total number of patient-days in the week	In multivariate linear regression analysis, CP was the only significant predictor, p=0.0002	
Muller 2003 [20]	France, Spain	Prospective 1-year cohort	59 hospital units (35 medical, 21 surgical, 3 ICU)	1) surveillance nasal samples on admissions, and 2) clinical samples	The ratio of the number of MRSA-positive patient-days to the total number of patient-days for each hospital unit	In multivariate Poisson-regression analysis, RR for CP = 1.02 (1.02-1.03), p<0.001.	Type of hospital unit, and types of antibiotics exposure during ICU stay
Ho 2003 [17]	Hong Kong	Prospective 4-month cohort	10 ICUs	1) surveillance samples from nares, throat and rectum on admissions and at discharge, and 2) clinical samples	Proportion of MRSA-positive patients at the ICU entry	There was significant correlation between each ICU CP and acquisition rates.	By logistic regression analysis, illness severity, length of stay,

							number of antibiotics use, use of medical device increased acquisition risk.
Lucet 2005 [21]	France	Prospective 7-year cohort	3 ICUs	1) surveillance nasal samples on admission and weekly, and 2) clinical samples	The ratio of case-days over patient-days in the unit during the week preceding either MRSA acquisition or discharge	In multivariate logistic regression analysis, adjusted OR = 1.019, $p<0.0001$.	Age, ICU length of stay, illness severity.
Cepeda 2005 [22]	UK	Prospective 1-year cohort	3 ICUs from 2 hospitals	1) surveillance samples from nares and groin on admission, weekly and at discharge, and 2) clinical samples	For every patient, CP was derived from the number of other MRSA-positive patients present on a given day	In Cox proportional hazard analysis, HR = 1.19 (95% CI, 0.86-1.65)	
Dancer 2006 [23]	Scotland	Retrospective 5-month study	8-bed ICU	1) surveillance samples on admission and alternate days thereafter, 2) blood samples and 3)	The total number of MRSA-positive patient-days per week divided by the total number of patients in the ICU for that week	No association of CP and MRSA acquisition. No reported detailed	Reduced number of trained nurses and hand-touch environment

				weekly environmental surface samples	per 1000 patient-days	analysis.	al surface hygiene failures
Williams 2009 [11]	Canada	Prospective 2-year cohort	36-bed general medical unit	1) surveillance samples from nares, perineum, any lesions, exit sites of any indwelling device, 2) clinical samples	The monthly number of MRSA patient-days x 100/total number of patient-days	The relative risk of MRSA acquisition increased as CP increased above the median (RR,7.6; 95%CI, 1.1-52.6; p=0.008)	
Bloemendal 2009 [18]	The Netherland, France, Portugal, Spain, Italy, Greece	Prospective cohort for a period of 3 months per center	6 ICUs in 6 universit y hospitals	1) surveillance samples from nares and perineum on admissions, twice weekly and at discharge, and 2) clinical samples	1) mean CP per ICU was the mean percentage of patients colonized with *S.aureus* during study period, 2) preacquisition CP was the mean percentage of patients colonized with *S.aureus* during the 3 days preceeding acquisition	1) mean CP varied greatly between centers, 2) preacquisition CP was significantly higher for patients who acquired *S.aureus* (p=0.002 for MSSA and p=0.005 for MRSA)	The number of beds per nurse and the treatment of all patients in private rooms

Wang 2011 [16]	China	Prospective 1-year study	23-bed emergency ward (EW) and 7-bed respiratory ICU (RICU)	1) surveillance samples from nares, throat, axilla, groin and any wound. For EW: on admission, every 3 days (if in the open-bay ward with a MRSA patient) otherwise weekly, for RICU: on admissions, every 2 days until discharge, 2) clinical samples, and 3) environmental surface samples	Weekly CP (WCP) $= \sum_{i=1}^{m} n_i / \sum_{j=1}^{k} n_j$ and WCP adjusted for degree of environmental contamination (WCPe) $= \sum_{i=1}^{m} n_i \theta_i / \sum_{j=1}^{k} n_j$ where n_i and m denote hospitalization days of each MRSA-positive patients and the total number of MRSA-positive patients in the week, n_j and k denote hospitalization days of each patients and total number of patients during the week, for each MRSA positive patients, θ_i denote the percentage of immediate environmental sites contaminated by MRSA.	1) by comparing the areas under the receiver operating characteristic curve, WCPe showed moderate predictor accuracy for MRSA acquisition in the subsequent weeks, $p<0.01$, 2) Spearman's correlation coefficient ($r = 0.45$ for EW, 0.51 for RICU, p0.001) showed a positive and significant correlation between WCPe and MRSA acquisition in the subsequent weeks.	

Note: MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*, ICU, intensive care unit; CP, colonization pressure; WCP, weekly colonization pressure; WCPe, weekly colonization pressure adjusted for environmental contamination degree; OR, odds ratio; HR, hazard ratio; RR, risk ratio or relative risk

[a] Finding interpretation: An OR = 1.01 means for each 1% increase of CP, the odds of MRSA acquisition increases by 1%; A HR of 1.01 means for each 1% increase of CP, the hazard of MRSA acquisition increases by 1%, A RR of 1.01 means for each 1% increase of CP , the risk of MRSA acquisition increases by 1%.

[b] Other factors that were found to be associated with MRSA acquisition

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CHAPTER V

The Effect of Continual MRSA Shedding on Exposure Patterns and Surface Contamination

5.1 Introduction

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) continue to increase in the U.S. and Europe [1-5]. Recommendations and guidelines for MRSA control originate from many professional organizations and national institutions [6-8]. In these documents, hand hygiene has indisputably been an integral part of infection-control measures, but the effect of environmental cleaning, if any, remains to be demonstrated [9].

The presence of MRSA-positive patients affect the acquisition risk of other susceptible patients bedded in the same hospital unit [10]. However, the mechanisms through which such exposure affects the acquisition are not well characterized. Individuals who are colonized or infected with MRSA can shed MRSA via contaminated skin scales, even through clothing [11, 12]. As many as 10^6 to 10^7 of these 8 to 20 μm skin particles can be dispersed from the body in 24 hours [13]. These aerial skin scales sediment onto surfaces, become airborne when mechanically disturbed, and redeposit back on surfaces again [14]. Thus, the possible exposure pathways from a MRSA-positive patient to the healthcare worker may be from direct skin-to-skin contact with MRSA-positive patients, or indirectly through the environment, such as from touching

contaminated environmental surfaces [15, 16]. Similarly, the exposure pathways to susceptible patients may be from direct skin-to-skin contact with a healthcare worker, or indirectly from touching contaminated room surfaces.

Healthcare workers (HCWs) touch room surfaces more frequently than they touch patients. In a prospective study in a 12-bed intensive care unit, the frequency of direct contact (i.e. HCWs made contact with patients), and indirect contact (i.e. HCWs made contact with patients' environment), and subsequent hand hygiene, were measured over 120 hourly periods [17]. The study showed that each patient was contacted indirectly more frequently than directly (191 vs 159 times/day, respectively). Furthermore, healthcare workers who contacted more than one patient and were thus more likely to spread the pathogens had, on average, 22 direct and 107 indirect contacts without adequate hand hygiene per patient per day.

Touching contaminated surfaces may result in contaminated hands. A study in 8 general wards and ICUs evaluated hand imprint cultures after contact with environmental surfaces in patients' rooms [16]. *S.aureus*, the most commonly identified pathogen, was found in 30% of hand imprint cultures in the randomly chosen occupied rooms, and 8% of cultures in clean rooms following terminal cleaning after patient discharge. Given the shedding of MRSA-positive patients onto surfaces, touching surfaces in MRSA-positive patients' rooms may lead to more hand contamination.

Moreover, hand hygiene compliance after indirect contact by touching surfaces was consistently less frequent than hand hygiene compliance after patient contact [17, 18]. Therefore, collectively the indirect exposure may be high-risk exposure sources to healthcare workers, who may subsequently transfer MRSA to susceptible patients. This

underlies the need to improve our understanding of exposure patterns to healthcare workers and patients.

The continuous dispersal of skin scales also means continuous environmental contamination, which presents challenges for maintaining adequate hand hygiene practices as well as ensuring adequate surface decontamination. These contaminated areas include various near-patient locations in patients' rooms such as bed linen, over-bed tables, bedrails, floors, as well as in other common areas [19]. A study reviewing the thoroughness of hygiene cleaning in healthcare settings showed that only 40% of near-patient surfaces are being cleaned in accordance with existing hospital policies [20]. Furthermore, when rooms were thoroughly cleaned following the use of hydrogen hydroxide vapor, MRSA recontamination on surfaces could occur within 24 hours after readmitting patients [21]. We hypothesize that the continual dispersal from a MRSA-positive patient, resulting in rapid recontamination of surfaces, may impair the long-term benefit of surface decontamination. Similarly, this recontamination of surfaces may also lead to recontamination of cleaned hands and impair the potential benefits of hand hygiene.

Many surface decontamination studies have focused on the efficacy of cleaning methods, while the frequency of decontamination has not received much attention [22-24]. Surface decontamination generally refers to thorough, entire-surface disinfection. Its efficacy depends on its microbicidal activity, the quantity that is used, and the contact time on surfaces [22]. At this time, there is more effort in using wiping as a means for decontamination [25, 26]. Wipes use formulations with weak and/or limited microbicidal

activity; however, the mechanical action of wiping can substantially enhance the process of decontamination [22].

In this study, we developed an MRSA fate and transport model to determine the effect of MRSA continual shedding i) on the direct and indirect exposure patterns of nurses and uncolonized patients, and ii) on the surface contamination levels following decontamination interventions. The surface decontamination interventions included daily surface decontamination, and decontamination by wiping after each nurse touches the nonporous surfaces. We also examined the effect of hand hygiene and its joint effects with two surface decontamination methods.

5.2 Methods

5.2.1 The exposure pathway model

Based on an Environmental Infection Transmission System (EITS) framework, we constructed and analyzed a deterministic compartmental model of MRSA fate and transport between two hypothetical hospital rooms including (1) MRSA shedding from a colonized patient, (2) MRSA transfer, deposition, and die-off on skin and hands and on room surfaces, (3) MRSA exposure to an uncolonized patient, and (4) two MRSA interventions: surface decontamination and hand hygiene.

This model is an ordinary differential equation-based model consisting of 9 compartments. These compartments include 1) the colonized patient (PT_c), 2) the porous surface in the colonized patient's room (P_c), 3) the nonporous surface in the colonized patient's room (NP_c), 4) the uncolonized patient (PT_u), 5) the porous surface in the uncolonized patient's room (P_u), 6) the nonporous surface in the uncolonized patient's room (NP_u), 7) the nurses (NS), 8) the uncolonized patient's nose (PT_{un}) and 9) the

nurses' noses (NS_n). Given the colonization status, the colonized patient's nose is assumed to approximate a constant MRSA concentration.

5.2.1.1 Model description

Upon admission, the colonized patient sheds onto the environment through two pathways: (1) by continuously dispersing MRSA via skin squamous cells into the air, and (2) by touching environmental surfaces with contaminated hands. MRSA in the air is assumed to instantaneously settle on environmental surfaces. While on environmental surfaces, some MRSA may naturally die off. Nurses and patients who touch these surfaces will then pick up a fraction of MRSA that survive desiccation onto their hands. This fraction varies depending on transfer efficiency and MRSA quantity on the hands and the surfaces. Both nurses and patients touch the room surfaces, which may result in either hand contamination or surface contamination. They also touch their noses, which may lead to self-inoculation when their hands are contaminated. Nurses work in eight-hour shifts; for each shift there is one nurse who visits a colonized patient's room for the first 20 minutes, an uncolonized patient's room for the next 20 minutes, and the nurses' center for the last 20 minutes where there is no touching event. The cycle repeats hourly throughout their shift. In each room visit, the nurse touches the patient and the two environmental surfaces at specified touch rates.

This model keeps tracks of changes in MRSA concentrations in each compartment. Model events are described in section 5.2.3. Model parameters are presented in Table 5.1. The literature review for parameterization was presented in Chapter III.

5.2.2 Model assumptions

1. The only MRSA source is the MRSA colonized patient. This colonized patient sheds onto environmental surfaces by aerially dispersed MRSA-contaminated skin squamous cells that deposit on surfaces and by surface touching with contaminated hands.

2. The MRSA exposure pathways to a susceptible patient are either by touching contaminated room surfaces or being touched by contaminated nurses' hands.

3. Nurses are not colonized with MRSA and do not shed MRSA. Their hands serve as vectors of the transmission process.

4. MRSA instantaneously and homogenously mixes on surfaces, skin, and hands.

5. Transfer efficiency is symmetrical. For example, in an event when a hand touches a nonporous surface, 40% of MRSA per 150 cm^2 is transferred from that hand to the nonporous surface and 40% of MRSA per 150 cm^2 from the nonporous surface is transferred to the hand.

Table 5.1: Model parameters and their values.

	Symbol	**Values**	**Reference**
SHEDDING PARAMETERS:			
Shedding (air dispersal) rate (cfu/cm^2/min)	α	0.01	[12, 27, 28]
SURVIVAL PARAMETERS:			
Die off rate on skin and hand (min^{-1})	μ_{sk}	0.00353	[29]
Die off rate on porous surface (min^{-1})	μ_p	0.000632	[30]
Die off rate on nonporous surface (min^{-1})	μ_{np}	0.0002	[31]
CONTACTS PARAMETERS:			
Rate of patient touches surfaces (min^{-1})	$\tau_{pt\text{-}sf}$	0.134	
Rate of nurse touches patient (min^{-1})	$\tau_{ns\text{-}pt}$	0.4	
Rate of nurse touches surfaces (min^{-1})	$\tau_{ns\text{-}sf}$	0.4	
Rate of touching nose (min^{-1})	τ_n	0.025	
Rate of nurse wipes nonporous surface	ω_{np}	0.4	

(min^{-1})			
TRANSFER EFFICIENCY PARAMETERS:			
Transfer efficiency from porous surface to fingertip	ρ_p	0.1	[32]
Transfer efficiency from nonporous surface to fingertip	ρ_{np}	0.4	[33]
Transfer efficiency from hand to skin	ρ_{sk}	0.35	[33]
Transfer efficiency from finger to nose	ρ_n	0.2	
SURFACE AREA PARAMETERS:			
Total exposed skin and hand surface area of patients (cm^2)	A_{pt}	2000	
Total exposed skin and hand surface area of nurses (cm^2)	A_{ns}	2000	
Total porous surface area (cm^2)	A_p	2000	
Total nonporous surface area (cm^2)	A_{np}	2000	
Nose surface area (cm^2)	A_n	4	
Hand contact surface area (cm^2)	A_h	300	
Fingertip contact surface area (cm^2)	A_f	1	
INTERVENTIONS:			
Daily surface decontamination efficacy	ε_d	0-100%	
Wiping efficacy	ε_w	0-100%	
Hand hygiene efficacy	ε_h	58%, 83%	[34, 35]

5.2.3 Model events

5.2.3.1. Shedding

The colonized patient sheds MRSA continuously onto porous and nonporous surfaces in the colonized patient's room. This shedding quantity is governed by αA_p or αA_{np}. Shedding rate is assigned at 0.01 $cfu/cm^2/min$. We assume shedding only affects the colonized patient's room surfaces. There is no MRSA aerial dispersal into the uncolonized patient's room.

5.2.3.2 Nurse visiting patient rooms

At the beginning of each hour, a nurse will first visit the colonized patient for 20 minutes and then visit the uncolonized patient for the next 20 minutes. Before and after a

visit, nurses may perform hand hygiene. While in a patient's room, nurses touch the patient and the two room surfaces at given rates in Table 5.1.

5.2.3.3 Touching

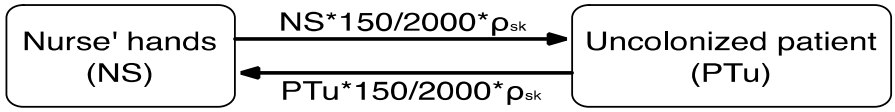
Touching or contact is one of the main events that determine the changes of pathogen concentrations in each compartment. In this model, there are i) direct contacts, where nurses touch patients, ii) indirect contacts, where either nurses or patients touch the room surfaces, and iii) self-inoculation, where nurses and patients touch their noses with a fingertip. Each type of contact is governed by contact rate as in Table 5.1.

For each contact event, there are bidirectional flows of pathogen transfers to and from the two contacting surfaces. The fraction of pathogens that is transferred from one contacting surface to another is called transfer efficiency. In this model, we assume symmetrical transfer efficiency.

To illustrate these contact mediation processes, consider an example of a direct contact event where a nurse touches an uncolonized patient as seen in Table 5.2. For each touch, there is a quantity of MRSA transferred from the nurse's hand to the patient ($NS*150/2000*\rho_{sk}$) and, as well, there is a quantity of MRSA transferred from the patient to the nurse's hand ($PT_u*150/2000*\rho_{sk}$). These MRSA quantities depend on 1) the bacterial concentrations at both contacting surfaces (i.e. NS and PT_u), 2) the contact surface area (i.e. 150 sq.cm.), 3) the total surface area (i.e. 2000 sq.cm.), and 4) transfer efficiency. The assumption of symmetrical transfer efficiency in this case means the fraction of pathogen that is transferred from the nurse to the uncolonized patient is the same fraction that is transferred from the uncolonized patient to the nurse, which is the transfer efficiency of hands to skin or skin to hands, i.e. 0.35 (ρ_{sk}).

The net quantity of pathogens transferred and the result of the contact depend on the contamination levels on contacting surfaces. In this case, nurses are the only sources of MRSA into the uncolonized patient's room. Thus, the direct contact of nurses and the uncolonized patient results in an increase in contamination of the uncolonized patient.

Table 5.2: A direct contact event between nurses' hands (NS) and the uncolonized patient (PT_u). NS represents the concentration of MRSA cfu on nurses (MRSA cfu/2000 sq.cm.). PT_u represents the concentration of MRSA cfu on the uncolonized patient (MRSA cfu/2000 sq.cm.). Contact surface area is 150 sq.cm. Transfer efficiency for the direct contact event (ρ_{sk}) is 0.35. The transfer efficiency of MRSA from nurses' hands to the uncolonized patient's skin is assumed to be the same as transfer efficiency from the uncolonized patient's skin to nurses' hands. Thus, MRSA quantity that is transferred from nurses' hands to the uncolonized patient's skin is NS*150/2000*0.35. MRSA quantity transferred from the uncolonized patient's skin to nurses' hands is PT_u*(150/2000)*0.35.

	Nurses (NS)	**Uncolonized patient (PT_u)**
Total surface area (sq.cm.)	2000	2000
Contact surface area (sq.cm.)	150	150
Transfer efficiency (ρ_{sk})	0.35	0.35
MRSA concentration per total surface area (cfu/2000 sq.cm.)	NS	PT_u
Bidirectional flows between the two contacting surfaces: 		

5.2.3.4 Natural die-off event or survivability of MRSA

MRSA on surfaces, patients, and nurses continuously decreases with fixed die-off rates depending on whether they are on the skin and hands, porous surfaces, or nonporous surfaces.

5.2.4 Model interventions

We studied the effect of three interventions separately and jointly: daily surface decontamination, surface decontamination by wiping, and hand hygiene.

5.2.4.1 Daily surface decontamination

Daily surface decontamination affects both porous and nonporous surfaces and is scheduled every 24 hours. Following each decontamination event, a fraction of MRSA will be removed depending on surface decontamination efficacy (ε_d).

5.2.4.2 Surface decontamination by wiping.

Wiping only affects nonporous surfaces. After each nonporous surface touch, nurses wipe the surface. Thus, the wiping rate is the same as the rate that nurses touch the nonporous surfaces. Following each wipe, a fraction of MRSA will be removed depending on wiping efficacy (ε_w).

5.2.4.3 Hand hygiene

Hand hygiene is scheduled before and after each nurse visit, i.e. at time 0 and 20 minutes of the hour for the colonized patient's room visit and time 21 and 40 minutes for the uncolonized patient's room visit. Following a hand-hygiene event, a fraction of MRSA will be removed depending on hand hygiene efficacy (ε_h). The two efficacy parameters depend on the hygiene methods. Hand hygiene efficacy for soap and water is 58%, and for alcohol hand gel rub is 83% [34, 35].

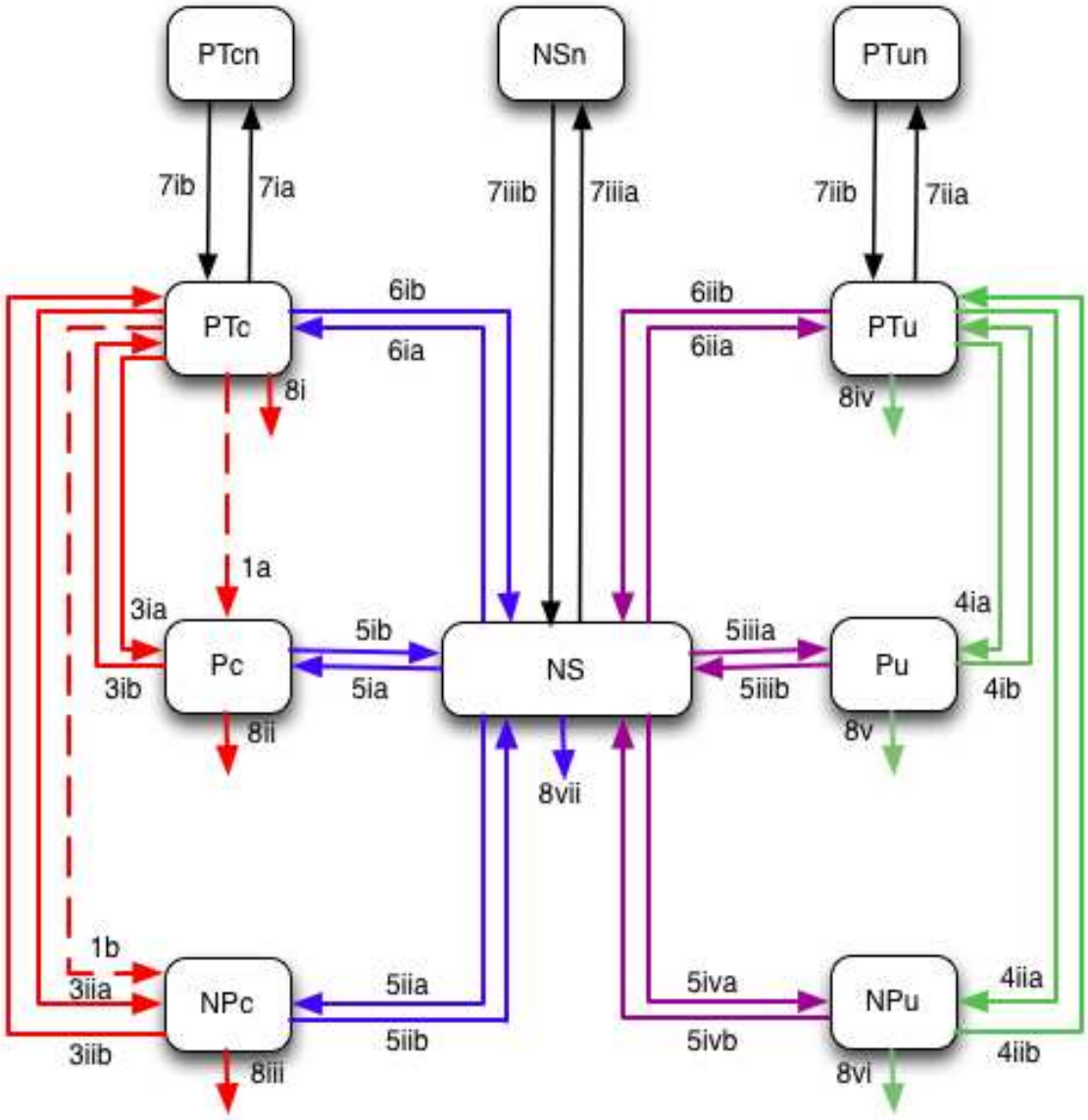


Figure 5.1: A diagram of the compartmental model with ten compartments. These are exposed skin and hands of the colonized patient (PT_c), porous surface in the colonized patient's room (P_c), nonporous surface in the colonized patient's room (NP_c), exposed skin and hand of the uncolonized patient (PT_u), porous surface in the uncolonized patient's room (P_u), nonporous surface in the uncolonized patient's room (NP_u), exposed skin and hand of the nurse (NS), the colonized patient's nose (PT_{cn}), the uncolonized patient's nose (PT_{un}), and the nurse's nose (NS_n). Solid arrows are pathogen flows due to touching events or due to the natural die off. Dashed arrows are shedding from colonized patient to the porous and nonporous surfaces in the room. Red arrows are flows within colonized patient's room that are independent of time. Green arrows are flows within uncolonized patient's room that are also independent of time. Nurses' flows are, however, time-dependent. Blue arrows indicate flows in and out of the nurse compartment during the first 20 minutes of the hour. Purple arrows indicate flows in and out of the nurse compartment during the next 20 minutes of the hour. Black arrows

indicate flows resulting from touching noses, which are time-independent. The flow numbers are row numbers correspondent to Table C.1. in Appendix C.

5.2.5 Differential equations

Figure 5.1 shows the diagram of this compartmental model. The mathematical flow descriptions between compartments are in Table C.1: Appendix C.

5.2.5.1 The colonized patient (PT_c)

We assume that the colonized patient maintains a steady MRSA concentration on the exposed skin and hands (PT_c). This balance is achieved by the gain and loss in MRSA. The colonized patient gains MRSA from the replenishment of the contaminated skin scales and from touching the nose. The replenishing rate is assumed to be the same as the dispersal rate. A concentration of MRSA in the nose (PT_{cn}) is assigned at a constant of 1000 cfu/4 cm^2. On the other hand, the colonized patient loses MRSA from natural die-off, and from pathogen flows out to surfaces and nurses after touching events. The colonized patient is touched by the nurses only during the first 20 minutes of the hour.

The change of MRSA on the skin and hand of the colonized patient (PT_c) are given by:

$$\frac{dPT_c}{dt} = \alpha A_{pt} - PT_c \frac{A_f}{A_{pt}} \rho_n \tau_n + PT_{cn} \frac{A_f}{A_n} \rho_n \tau_n - PT_c \frac{A_c}{A_{pt}} \rho_p \tau_{pt-p} + P_c \frac{A_c}{A_p} \rho_p \tau_{pt-p} - PT_c \frac{A_c}{A_{pt}} \rho_{np} \tau_{pt-np} + NP_c \frac{A_c}{A_{np}} \rho_p \tau_{pt-np} - PT_c \frac{A_c}{A_{pt}} \rho_{sk} \tau_{ns-pt} f(t) + NS \frac{A_c}{A_{ns}} \rho_{sk} \tau_{ns-pt} f(t) - PT_c \mu_{sk} \quad (5.2.1)$$

Where n ∈ Z+, and

$$f(t) = \begin{cases} 1, & t \in [n-1, n-\frac{2}{3}) \\ 0, & t \in \left[n-\frac{2}{3}, n\right) \end{cases} \quad (5.2.2)$$

The function f(t) is a time indicator function for a nurse's visit in the colonized patient's room. F(t) is equal to one during the first 20 minutes, allowing a nurse's touching events to occur and equal 0 during other times.

PT_c is initialized at the equilibrium MRSA level of 6,000 cfu/2000 cm^2. MRSA concentration in a colonized patient's nose is set at an equilibrium level of 1000 cfu/4 cm^2.

5.2.5.2 The porous surface in the colonized patient's room (P_c)

Changes of MRSA on the porous surface in the colonized patient's room (P_c) as seen in equation 5.2.3 are driven by the deposition of MRSA dispersal on the surface, surface touches by the colonized patient, surfaces touches by the nurses during the first 20 minutes of the hour, the natural die-off, and the daily surface decontamination.

$$\frac{dP_c}{dt} = \alpha A_p - P_c \frac{A_c}{A_p} \rho_p \tau_{pt-p} + PT_c \frac{A_c}{A_{pt}} \rho_p \tau_{pt-p} - P_c \frac{A_c}{A_p} \rho_p \tau_{ns-p} f(t) + NS \frac{A_c}{A_{ns}} \rho_p \tau_{ns-p} f(t) - P_c \epsilon_d h(t) - P_c \mu_p \quad (5.2.3)$$

where n ∈ Z+, and

$$h(t) = \begin{cases} 1, & t = n * 24 \\ 0, & otherwise \end{cases} \quad (5.2.4)$$

The function h(t) is a time indicator function for the every 24 hours decontamination schedule.

5.2.5.3 The nonporous surface in the colonized patient's room (NP_c)

Changes of MRSA on the nonporous surface in the colonized patient's room (NP_c) as seen in equation 5.2.5 are driven by the deposition of MRSA dispersal on the surface, surface touches by the colonized patient, surfaces touches by the nurses during the first 20 minutes of the hour, the natural die-off, and the daily surface decontamination. The structural changes of the nonporous surface is similar to the porous surface, except that only the nonporous surfaces can be wiped off following each nurse touch. The wiping rate is as frequent as the rate that nurses touch the nonporous surface. The efficacy of the wipes and the wiping rate is denoted by ε_w and $\omega_{ns\text{-}np}$.

$$\frac{dNP_c}{dt} = \alpha A_{np} - NP_c \frac{A_c}{A_{np}} \rho_{np} \tau_{pt-np} + PT_c \frac{A_c}{A_{pt}} \rho_{np} \tau_{pt-np} - NP_c \frac{A_c}{A_{np}} \rho_{np} \tau_{ns-np} f(t) + NS \frac{A_c}{A_{ns}} \rho_{np} \tau_{ns-np} f(t) - NP_c \frac{A_c}{A_{np}} \epsilon_w \omega_{ns-np} f(t) - NP_c \epsilon_d h(t) - NP_c \mu_{np} \tag{5.2.5}$$

5.2.5.4 The uncolonized patient (PT_u)

Changes of MRSA on the skin and hands of the uncolonized patient (PT_u) as seen in equation 5.2.6 are driven by contacts with nurses during the second 20 minutes of the hour, contacts with the two room surfaces, contact with own nose, and the natural die-off on the skin and hand.

$$\frac{dPT_u}{dt} = -PT_u \frac{A_f}{A_{pt}} \rho_n \tau_n + PT_{un} \frac{A_f}{A_n} \rho_n \tau_n - PT_u \frac{A_c}{A_{pt}} \rho_p \tau_{pt-p} + P_u \frac{A_c}{A_p} \rho_p \tau_{pt-p} - PT_u \frac{A_c}{A_{pt}} \rho_{np} \tau_{pt-np} + NP_u \frac{A_c}{A_{np}} \rho_p \tau_{pt-np} - PT_u \frac{A_c}{A_{pt}} \rho_{sk} \tau_{ns-pt} g(t) + NS \frac{A_c}{A_{ns}} \rho_{sk} \tau_{ns-pt} g(t) - PT_u \mu_{sk} \tag{5.2.6}$$

where $n \in Z+$, and

$$g(t) = \begin{cases} 1, & t \in [n - \frac{2}{3}, n - \frac{1}{3}) \\ 0, & t \in \left[n-1, n-\frac{2}{3}\right) or [n - \frac{1}{3}, n] \end{cases} \quad (5.2.7)$$

The function g(t) is a time indicator function for a nurse's visit in the uncolonized patient's room. g(t) is equal to one during the second 20 minutes of the hour, allowing a nurse's touching events to occur and equal 0 during other times.

5.2.5.5 The porous surface in the uncolonized patient's room (P_u)

Changes of the porous surface in the uncolonized patient's room as in equation 5.2.8 are similar to those of the porous surface in the colonized patient's room except that there is no MRSA dispersal and deposition in the uncolonized patient's room. Surface touches by nurses occur during the second 20 minutes of the hour.

$$\frac{dP_u}{dt} = -P_u \frac{A_c}{A_p} \rho_p \tau_{pt-p} + PT_u \frac{A_c}{A_{pt}} \rho_p \tau_{pt-p} - P_u \frac{A_c}{A_p} \rho_p \tau_{ns-p} g(t)$$
$$+NS \frac{A_c}{A_{ns}} \rho_p \tau_{ns-p} g(t) - P_u \epsilon_d h(t) - P_u \mu_p \quad (5.2.8)$$

5.2.5.6 The nonporous surface in the uncolonized patient's room (NP_u)

Changes of the nonporous surface in the uncolonized patient's room as in equation 5.2.9 are similar to those of the nonporous surface in the colonized patient's room except that there is no MRSA dispersal and deposition in the uncolonized patient's room. Surface touches by nurses occur during the second 20 minutes of the hour.

$$\frac{dNP_u}{dt} = -NP_u \frac{A_c}{A_{np}} \rho_{np} \tau_{pt-np} + PT_u \frac{A_c}{A_{pt}} \rho_p \tau_{pt-np} - NP_u \frac{A_c}{A_{np}} \rho_{np} \tau_{ns-np} g(t)$$
$$+NS \frac{A_c}{A_{ns}} \rho_{np} \tau_{ns-np} g(t) - NP_u \frac{A_c}{A_{np}} \epsilon_w \omega_{ns-np} g(t) - NP_u \epsilon_d h(t) - NP_u \mu_{np} \quad (5.2.9)$$

5.2.5.7 The nurses (NS)

Changes of MRSA on the exposed skin and hands of nurses (NS) as in equation 5.2.10 are driven by all nurses' activities and natural die-off on skin and hands. Nurses' activities include touching the colonized patient and the room surfaces during the first 20 minutes while in the colonized patient's room, touching the uncolonized patient and the room surfaces during the second 20 minutes while in the uncolonized patient's room, and touching own noses. Nurses wash hands before and after a patient's room visit. The time indicator for before and after the colonized patient's room visit are *u(t)* and *v(t)*. The time indicator for before and after the uncolonized patient's room visit are *x(t)* and *y(t)*. Nurses may also wipe the nonporous surfaces after surface touches. Nurses are assumed to have clean skin and hands at the beginning of each 8-hour shift. The time indicator for the beginning of the shift is *s(t)*.

$$\frac{dNS}{dt} = -NS\frac{A_f}{A_{ns}}\rho_n\tau_n + NS_n\frac{A_f}{A_n}\rho_n\tau_n - NS\frac{A_c}{A_{ns}}\rho_{sk}\tau_{ns-pt}f(t) + PT_c\frac{A_c}{A_{pt}}\rho_{sk}\tau_{ns-pt}f(t)$$
$$-NS\frac{A_c}{A_{ns}}\rho_p\tau_{ns-p}f(t) + P_c\frac{A_c}{A_p}\rho_p\tau_{ns-p}f(t) - NS\frac{A_c}{A_{ns}}\rho_{np}\tau_{ns-np}f(t) + NP_c\frac{A_c}{A_{np}}\rho_{np}\tau_{ns-np}f(t)$$
$$-NS\frac{A_c}{A_{ns}}\rho_{sk}\tau_{ns-pt}g(t) + PT_u\frac{A_c}{A_{pt}}\rho_{sk}\tau_{ns-pt}g(t) - NS\frac{A_c}{A_{ns}}\rho_p\tau_{ns-p}g(t) + P_u\frac{A_c}{A_p}\rho_p\tau_{ns-p}g(t)$$
$$-NS\frac{A_c}{A_{ns}}\rho_{np}\tau_{ns-np}g(t) + NP_u\frac{A_c}{A_{np}}\rho_{np}\tau_{ns-np}g(t) - NS\frac{A_h}{A_{ns}}\varepsilon_h u(t) - NS\frac{A_h}{A_{ns}}\varepsilon_h v(t)$$
$$-NS\frac{A_h}{A_{ns}}\varepsilon_h x(t) - NS\frac{A_h}{A_{ns}}\varepsilon_h y(t) - NSs(t) - NS\mu_{sk} \qquad (5.2.10)$$

where n ∈ Z+ and

$$u(t) = \begin{cases} 1, & t = n-1 \\ 0, & otherwise \end{cases}$$

$$v(t) = \begin{cases} 1, & t = n-2/3 \\ 0, & otherwise \end{cases}$$

$$x(t) = \begin{cases} 1, & t = n-\frac{2}{3}+dt \\ 0, & otherwise \end{cases}$$

$$y(t) = \begin{cases} 1, & t = n - 1/3 \\ 0, & otherwise \end{cases}$$

$$s(t) = \begin{cases} 1, & t = n * 8 \\ 0, & otherwise \end{cases} \quad (5.2.11)$$

The other two compartments are MRSA accumulated in the uncolonized patient's nose and nurse's nose. They are given by:

$$\frac{dPT_{un}}{dt} = -PT_{un}\frac{A_f}{A_n}\rho_n\tau_n + PT_u\frac{A_f}{A_{pt}}\rho_n\tau_n \quad (5.2.12)$$

$$\frac{dNS_n}{dt} = -NS_n\frac{A_f}{A_n}\rho_n\tau_n + NS\frac{A_f}{A_{ns}}\rho_n\tau_n \quad (5.2.13)$$

5.2.6 Model analysis

The analysis is divided in three parts. First, we examine the effect of continual shedding to the contamination levels or MRSA concentrations (cfu/2000 cm^2) in both patients' rooms at baseline scenario with no intervention. The initial condition is set to reflect clean room surfaces, nurses and the uncolonized patient with MRSA concentrations at zero. The initial MRSA concentration for the colonized patient and the colonized patient's nose are 6,000 cfu/2000cm^2, and 1000 cfu/4cm^2, respectively. The outcome measurements are 1) the MRSA contamination levels, which are the net MRSA concentrations in the compartments, 2) the MRSA exposure dose to the nurses in the colonized patient's room, which is the net flow to the nurses from the colonized patient and surfaces, and 3) the MRSA exposure dose to the uncolonized patient, which is the net flow to the uncolonized patient from nurses and surfaces. According to the diagram, the net flow to the nurses (the blue flows) is (6ib-6ia)+(5ib-5ia)+(5iib-5iia). The net flow to the uncolonized patient (the purple and green flows) is (6iia–6iib) + (4ib-4ia) + (4iib-

4iia).We compared these direct and indirect exposure sources to the nurses in the colonized patient's room and to the uncolonized patient. For example, direct exposure to the uncolonized patient is the net flow resulting from the skin-to-skin contact with nurses, which is (6iia-6iib). Indirect exposure to the uncolonized patient is the net flow to the uncolonized patient from touching the two surfaces in the room, which is (4ib-4ia) + (4iib-4iia).

Second, we evaluated and compared the effect of two surface decontamination methods. The once daily surface decontamination was evaluated with 0%, 50% and 100% efficacy. The decontamination by wiping following each nurse touch was also examined with 0%, 50% and 100% efficacy. These two decontamination methods may differ in three aspects, which are the surface area that is cleaned each time, the cleaning efficacy, and the frequency of cleaning. We compared the effect of the decontamination frequency while the efficacy is 100%. The effect of the surface-decontamination frequency every 24, 12 and 8 hours was examined. Surface wiping frequency is at the same rate a nurse touches the nonporous surface, which is eight times every hour while the nurse is in the room. The outcome measure for this comparison is the total MRSA exposure dose to the uncolonized patient.

Third, we examined the effect of hand hygiene when compliance is ideally at 100%, while the efficacy varies according to the hygiene method. Hand hygiene efficacy for soap with water is 58%, and for alcohol hand gel rub is 85% [34, 35]. We then examined the joint effect of surface decontamination and hand hygiene when compliance is 100% and efficacy for each intervention varies at 0%, 50%, and 100%.

5.2.7 Sensitivity analysis

We explore how sensitive the system is to various model parameters including contact surface area, total exposed surface area, transfer efficiency, survivability, and contact rates using parameter plots and the total MRSA exposure dose to the uncolonized patient. To relax the assumption of instantaneous and homogenous mixing of the pathogen, we create and compare models where contact and total surface areas are equal and do not require the instantaneous and homogenous mixing and models where there is a difference between the two areas and the above assumption is needed. To relax the assumption of continuous touches modeling using touch rates, we explicitly define touch and wipe time points and create a discrete event model for direct comparison with the original model.

5.3 Results

5.3.1 Baseline scenario with no intervention

At baseline scenario with no intervention, Figure 5.2 shows that regular patterns of MRSA concentrations with constant averages are reached in each compartment by 24 to 48 hours. Comparing the patient and the two surfaces in the colonized patient's room, surfaces accumulate higher MRSA concentration than the colonized patient. This is largely due to the longer survival time on surfaces than on skin and hands. All lines show jagged patterns, which correspond to changes of MRSA concentrations due to the nurse's hourly room visits and the change of shift every eight hours.

Comparing the two surface compartments (P_c and NP_c) in the colonized patient's room, the porous surface accumulated a higher concentration than the nonporous surface. As seen in equations 5.2.3 and 5.2.5, the surfaces share the same structure of flows in and out of the compartments; the same total surface area, which results in the same MRSA

deposition; and the same patient's and nurse's touch rates. The two surfaces have different die-off rates, but are still within the same order of magnitude. Of the four main events on the surfaces, (1) MRSA deposition on surfaces, (2) contact with the colonized patient, (3) contact with nurses, and (4) natural die-off (survivability), contact with nurses is the most influential in determining the MRSA levels in the compartment, since it results in the largest net transfer. Thus, the porous surface has smaller transfer efficiency; lower net MRSA transfers from the porous surface to nurses, resulting in a higher MRSA concentration remaining in the porous surface compartment (P_c).

In the uncolonized patient's room, the nonporous surface (NP_u) has a higher concentration than the porous surface (P_u) and the uncolonized patient (PT_u). As seen in equations 5.2.8 and 5.2.9, the two surfaces share the same flows due to patient contacts, nurse contacts, and natural die-off. The uncolonized patient receives no direct deposition from the shedding process. The only source of MRSA to the uncolonized patient is from nurses, either directly by being touched by nurses or indirectly by touching surfaces that were contaminated by nurses. The nonporous surface has a higher transfer efficiency—that is, more MRSA transfers into the nonporous surface compartment, which leads to a higher accumulated concentration on the nonporous surface (NP_u).

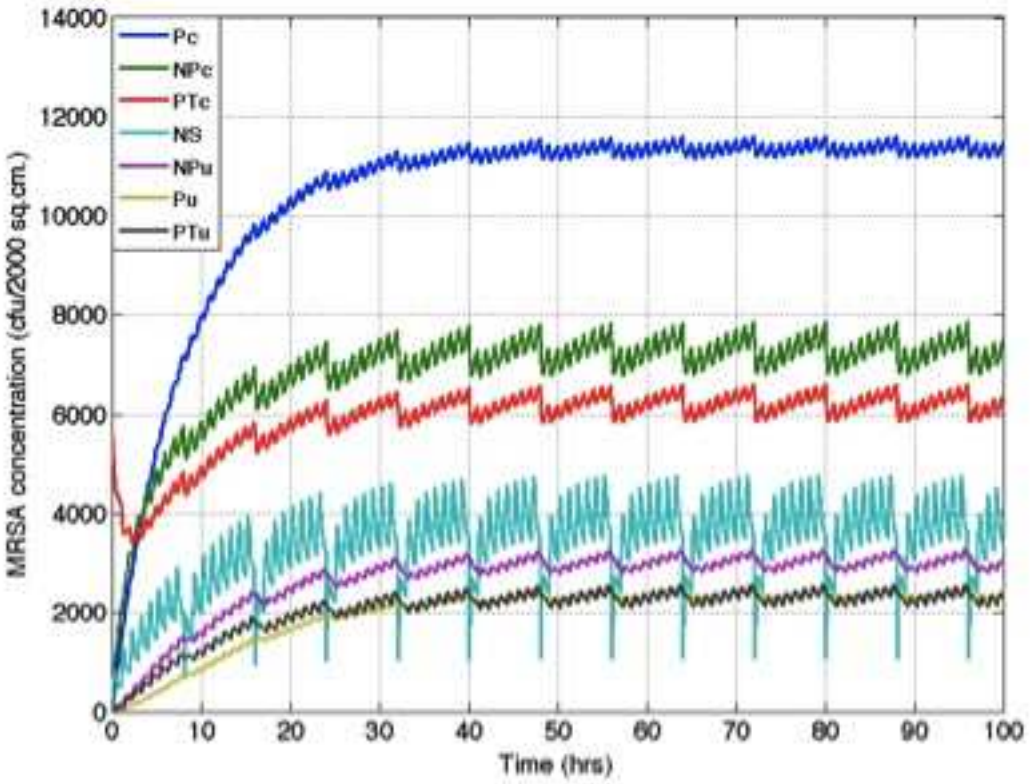
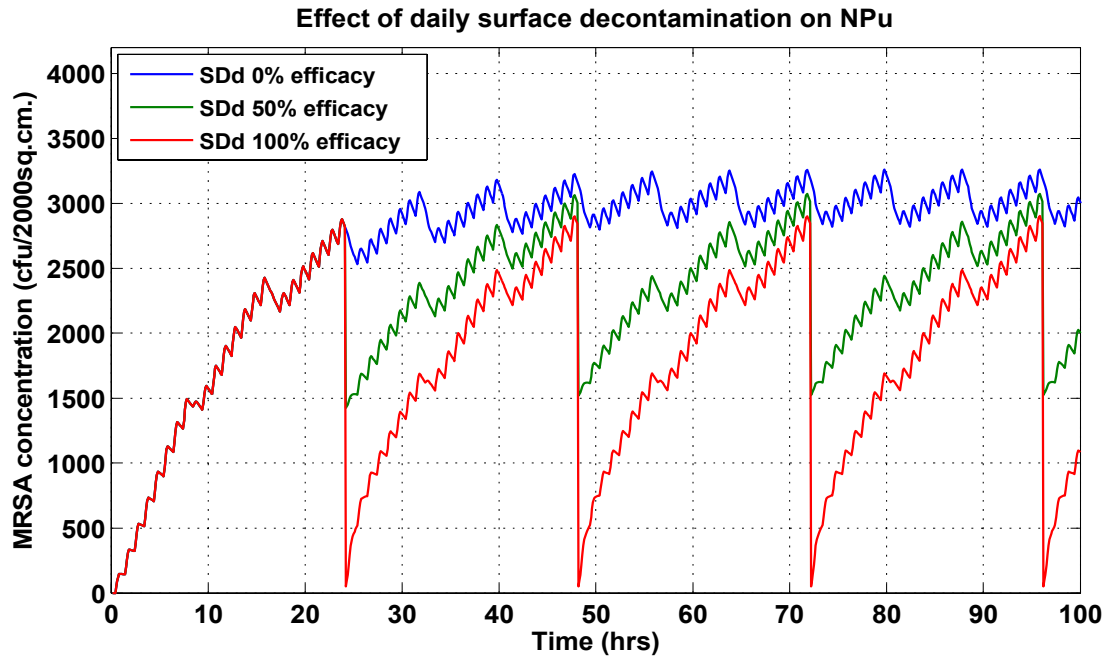


Figure 5.2: MRSA quantity at baseline scenario without intervention. The Y-axis represents MRSA quantity on the entire surface area (cfu/2000 cm^2) from the seven compartments including the exposed skin and hand of the colonized patient (PT_c), the porous surface in the colonized patient's room (P_c), the nonporous surface in the colonized patient's room (NP_c), the exposed skin and hand of the uncolonized patient (PT_u), the porous surface in the uncolonized patient's room (P_u), the nonporous surface in the uncolonized patient's room (NP_u), and the exposed skin and hand of the nurse (NS).

a)



b)

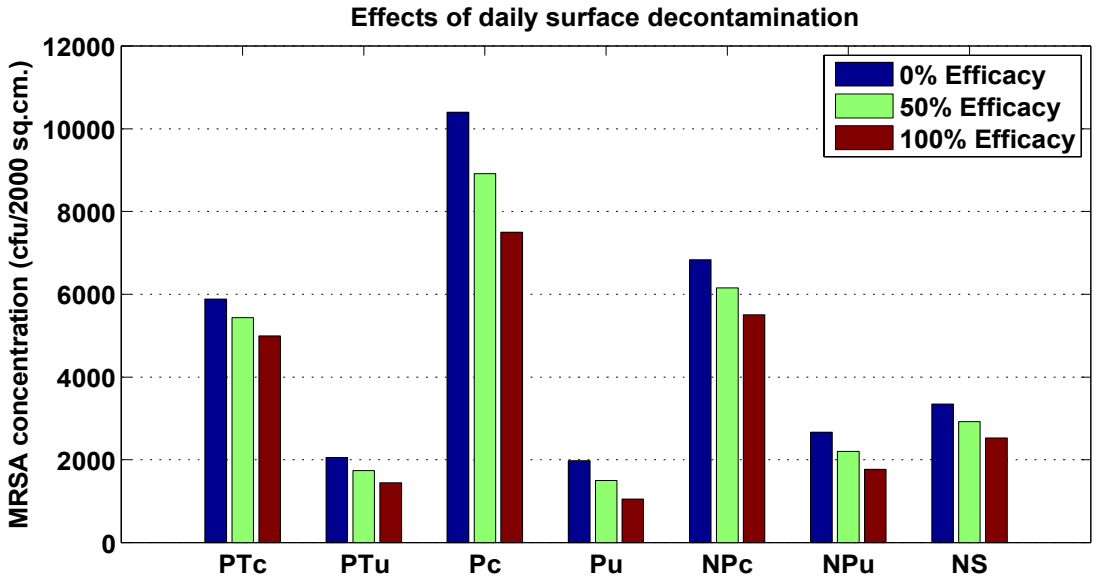


Figure 5.3: Effects of daily surface decontamination (SDd) at 0%, 50% and 100% efficacy levels. Figure 5.3a shows the effects of SDd on the nonporous surface in the uncolonized patient's room. Figure 5.3b shows the average MRSA concentrations on the seven compartments, which are the exposed skin and hand of the colonized patient (PT_c), the porous surface in the colonized patient's room (P_c), the nonporous surface in the colonized patient's room (NP_c), the exposed skin and hand of the uncolonized patient (PT_u), the porous surface in the uncolonized patient's room (P_u), the nonporous surface in the uncolonized patient's room (NP_u), and the exposed skin and hand of the nurse (NS).

5.3.2 Daily surface decontamination

Figure 5.3 shows the effect of daily room surface decontamination at 0%, 50%, and 100% efficacy. Figure 5.3a shows changes of MRSA concentrations on the nonporous surface in the uncolonized patient's room (NP_u) through time and demonstrates a distinct pattern, which is also noted throughout other compartments. The pattern shows the short-lived effect of the daily decontamination in decreasing MRSA. After 24 hours MRSA concentration quickly returned to the level prior to the cleaning.

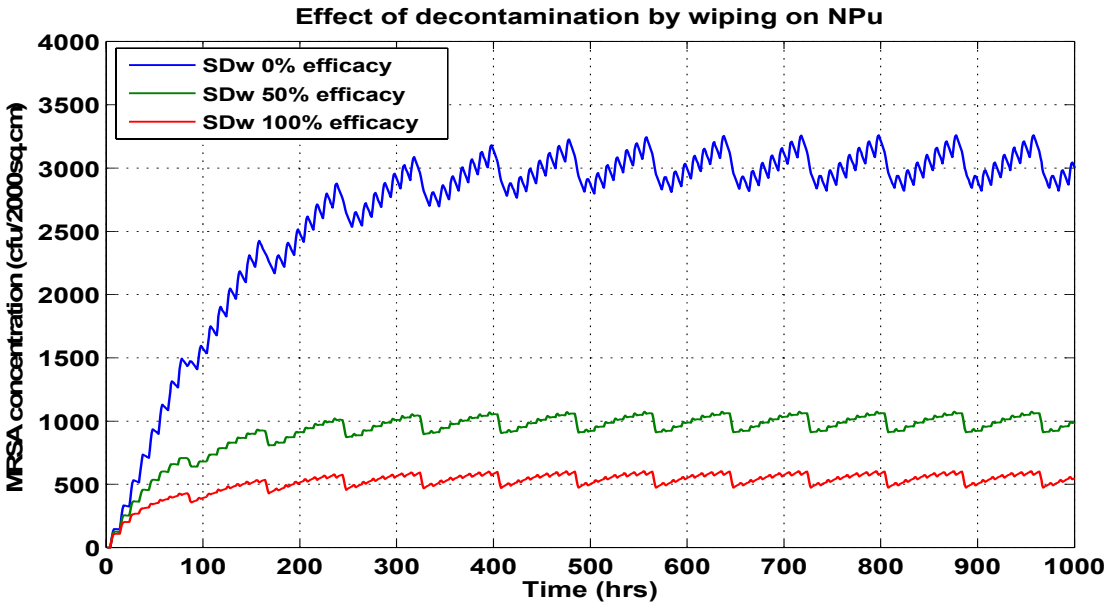
Figure 5.3b shows the effects of daily decontamination to the average MRSA concentrations in the seven compartments. On an absolute scale, the porous surface in the colonized patient's room (P_c) was the most affected with the largest decrease in MRSA concentration. Interestingly, both the colonized and the uncolonized patients as well as nurses also demonstrated the decreasing patterns corresponding to the cleaning of the porous and nonporous surfaces. The pattern is due to the sudden decrease in pathogen flows from both surfaces to the patients and nurses.

5.3.3 Decontamination by wiping after each nurse touches nonporous surfaces

Figure 5.4a shows the effect of decontamination by wiping at 0%, 50%, and 100% efficacy. Here, there is no fluctuation pattern as was seen with daily decontamination. In Figure 5.4b, with 50% efficacy, the total exposure dose to the uncolonized patient is reduced by 54%, while 100% efficacy decontamination decreased the exposure dose further to 63%. This shows that increasing efficacy from 50% to 100% does not linearly decrease exposure to the uncolonized patient. This is due to the fact that only the contact surface area is wiped each time, not the entire surface area. Nevertheless, wiping with

50% efficacy decreases the concentration of MRSA more substantially than daily decontamination with 100% efficacy.

a)



b)

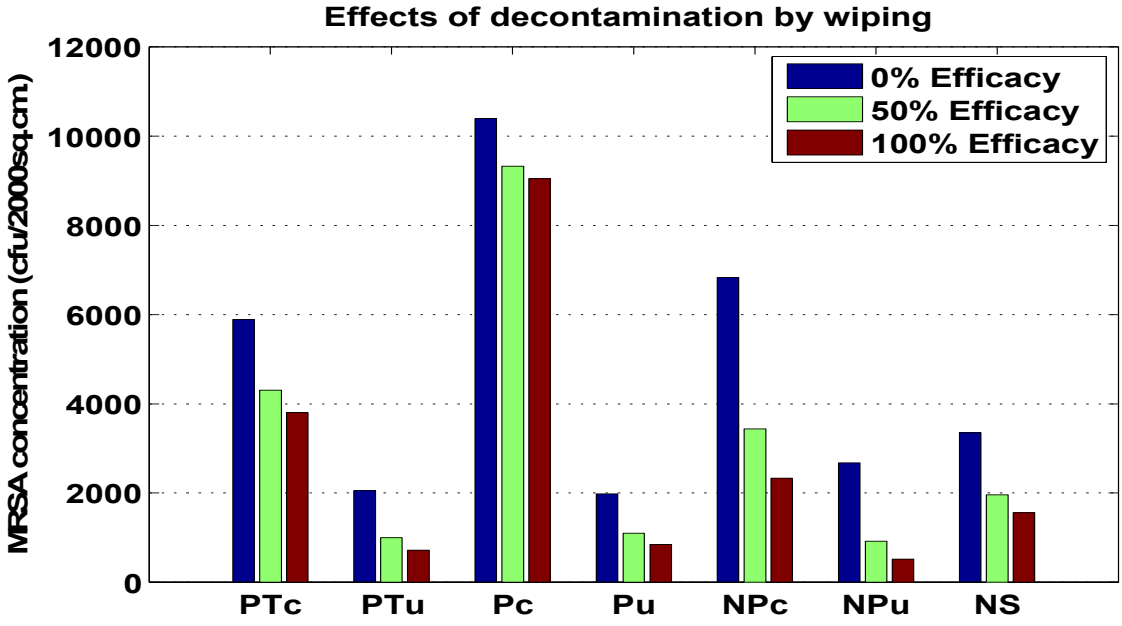


Figure 5.4: Effects of surface decontamination by wiping (SDw) at 0%, 50% and 100% efficacy levels. Figure 5.4a shows the effects of SDw on the nonporous surface in the uncolonized patient's room. Figure 5.4b shows the average MRSA concentrations on the seven compartments, which are the exposed skin and hand of the colonized patient (PT_c), the porous surface in the colonized patient's room (P_c), the nonporous surface in the colonized patient's room (NP_c), the exposed skin and hand of the uncolonized patient

(PT_u), the porous surface in the uncolonized patient's room (P_u), the nonporous surface in the uncolonized patient's room (NP_u), and the exposed skin and hand of the nurse (NS).

Table 5.3: Comparison of the frequency of the two decontamination methods and the affected surface area.

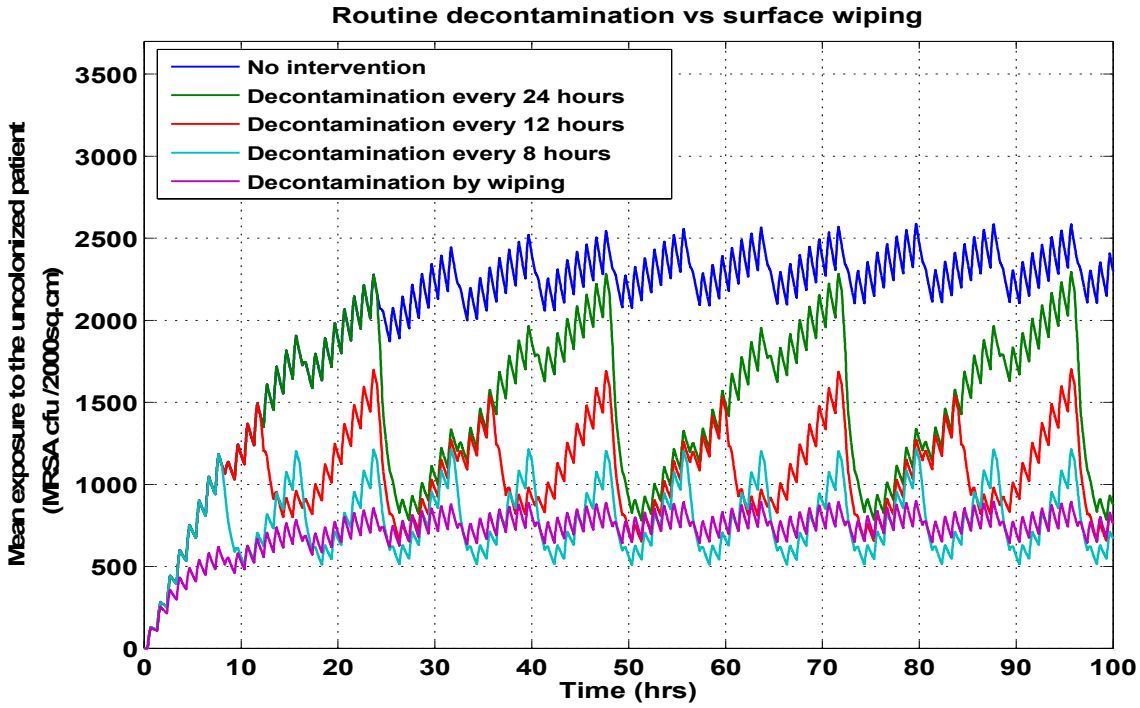
	Routine surface decontamination (SDd)			**Surface decontamination by wiping (SDw)**	
Comparison for each surface	a. Every 24 hours	b. Every 12 hours	c. Every 8 hours	d. Nurses touch 8 times/hour	e. Nurses touch 3 times/hour
Total surface area decontaminated (cm^2 per each cleaning event)	8000	8000	8000	300	300
Number of cleaning events per hour	0.0417	0.0833	0.125	8	3.333
Numbers of cleaning events per day	1	2	3	192	80
Total surface area decontaminated per hour	333.36	666.67	1000	2400	1000
Total surface area decontaminated per day	8000	16000	24000	57600	24000

5.3.4 Comparison of daily surface decontamination and decontamination by wiping

To further understanding of the effects of decontamination in relation to the mechanical process, the surface area cleaned and the frequency of decontamination are compared in Table 5.3. While daily decontamination affects a larger surface area (2000 cm^2) each time and decreases total concentration on surfaces to zero with 100% efficacy, MRSA can quickly redeposit on the surfaces. On the other hand, wiping affects a much

smaller surface area (150 cm^2), but due to the wiping frequency the accumulated total surface area per day is much larger. The surface area cleaned by daily decontamination is 8000 cm^2/day, while by wiping it is 57600 cm^2/day. Figure 5.5a compares the two decontamination methods with 100% efficacy: Decontamination by wiping was superior to the daily decontamination (even when increased to twice per day), leading to much less total exposure dose to the uncolonized patient.

a)



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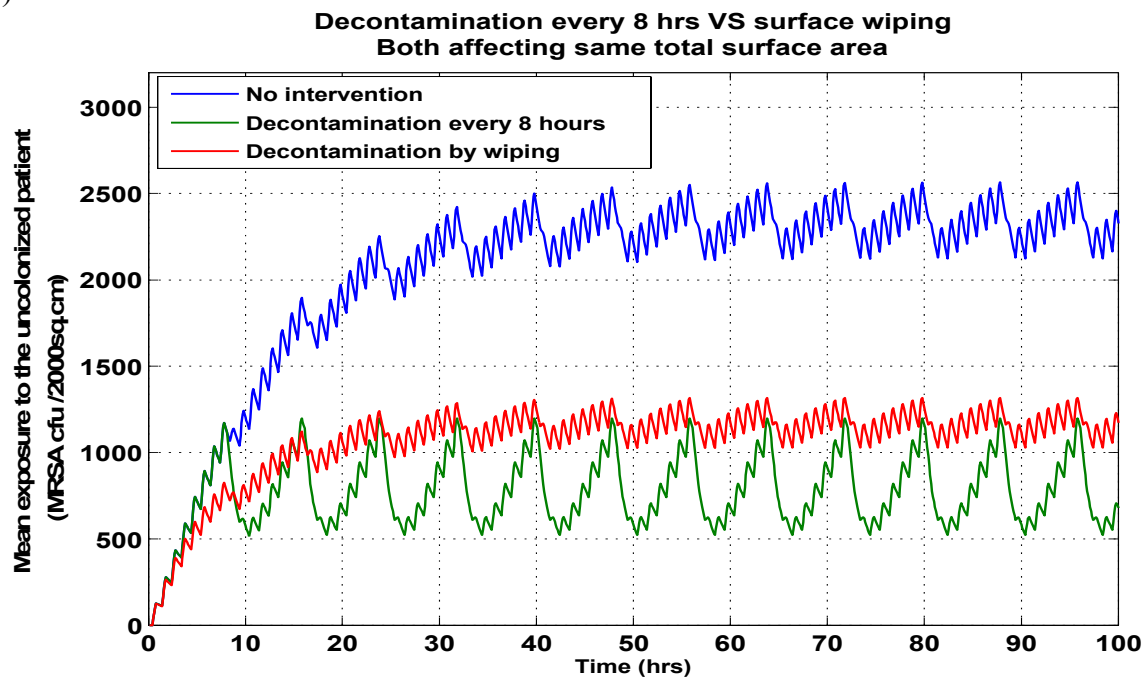


Figure 5.5: The effect of the routine surface decontamination and decontamination by wiping to the mean exposure dose to the uncolonized patient (MRSA cfu/2000 cm^2). Figure 5.5a compares three different schedules of the surface decontamination and decontamination by wiping. The three schedules are decontamination (SDd) every 24, 12, and 8 hours. All lines overlap in the beginning since there is no intervention until their scheduled time. Figure 5.5b compares the every 8 hours decontamination and decontamination by wiping when both affect the same total surface area (scenario c and e from Table 5.3).

Figure 5.5a also shows that in order to decrease MRSA exposure to the uncolonized patient to the same level decreased by wiping, the routine daily decontamination needs to increase its frequency to every eight hours. In comparison to the total surface area cleaned in Table 5.3, cleaning every eight hours affects less than half the surface area decontaminated by wiping (column c - 24,000 versus column e - 57,600 cm^2). When adjusting the two cleaning methods to affect the same total surface area cleaned by decreasing the nurse touching rate (Table 5.3 column c and e), the

thorough cleaning every eight hours is superior to decontamination by wiping, leading to less exposure dose to the uncolonized patient as in Figure 5.5b.

5.3.5 Hand hygiene

In addition to surface decontamination, we examined the effect of hand hygiene in decreasing the total exposure dose of MRSA to the uncolonized patient. Figure 5.6 compared the effect of the two hand-hygiene methods [34, 35], assuming 100% compliance. Hand hygiene with soap and water, where efficacy is 58%, decreases the total dose exposed by approximately 38% in comparison to no intervention. Alcohol hand gel rub, where efficacy is 83%, decreases the total dose exposed further to 48%.

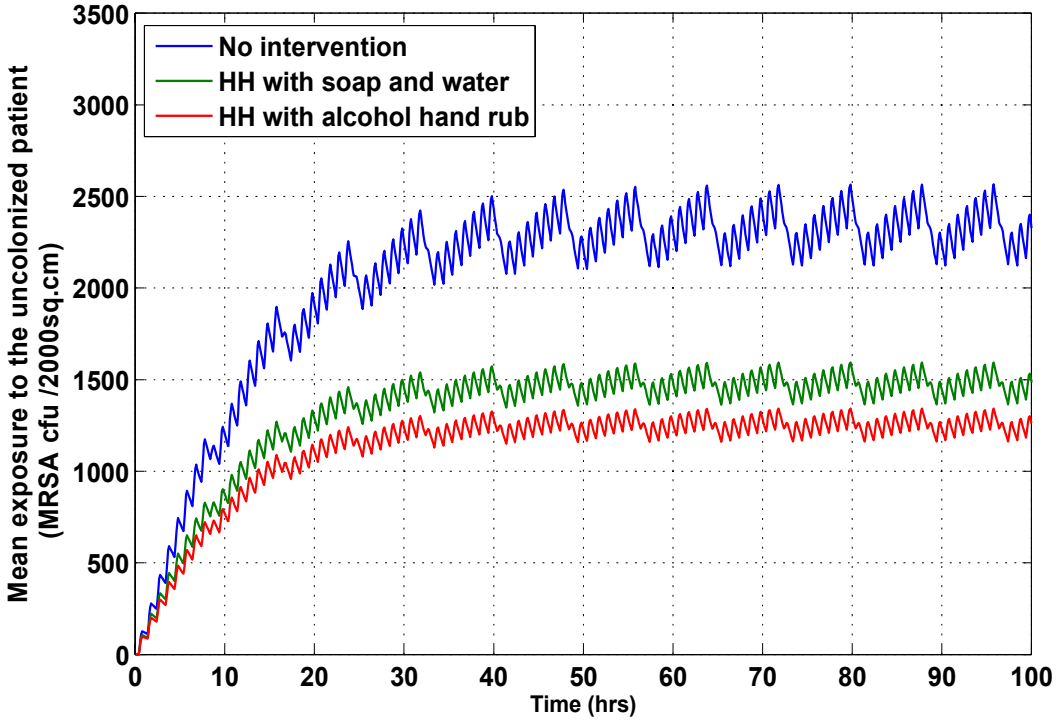
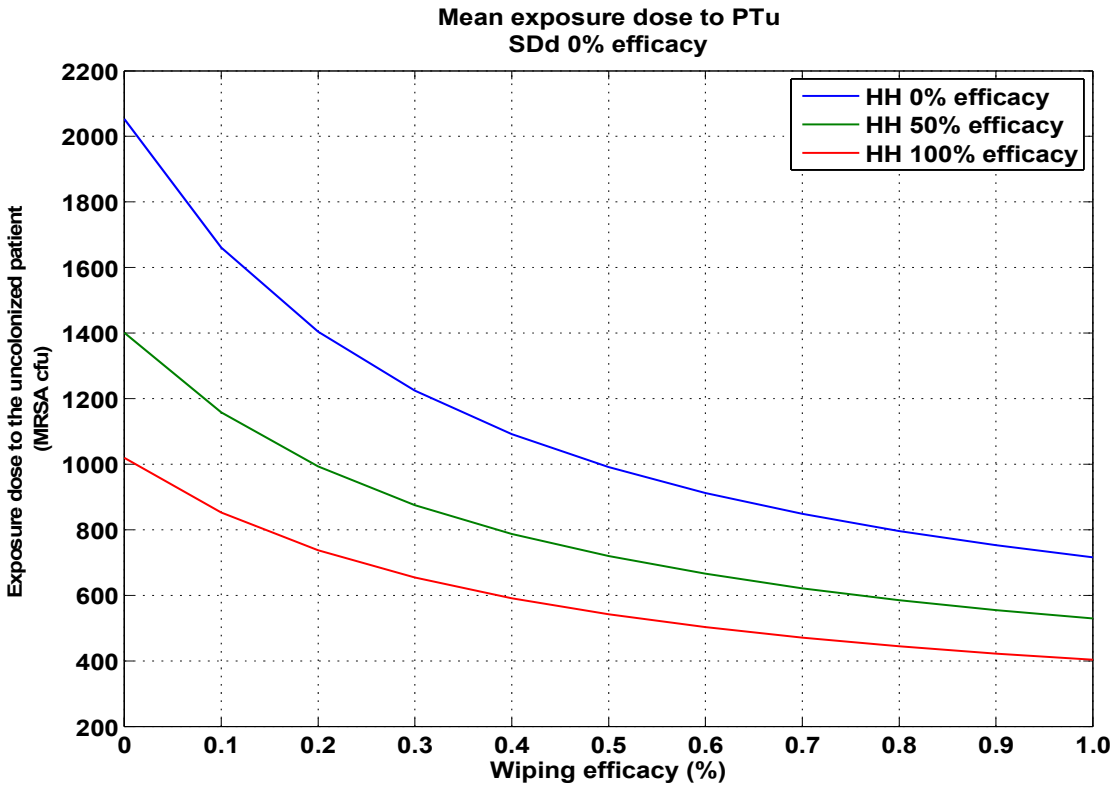


Figure 5.6: Effects of hand hygiene to the mean exposure dose to the uncolonized patient (MRSA cfu/2000 cm^2). The figure compares the effects of hand hygiene when using soap and water, where efficacy is 58% versus alcohol hand gel rub, where efficacy is 83%.

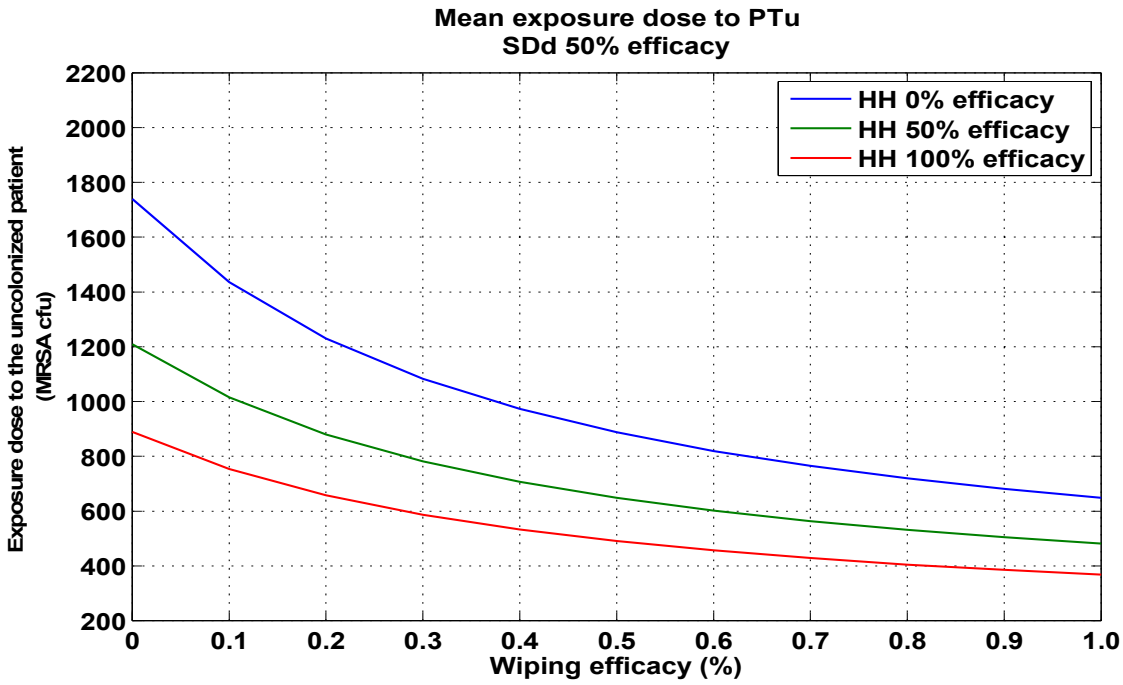
5.3.6 Joint effect of surface decontamination and hand hygiene

While studying the effect of interventions separately in the model is essential, in the real world, infection-control strategies always incorporate multiple interventions. As in this model, we found that MRSA concentrations on room surfaces and on nurses are positively correlated. For example, when wiping efficacy increases, MRSA concentrations both on the surfaces as well as on nurses decrease. Applying intervention to one contamination site, either to the surface or to the hand, will likely affect the other. Here we examined joint effects of the two surface decontamination methods in conjunction with hand hygiene.

a)


Mean exposure dose to PTu
SDd 0% efficacy
HH 0% efficacy
HH 50% efficacy
HH 100% efficacy
Exposure dose to the uncolonized patient (MRSA cfu)
Wiping efficacy (%)
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

b)


Mean exposure dose to PTu
SDd 50% efficacy
HH 0% efficacy
HH 50% efficacy
HH 100% efficacy
Exposure dose to the uncolonized patient (MRSA cfu)
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Wiping efficacy (%)

c)

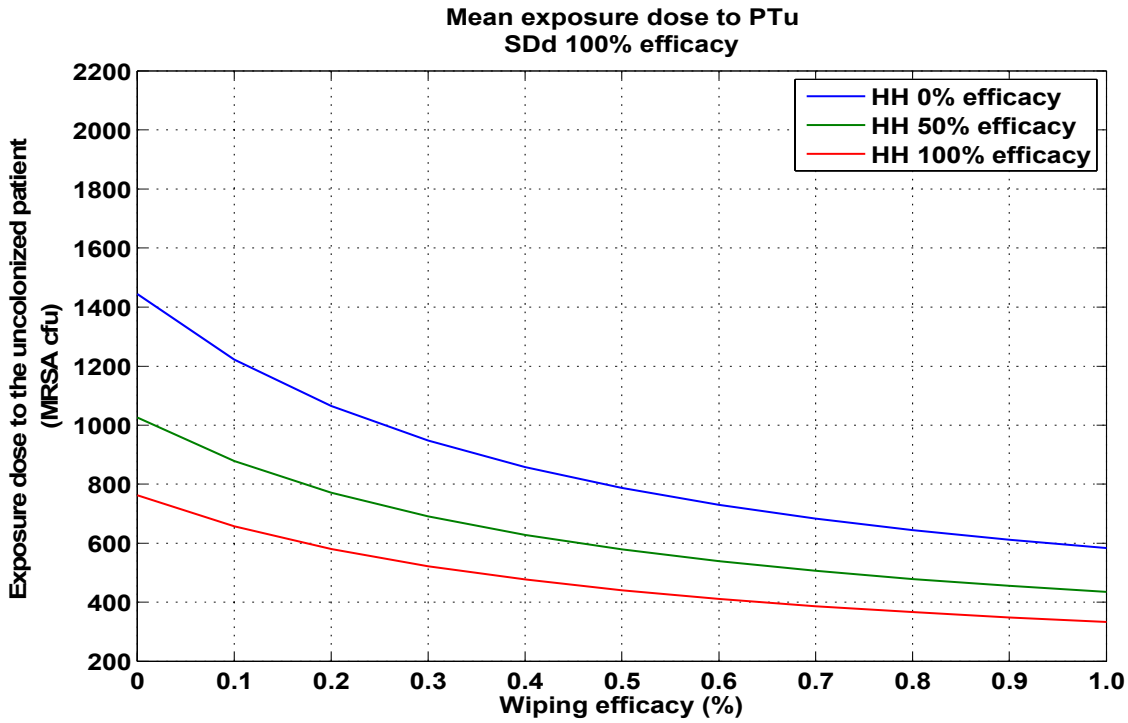


Figure 5.7: Joint effects of the two surface decontamination methods and hand hygiene to the total MRSA mean exposure dose to the uncolonized patient. Figure 5.7a, 5.7b and 5.7c represent three efficacy levels of daily surface decontamination (SDd) at 0%, 50% and 100%, respectively. The three lines in each figure represent three hand hygiene (HH) efficacy levels at 0%, 50% and 100%.

In Figure 5.7a-c, we examined the joint effects of interventions on hands and surfaces to exposure dose to the uncolonized patient. All three figures show the same patterns: as wiping efficacy increases, the exposure dose to the uncolonized patient decreases. Similarly, As hand-hygiene efficacy increases, the exposure dose to the uncolonized patient decreases. However, the benefit of hand hygiene varies at different levels of surface contamination. Increasing hand hygiene efficacy from 0% to 100% results in greater absolute reduction of exposure dose when decontamination efficacy is low compared to when efficacy is high. In a scenario when decontamination and hand-hygiene efficacies are zero and both surfaces and nurses are highly contaminated,

performing surface decontamination or hand hygiene will suppress exposure to the uncolonized patient. However, all three figures show that increasing hand-hygiene efficacy from 0% to 100% decreased the exposure dose less than increasing wiping efficacy from 0% to 100%.

5.4 Discussion

With our applied EITS model that integrates host, pathogen, and environment parameters, we demonstrate a significant role of environmental surfaces in contaminating and recontaminating nurses, who are the only vectors that transfer MRSA from the colonized patient's room into the uncolonized patient's room. The model also revealed the effect of *S. aureus* continuous shedding from the colonized patient onto room surfaces. The surfaces were quickly recontaminated with MRSA even after the most efficacious decontamination. It highlights the importance of decontamination frequency in addition to decontamination efficacy.

Over 30 years ago, E. H. Spaulding devised an approach to disinfection and sterilization of patient-care instruments and equipment. These patient-care items were divided into three categories of critical, semicritical, and noncritical based on the degree of the potential infection risk involved in their uses. Items and surfaces are considered noncritical if they come in contact with intact skin [36]. While noncritical surfaces are viewed as uncommonly associated with infection transmission, they are required to be cleaned and disinfected on a regularly scheduled basis. Nevertheless, the frequency of cleaning has been emphasized less than the efficacy of cleaning. An example of the current cleaning recommendations for bed frames, and nonporous surfaces includes decontamination between room occupancy, or once weekly if the room is occupied by the

same patient [37]. This frequency is far from what appears most effective based on the results from our model.

In our study, daily surface decontamination was not able to maintain a constant low level of contamination because of continual shedding from the MRSA-positive patient. Our finding supports the results from a hydrogen peroxide vapor (HPV) intervention study [21]. The study was a prospective study in a 9-bed open-plan ICU without isolation facilities. Environmental screening was monitored monthly for 3 months and weekly for 4 weeks and immediately prior to the HPV intervention. Thereafter, environmental screening was carried out immediately after, daily for 2 days, and weekly for 8 weeks after the HPV use. Patients were removed from the ICU prior to the HPV use and returned to the unit the next morning. After the HPV decontamination and before patient readmission into the ICU, no MRSA was isolated from the environment. However, 24 hours after readmitting patients, including two colonized with MRSA, MRSA was isolated from the environmental surfaces. These environmental strains were indistinguishable from a strain from one of the colonized patients, and were not all confined to the immediate vicinity of the colonized patient. The authors of that intervention study concluded that HPV is effective in eliminating MRSA from the environment, but the rapid recontamination suggested that, by itself, it is not an effective means of maintaining low levels of contamination. Our data supports this finding and also suggests that for an intervention to be effective, it needs to be both efficacious in eradicating the pathogen and as well as implemented with adequate frequency.

While our model suggested that the cleaning frequency is an important aspect of decontamination, the practical aspects of its implementation are also important to

consider. A previously published review of environmental hygiene in healthcare settings showed an imperfect thoroughness of cleaning [20]. Eight studies were included in this review, and by using direct covert observations or a fluorescent targeting method showed that only 40% of near patient surfaces were being thoroughly cleaned in accordance with existing policies [20]. In conjunction, using an environmental cleaning monitoring system could improve the thoroughness of disinfection cleaning [38-40]. Further analysis showed that such improvement was associated with an average decrease of MRSA infection [41, 42]. In one study, the enhanced cleaning required an extra person to clean, who performed more frequent cleaning of hand touch sites at near-patient locations and the nurses' station. This enhanced cleaning was associated with a 32.5% reduction in environmental contamination sites and 26.6 % reduction of new MRSA infections [42].

In contrast to routine surface decontamination where sufficient disinfectant quantity and adequate contact time is required, surface wiping may require a lower concentration of disinfectants [22]. A recent study to evaluate efficacy of various commercially available wipes in comparison with normal saline wipes, when used to wipe across plastic one, three, and five times, showed that wiping with any type of moist wipe decreased bacterial burden. Furthermore, a saline-moistened wipe appeared to be as effective as wipes containing disinfectant [25].

Even though introducing surface wiping to healthcare workers' responsibilities may appear as a threat to success given the potential compliance obstacle, providing surface wipes in patients' room may offer the opportunities for patients and visitors to partake in the decontamination responsibility. A 24-hour observational study of hand

hygiene showed that patients and visitors had better hand hygiene compliance than doctors (57% vs. 47%), but less than nurses (75%) [43].

Our model showed the interaction of hand hygiene and surface decontamination can work together to decrease the total exposure dose of MRSA to the uncolonized patient, and confirmed the necessity to clean environmental surfaces. Furthermore, MRSA levels on nurses were directly correlated with MRSA levels on the nonporous surfaces. In order to keep the surfaces in the uncolonized patient's room less contaminated, both increasing hand-hygiene effects and decreasing the exposure dose to the uncolonized patient were required to reduce MRSA transmission when the patient touched contaminated surfaces.

Although this model is more realistic than the original model based on the Environmental Infection Transmission System (EITS) framework [44], it is still rather abstract. We kept it simple to provide insight to model behaviors, but is strictly a fate and transport model with no prediction of risk or interpretation of the total exposure dose to the uncolonized patient. However, conventional wisdom would suggest that the smaller dose exposed to the patient the better. According to the quantitative risk assessment paradigm, exposure assessment is an initial and essential step toward improving our understanding in transmission systems. To develop a full transmission system model, we also require a dose response function. However, at this point, existing applicable dose response studies for *S. aureus* are limited to artificial colonization studies in newborns in 1963 and an artificial inoculation study in healthy adults using over 10^7 cfu, which is much higher than the total exposure dose to the uncolonized patient in our model [45-47]. These studies revealed substantial information regarding single exposure dose-response

relationship. However, they are not readily applicable for modeling where exposure may be continuous and cumulative over time [48]. A full risk assessment model will require additional assumptions. In our model, patients are exposed to pathogens continuously by direct contact with nurses and indirect contact with contaminated surfaces. Future dose response studies may need to consider cumulated time-dependent dose responses as well as more realistic dose exposure pathways.

Our model identified several parameters which have a high impact in the system. Transfer efficiency is the main parameter that differentiates behavior of the two environmental surfaces. Also, according to our sensitivity analysis, survivability on surfaces has a high impact on the constant averages of MRSA concentration when contact surface area is much smaller than total surface area.

One of the main assumptions inherent to the deterministic model is the homogenously mixed assumption. This leads to the instantaneous equilibration of MRSA in each compartment following each event. To evaluate the effect of this assumption, we performed experiments to compare different levels of equilibration by stratifying nonporous surface area and nonporous touching rates, while keeping the strata sums of surface areas and touch rates the same as the original scenario. The analysis showed unchanged qualitative model behaviors. However, quantitatively there are small differences of the total exposure dose to the uncolonized patient. Future studies that relax this assumption to evaluate the effect of surface touching will be needed. For decontamination purposes, much attention is on frequently touched surfaces; however, with the survivability of *S. aureus* and its presence in dusty, inaccessible, high surfaces, one cannot ignore the less frequently touched surfaces [49].

Overall, this model demonstrates how using EITS framework can provide insight into the dynamics of host, pathogen, and environment. It reveals the importance of surface decontamination frequency in addition to decontamination efficacy given the continual pathogen exposure. It shows how healthcare workers' hands and contaminated surfaces are directly correlated, resulting in various levels of hand-hygiene effect dependent on surface contamination levels. It indicates expected benefits of surface wiping in conjunction with our current MRSA infection-control armamentarium.

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CHAPTER VI

The Effect of Hand Hygiene at the Entry and Exit of a Patient's Room Visit on the Exposure of MRSA to the Uncolonized Patient

6.1 Introduction

The hands of healthcare workers are key vectors for nosocomial transmission of MRSA [1]. For hands to transmit these pathogens, several sequential events must occur. First the healthcare workers must acquire the pathogen either by directly touching a patient's skin, wound or body fluid where MRSA is present, or by indirectly touching the contaminated surrounding surfaces where the pathogens have been shed. In addition, the pathogens must survive for at least several minutes on the hands and hand hygiene must be inadequate. Finally, these contaminated hands must transfer the pathogen by touching another patient or a surface that the patient subsequently touches [1-3]. Since the exposure pathways can be through hands, environment, or both, these interdependent pathways will need to be addressed together when hand hygiene efficacy is evaluated.

Hand hygiene is undoubtedly the cornerstone of infection control. Several aspects of hand hygiene can influence its effectiveness, including the timing of when hand hygiene is performed, the efficacy of the techniques, and importantly, the compliance. A concept called 'My five moments for hand hygiene', developed for the Swiss Hand Hygiene Campaign in 2005, described the fundamental reference points for healthcare workers (HCW) in a time-space framework, and designated the moments when hand

hygiene is required to effectively interrupt microbial transmission during patient care [4]. This concept has been adopted and adapted by the World Health Organization (WHO) for inclusion in the implementation strategy proposed in the 2009 WHO Guidelines on Hand Hygiene in Healthcare [4, 5]. These 'five moments', or opportunities to initiate hand hygiene, are 1) before touching a patient, 2) before a clean/aseptic procedure, 3) after body fluid exposure, 4) after touching a patient, and 5) after touching patient surroundings [5]. Currently, direct observation of hand hygiene by trained observers is considered the gold standard for determining hand hygiene compliance among HCWs [6]. However, the direct observation method has several limitations, including the fact that they are time-consuming and costly [6]. They provide only a small proportion of all hand hygiene opportunities in healthcare [7]. Direct observation may also spuriously result in high compliance related to the Hawthorne effect [8], and may also be inaccurate unless performed by trained personnel [9].

As a result of these limitations, several alternative techniques for monitoring compliance have been developed and include sophisticated electronic hand hygiene monitoring systems [6]. These monitoring systems provide great potential in capturing large quantities of hand hygiene opportunities with less human and time resources, while avoiding an observation bias. However, the systems are not readily adapted to capture all five moments. Some require a compromise to include only before and after patient care (moments 1 and 4), or on entry and exit of a patient room [10, 11]. This raises several concerns whether this compromise is appropriate, and what should be the outcome measure of hand hygiene effectiveness. We argue that the outcome for effective hand

hygiene should be the lowest microbial exposure dose that delivers minimum risk to susceptible patients from each contact with HCWs.

From the previous chapter using the environmental infection transmission system (EITS) framework in two hypothetical hospital rooms, we demonstrated that the contaminated nonporous surface was an important source of contamination to nurses in the colonized patient's room. This model assumed no dispersal across rooms, so nurses were the only sources of MRSA to the uncolonized patient's room. We found that the contaminated surfaces in the uncolonized patient's room, which resulted from nurses' touching, also contributed to contamination to the uncolonized patient. While this previous deterministic model provided insights into the interdependency among the colonized patient's shedding, nurses' hands contamination and environmental surfaces contamination, it was not appropriate to examine hand hygiene compliance.

In this chapter, we will examine the effects of hand hygiene compliance by using a stochastic model with more realistic features. The model structure remains the same as was described for the two hypothetical hospital rooms. The comparison of chapter V and VI is in Table 7.1. Hand hygiene opportunities are at the entry and exit of a patient room, while nurses can touch the patients and the surfaces in a random order. To examine the role of environmental contamination on MRSA transmission, we vary the shedding magnitude that the colonized patient sheds MRSA to the room environment. We evaluate the effect of dispersal across rooms by allowing MRSA dispersal and deposition on both patients' room surfaces. We also perform sensitivity analysis of the model parameters, as well as of the symmetrical transfer efficiency assumption.

6.2 Methods

6.2.1 The exposure pathway model

Based on an Environmental Infection Transmission System (EITS) framework, we constructed and analyzed an individual-based model of MRSA fate and transport between two hospital rooms including (1) MRSA shedding from a colonized patient, (2) MRSA transfer, deposition, and die-off on skin and hands and on room surfaces, (3) MRSA exposure to an uncolonized patient, and (4) two MRSA interventions: surface decontamination and hand hygiene.

The model consists of three types of entities: patients, nurses, and environmental surfaces in the patient rooms (Table 6.1). A colonized patient resides in one room, while an uncolonized patient resides in the other. Patients remain in the same rooms throughout the simulation. In each room, there is one porous surface and one nonporous surface. There are three nurses (NS). Each nurse works an eight-hour shift. Nurses stay in a nurses' center during shifts when they are not in a patient's room. The model keeps track of MRSA pathogen concentration (MRSA cfu per surface area) on each entity (patient, nurse, and surface). We use 2000 cm^2 to represent exposed skin and hands surface area for patients and room surfaces, and 300 cm^2 to represent a surface area for both hands of nurses.

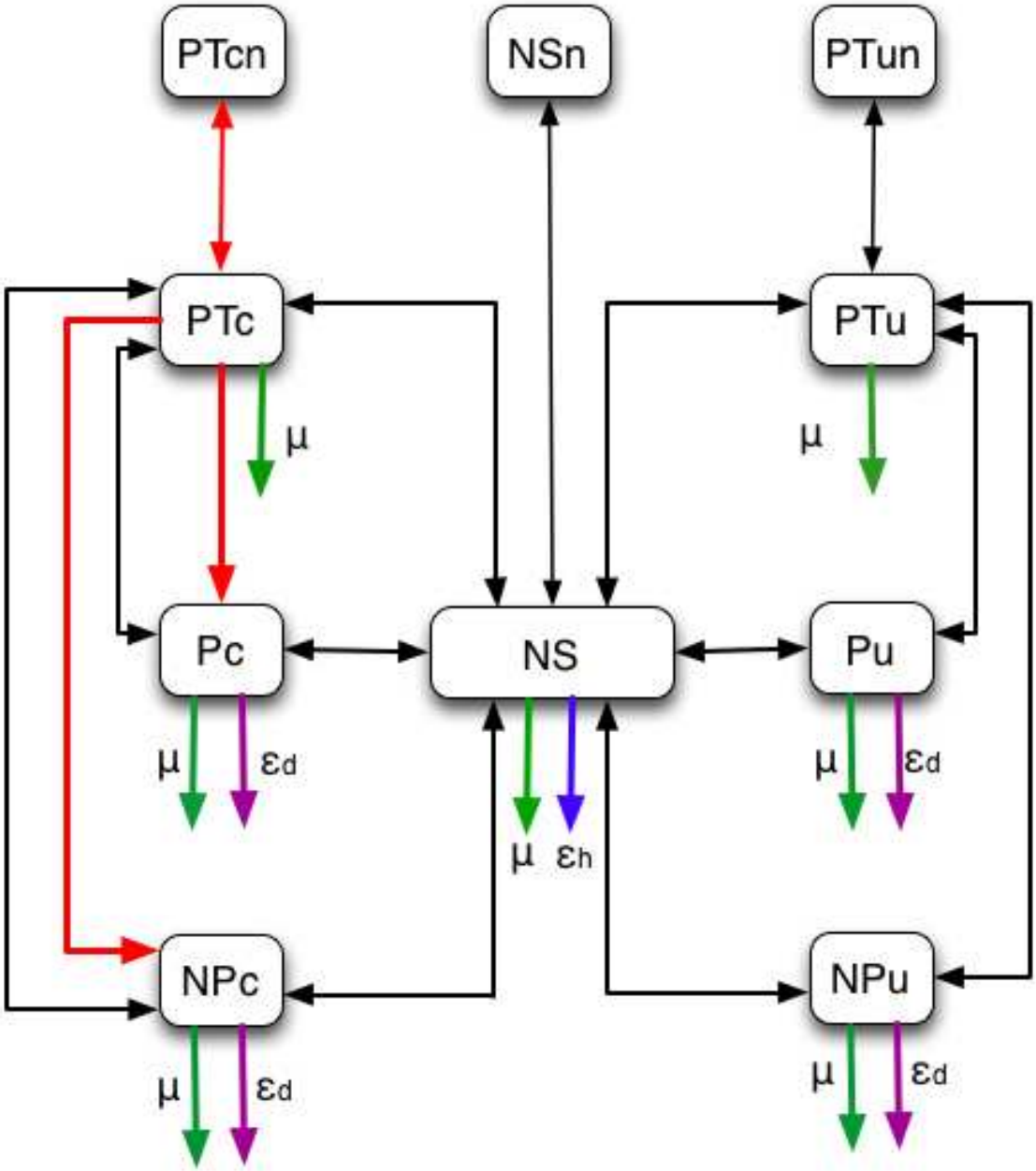
The model runs with discrete fixed time steps. It starts at the beginning of a nurse's shift at 8:00 a.m. and terminates after seven days. Each time step corresponds to two minutes. The simulation is repeated 100 times for each scenario. In the following sections, we describe in greater detail the model entities, events, and assumptions

inherent to this model. The model diagram, which captures the main entities and events, is in Figure 6.1.

Table 6.1: Model entities and their events

Entity	Sub-entity	Variables	Events
Patients	Colonized patient (PT_c)	1. MRSA concentration on the skin and hands 2. MRSA concentration in the nose	1. Shedding 2. Touching surfaces 3. Touching nose 4. Natural die off
	Uncolonized patient (PT_u)	1. MRSA concentration on the skin and hands 2. MRSA concentration in the nose	1. Touching surfaces 2. Touching nose 3. Natural die off
Nurse (NS)		1. MRSA concentration on both hands 2. MRSA concentration in the nose	1. Visiting patient's room 2. Touching surfaces 3. Touching the patient 4. Touching nose 5. Natural die off 6. Hand hygiene
Surfaces	1. The porous surface in the colonized patient's room (P_c) 2. The nonporous surface in the colonized patient's room (NP_c) 3. The porous surface in the uncolonized patient's room (P_u) 4. The nonporous surface in the uncolonized patient's room (NP_u)	1. MRSA concentration on the surface	1. Natural die off 2. Surface decontamination

Figure 6.1: The model diagram including the model entities and main events. The model entities include the exposed skin and hands of the colonized patient (PT_c), the porous surface in the colonized patient's room (P_c), the nonporous surface in the colonized patient's room (NP_c), the exposed skin and hand of the uncolonized patient (PT_u), the porous surface in the uncolonized patient's room (P_u), the nonporous surface in the uncolonized patient's room (NP_u), the nurses' hands (NS), the colonized patient's nose (PT_{cn}), the uncolonized patient's nose (PT_{un}), and the nurse's nose (NS_n). Black double arrowed lines are pathogen flows in and out of model entities due to touching events. Red double and single arrowed lines represent shedding and contamination process when the colonized patient touches the porous and nonporous surfaces and the nose. Green single arrowed lines represent pathogen flows out of the model entities due to the natural die off process, which are governed by the parameter, μ. Purple single arrowed lines arrows represent pathogen flows out of the entities due to the daily surface decontamination process, governed by the parameter, ε_d. The decontamination process affects the entire surface area of each surface. Blue single arrowed lines represent pathogen flows out of nurses following each hand hygiene event, governed by the parameter ε_h. This hand hygiene event affects both hands surface area, which is 300 cm^2.


PTcn
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6.2.2 Model entities

6.2.2.1 Patients

In this model each patient is described by two properties: (1) MRSA concentration on the exposed skin and hands and (2) the MRSA concentration in the nose.

The colonized patient can shed MRSA or touch room surfaces and their nose, while the MRSA naturally die-off on the skin and hands. The uncolonized patient cannot shed MRSA, but can perform the other events the same way as the colonized patient.

6.2.2.1.1 Initialization and the balance of colonized state

At the start of the simulation, the colonized patient is assigned an MRSA concentration in the nose and on the skin and hands. A number representing initial MRSA concentration in the nose is randomly drawn from a uniform distribution from 10 and 2,000 cfu/4 cm^2 [12, 13]. This concentration remains constant assuming the continual MRSA proliferation in the nose of the colonized patient. Another number representing initial MRSA concentration on the exposed skin and hand is randomly drawn from a uniform distribution between 10 and 100,000 cfu/2000 cm^2 to be the MRSA concentration on the skin and hands [26]. This concentration, however, changes during model execution due to touching events and die-off.

We assume that MRSA replenishes itself on the colonized skin with the same rate at which the colonized patient sheds MRSA onto the environmental surfaces. Shedding rate is assigned at 0.004 cfu/cm^2/min to represent a low shedding scenario and 0.04 cfu/cm^2/min as a high shedding scenario. The colonized patient loses MRSA through natural die-off and touching surfaces or nurses.

6.2.2.2 Nurses

Nurses are also described by two properties: (1) the MRSA concentration on both hands, and (2) the MRSA concentration in their noses.

Nurses work in eight-hour shifts, and at the beginning of each shift are assumed to have no MRSA on their hands and in the nose. Nurses can enter patient rooms, touch the patients, touch room surfaces, touch their own noses, and perform hand hygiene, while MRSA naturally dies off on their hands. When nurses are not in patient rooms, they are at the nurses' center, where we assume there is no surface touching occurring.

6.2.2.3 Room surfaces

Both porous and nonporous surfaces are described by their MRSA concentration levels. These levels change with the touching events and natural die-off. The surfaces may also be decontaminated daily at 8:00 a.m.

6.2.3 Model parameters

Literature review for model parameterization is in the Chapter III. Table 6.2 shows the model parameters and their values.

Table 6.2: Model parameters and their values in the baseline scenario.

	Symbol	Values	Reference
SHEDDING PARAMETERS:			
Shedding rate (cfu/cm^2/min)	α	0.004-0.04	[14-16]
SURVIVAL PARAMETERS:			
Die off rate on skin and hand (logcfu /min)	μ_{sk}	0.00353	[17]
Die off rate on porous surface (logcfu /min)	μ_{p}	0.000632	[18]
Die off rate on nonporous surface (logcfu /min)	μ_{np}	0.0002	[19]
CONTACTS PARAMETERS:			

Rate of patient touches surfaces (min^{-1})	$\tau_{pt\text{-}sf}$	0.134	
Rate of nurse touches patient (min^{-1})	$\tau_{ns\text{-}pt}$	0.4	
Rate of nurse touches surfaces (min^{-1})	$\tau_{ns\text{-}sf}$	0.4	
Rate of touching nose (min^{-1})	τ_n	0.025	
TRANSFER EFFICIENCY PARAMETERS:			
Transfer efficiency from porous surface to fingertip	ρ_p	0.1	[20]
Transfer efficiency from nonporous surface to fingertip	ρ_{np}	0.4	[21]
Transfer efficiency from skin to skin	ρ_{sk}	0.35	
Transfer efficiency from finger to nose	ρ_n	0.2	
SURFACE AREA PARAMETERS:			
Total exposed skin and hand surface area of patients (cm^2)	A_{pt}	2000	
Both hands surface area of nurses (cm^2)	A_{ns}	300	
Total porous surface area (cm^2)	A_p	2000	
Total nonporous surface area (cm^2)	A_{np}	2000	
Anterior nares (nose) surface area	A_n	4	
Hands surface area (cm^2)	A_h	300	
Fingertip surface area (cm^2)	A_f	1	
INTERVENTIONS:			
Hand-hygiene compliance	ε_{hc}	0.5	[1]
Hand-hygiene efficacy	ε_{he}	0.7	[22, 23]
Surface-decontamination efficacy	ε_d		

6.2.4 Model events

6.2.4.1 Shedding

The colonized patient sheds MRSA continuously onto the porous and nonporous surfaces in the colonized patient's room. This shedding quantity is governed by αA_p or αA_{np}. The shedding rate is assigned at 0.004 cfu/cm^2/min to represent a low shedding scenario and 0.04 cfu/cm^2/min as a high shedding scenario. In the baseline scenario, we assume shedding only affects the colonized patient's room. For sensitivity analysis we relax this assumption and allow MRSA dispersal and deposition in both room surfaces.

6.2.4.2 Nurse visiting patient rooms

At the beginning of each hour, a nurse will first visit the colonized patient and then visit the uncolonized patient. Once the nurse enters the colonized patient's room, a number representing the visit duration in minutes is randomly drawn from a uniform distribution between 10 and 30. When the visit ends, the nurse leaves the colonized patient's room to enter the uncolonized patient's room. Once in the room, another number is randomly drawn to determine the visit duration in the uncolonized patient's room.

6.2.4.3 Touching

While in the room, the nurse will touch the patient and the two surfaces in a random order. Similarly, the colonized patient and the uncolonized patients touch the two surfaces in a random order. Nurses and patients also touch their noses at a defined rate. All touching rates in Table 6.2 are converted to risks or probabilities of touching across two-minute periods.

In the model, there are two main types of contacts: direct contact when nurses' hands touch either the colonized or uncolonized patient, and indirect contact where hands of either nurses or patients touch the surfaces. For both types of contacts, we assume symmetric transfer efficiencies. For each touching event, there is a transfer of pathogens to and from the two contacting surfaces. For each direct contact as seen in Figure 6.2, there is a quantity of MRSA transferred from the nurse's hands to the patient ($NS*\rho_{sk}$) and vice versa there is a quantity of MRSA transferred from the patient to the nurse's hands ($PT_u*300/2000*\rho_{sk}$). These MRSA quantities depend on 1) the bacterial concentration at both contacting surface areas, 2) the contact surface areas (i.e. 300

sq.cm.), 3) the total surface areas (i.e. 2000 sq.cm. for patients and surfaces, and 300 sq.cm. for nurses' hands), and 4) transfer efficiency, which is a fraction of MRSA on the contacting surface area that is transferred. The net quantity and its direction depend on the difference of both quantities. Given that nurses are the source of MRSA in the uncolonized patient's room, the sum of these net quantities for all the direct contact events in an hour is then the hourly exposure dose to the uncolonized patient through direct contact route.

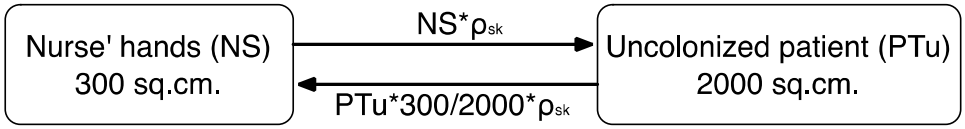


Figure 6.2: A direct contact event between nurses' hands (NS) and the uncolonized patient (PT_u). NS represents the concentration of MRSA cfu on nurses' hands (MRSA cfu/300 sq.cm.). PT_u represents the concentration of MRSA cfu on the uncolonized patient's skin (MRSA cfu/2000 sq.cm.). The contact surface area is 300 sq. cm. The transfer efficiency for the direct contact event (ρ_{sk}) is 0.35. The transfer efficiency of MRSA from nurses' hands to the uncolonized patient's skin is assumed to be the same as transfer efficiency from the uncolonized patient's skin to nurses' hands. Thus, MRSA quantity that is transferred from nurses' hands to the uncolonized patient's skin is NS*0.35. MRSA quantity transferred from the uncolonized patient's skin to nurses' hands is PT_u*(300/2000)*0.35.

For each indirect contact as seen in the example of the uncolonized patient (PT_u) touching the nonporous surface (NP_u) in Figure 6.3, there is a quantity of MRSA transferred from the uncolonized patient's hands to the nonporous surface (PT_u*300/2000*ρ_{np}), and vice versa there is a quantity of MRSA transferred from the nonporous surface to the uncolonized patient's hands (NP_u *300/2000*ρ_{np}). The net quantity and its direction depend on the difference of both quantities. This contact event

may result in an increase in contamination of the nonporous surface or increase exposure to the uncolonized patient.

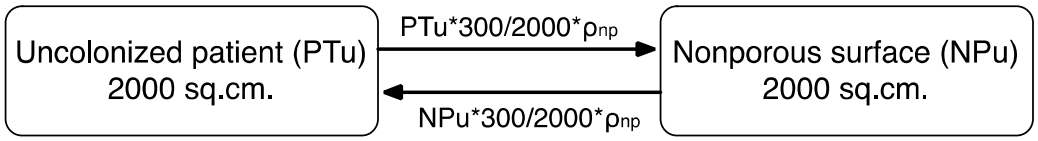


Figure 6.3: An indirect contact event between the uncolonized patient (PT_u) and the nonporous surface (NP_u). PT_u represents the concentration of MRSA cfu on the uncolonized patient's exposed skin and hands (MRSA cfu/2000 sq.cm.). NP_u represents the concentration of MRSA cfu on the nonporous surface (MRSA cfu/2000 sq. cm.). The contact surface area is 300 sq. cm. The transfer efficiency for contact event with nonporous surface (ρ_{np}) is 0.4. The transfer efficiency of MRSA from the uncolonized patient's hands to the nonporous surface is assumed to be the same as transfer efficiency from the nonporous surface to the uncolonized patient's hands. Thus, MRSA quantity that is transferred from the uncolonized patient's hands to the nonporous surface is PTu*300/2000*0.4. MRSA quantity transferred from the nonporous surface to the uncolonized patient's hands is NP_u*(300/2000)*0.4.

6.2.4.4 Natural die-off event or survivability of MRSA

MRSA on surfaces, patients, and nurses continuously decreases with fixed die-off rates depending on whether they are on the skin and hands, porous surfaces, or nonporous surfaces.

6.2.4.5 Hand-hygiene event

Nurses may perform hand hygiene before and after visiting a patient room. If a number randomly drawn from a uniform distribution between 0 and 1 is less than the defined hand-hygiene compliance probability, then hand hygiene will occur. When the event is executed, a fraction of pathogen (i.e. hand-hygiene efficacy) is removed from both hands surface area, which is 300 cm^2.

6.2.4.6 Surface decontamination

Surface decontamination may be scheduled on a daily basis at 8:00 a.m., affecting MRSA on both porous and nonporous surfaces of each room. For each decontamination event, a fraction of MRSA (i.e. decontamination efficacy) is removed from the entire surface area.

6.2.5 Model assumptions

1. The only MRSA source is the MRSA colonized patient. This colonized patient sheds MRSA onto environmental surfaces in the room by aerially dispersed MRSA contaminated skin squamous cells that instantaneously deposit on surfaces and by surface touching with contaminated hands.
2. The MRSA exposure pathways to the uncolonized patient are either by being touched by contaminated nurses' hands (hand-mediated route), or by touching contaminated room surfaces that result from nurses' touching (hand-to-surface contamination). In the sensitivity analysis, which allows MRSA aerial dispersal and deposition on the uncolonized patient's room surfaces, the uncolonized patient may also be exposed to MRSA by touching these environmental contaminations from MRSA dispersal and deposition (air-to-surface contamination).
3. Nurses are not colonized with MRSA and do not shed MRSA. Their hands only serve as vectors of the transmission process.
4. MRSA instantaneously and homogenously mixes on surfaces, skin, and hands.
5. Transfer efficiency is symmetrical for each contact. For example, in an event when hands touch a nonporous surface, 40% of MRSA presented on a 300 cm^2 contact

surface is transferred from hands to the nonporous surface, and 40% of MRSA presented on a 300 cm^2 from the nonporous surface is transferred to hands.

6. The total surface area for patients and surfaces is 2000 cm^2. The surface area for nurses' hands is 300 cm^2, which is a surface area of both palms and is used to represent contact surface areas in all types of contacts [37, 38].

6.2.6 Analysis of the simulated data

The initial conditions of all simulations are clean room surfaces, and no contamination on nurses or the uncolonized patient. The colonized patient has initial MRSA contaminations in the range of 10 to 100,000 cfu/2000 cm^2 on the exposed skin, and in the range of 10 to 2000 cfu/4 cm^2 in the nose. The shedding rate is assigned at 0.004 cfu/cm^2/min to represent a low shedding scenario and 0.04 cfu/cm^2/min as a high shedding scenario. Nurses have opportunities to perform hand hygiene at the entry and exit of a patient's room. Compliance may vary from 0%, 50% and 100%, and is the same at both hand hygiene opportunities. For each scenario, the reported measures are the averages from 100 simulations.

The simulation outcome is different in each room. In the colonized patient's room the outcomes are the contamination levels on nurses' hands, and the hourly-cumulated net MRSA quantities resulting from nurses' contacts with the colonized patient and the two room surfaces. Whereas in the uncolonized patient's room the outcome measures are the exposure dose to the uncolonized patient and the resulting contamination levels of the room surfaces from nurses' touching. The exposure dose is the hourly-cumulated net MRSA quantities resulting from the uncolonized patient's contacts with nurses and room surfaces.

6.2.7 Sensitivity Analysis

To examine the primary assumption that aerially dispersed MRSA only confines depositions within the colonized patient's room, we relaxed this assumption by allowing MRSA dispersal and deposition on the uncolonized patient's room surfaces. We examined two levels of dispersal, i.e. 1% and 10%. The reference scenario with no dispersal is a scenario where the colonized patient has a high shedding rate of 0.04 cfu/cm^2/min, and nurses perform 100% hand hygiene compliance with 70% efficacy. A scenario with 1% dispersal means there is 0.0004 cfu/cm^2/min MRSA deposition on the uncolonized patient's room surfaces, and 0.0396 cfu/cm^2/min MRSA deposition on the colonized patient's room. By relaxing this assumption, we can examine the effect of hand hygiene in settings where environmental contaminations originate from both 1) hands-to-surface contamination, as well as 2) air-to-surface contamination.

To examine the assumption of symmetrical transfer efficiency between hands and surfaces, we relaxed this assumption and set the transfer efficiency from hands to surfaces to be 1% and 10% of hand hygiene efficacy, i.e. 0.07 and 0.007. We keep the symmetrical assumption between hands and skin when nurses touch patients. The reference scenario with symmetrical transfer efficiency is the scenario where the colonized patient has a high shedding rate of 0.04 cfu/cm^2/min, and nurses perform 100% hand hygiene compliance with 70% efficacy.

Also, we perform sensitivity analysis of model parameters including die-off rates, transfer efficiencies, and contact rates, to the exposure dose to the uncolonized patient. We evaluate the effect of touching frequency by assigning the two room surfaces to be frequently touched and infrequently touched nonporous surfaces. Nurses and patients touch these two surfaces according to frequently and infrequently touch rates. We then

compare the MRSA concentration of these two surfaces in each room and their contributions to the uncolonized patient exposure.

6.3 Results

6.3.1 Effect of hand hygiene compliance to nurses' hands contamination levels in low (0.004 cfu/cm^2/min) and high (0.04 cfu/cm^2/min) shedding scenarios.

Figure 6.4 shows average MRSA contamination levels on nurses' hands in 6 different simulated conditions over time. In these simulations nurses touch the patient and the two room surfaces in a random order while in the room. All lines show small jagged patterns and large decreases every 24 hours. The 24 hour pattern corresponds with the scheduled daily surface decontamination, reflecting the correlation of surface contamination and the contamination on nurses' hands. In comparing the two shedding scenarios, as the colonized patient sheds with a higher rate and contaminates the colonized patient's room surfaces, the nurses' hands also become more contaminated compared to lower shedding scenarios. It is noted that in the high shedding scenario, with an idealistic 100% compliance, nurses' hands are even more contaminated than when shedding rate is low and compliance is 0%. This finding emphasizes the need for hand hygiene after touching patient's surrounding since contamination on hands can be influenced by the contextual contamination within the room as long as nurses continue to touch those surfaces.

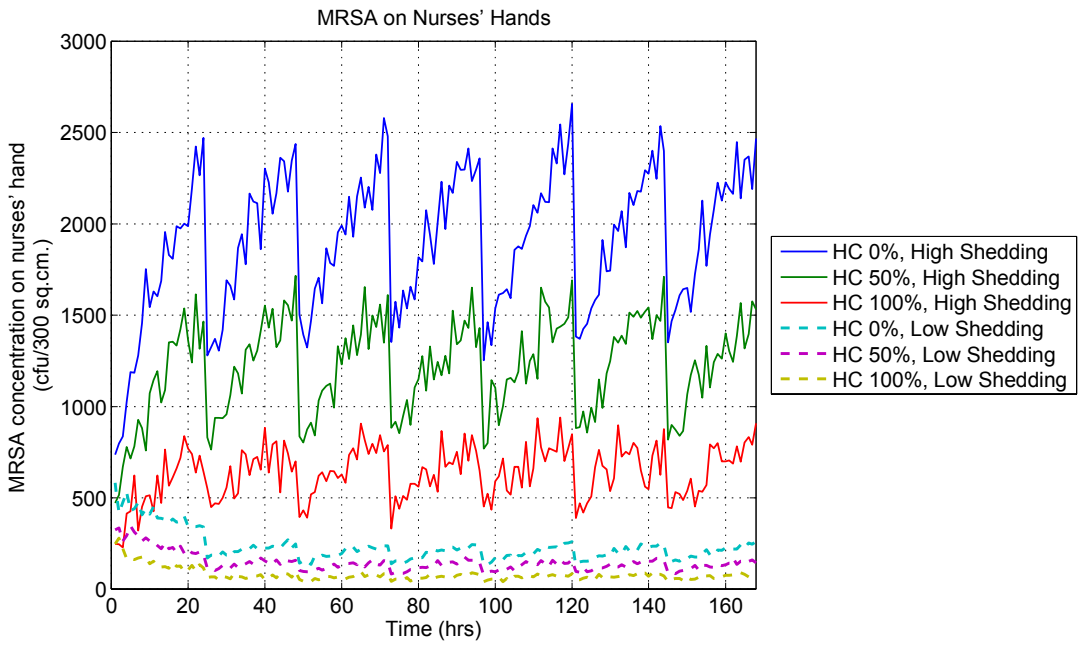


Figure 6.4: Comparison of MRSA contamination levels on nurses' hands in six simulation settings at low (0.004 cfu/cm^2/min) shedding rate in dashed lines and high (0.04 cfu/cm^2/min) shedding rate in solid lines and three hand hygiene compliance levels of 0%, 50% and 100%. Hand hygiene opportunities are at entry and exit of a patient's room. The simulation setting is in two hypothetical hospital rooms where nurses touch the patient and the room surfaces in a random order.

6.3.2 Comparison of the contamination sources to nurses' hands while nurses perform 100% hand hygiene compliance at the entry and exit of a patient's room

In this model, nurses touch the patient and the two room surfaces four times each in a random order during each patient visit. Each contact results in bidirectional flow of MRSA. One flow is from the patient or the surfaces to nurses' hands and the other is from the nurses' hands to the patient and the surfaces. The hourly sum of the net quantity of these bidirectional flows from each type of contact is displayed in Figure 6.5. A positive quantity represents a net MRSA flow to nurses from the patient or the surfaces. A negative quantity represents a net MRSA flow from the nurses to the patient or the surfaces. The spike of MRSA transferred to nurses from the colonized patient in the first

hour of simulation is due to the broad range of initial MRSA concentrations on the colonized patient's skin.

At the beginning of each day, which is the time of daily decontamination and the beginning of a nurse shift, there is a regular pattern of increase in MRSA quantity transfer to the nurse from the colonized patient. This finding is related to the largest difference of bidirectional flows between nurses' hands and the colonized patient. At this time, nurses' hands and the room surfaces have the least contamination levels. Therefore, nurses receive the largest net quantity from contacts with the colonized patient. Also, nurses contaminate the surfaces as a result of their contacts during these initial hours of the day as seen in the negative quantities of the Y-axis.

Also shown in this figure is that the room surfaces are the dominant contamination sources to nurses, more than the colonized patient. The net MRSA quantities to nurses from contacts with the nonporous surface are higher than from contacts with the porous surface and from contacts with the colonized patient. This finding is due to the differences in contamination levels on the colonized patient and the room surfaces, as well as the differences of transfer efficiency of the nonporous surface (i.e. 0.4), the porous surface (0.1) and the skin (0.35) to hands.

Figure 6.6 shows the comparison of MRSA concentrations per contact surface (cfu/300 sq.cm.). The colonized patient's room surfaces have higher MRSA concentrations than the colonized patient. Parameter sensitivity analysis suggested that this finding is contributed to the survivability, which is much longer on surfaces than on human skin. Die-off rates for porous, nonporous surfaces and the skin are 6.32×10^{-4}, 2×10^{-4} and 3.53×10^{-3} logcfu/min. When comparing the two surfaces, the porous surface

has a smaller transfer efficiency, so smaller net quantities transferred to nurses' hands allow higher accumulation of MRSA contamination over time.

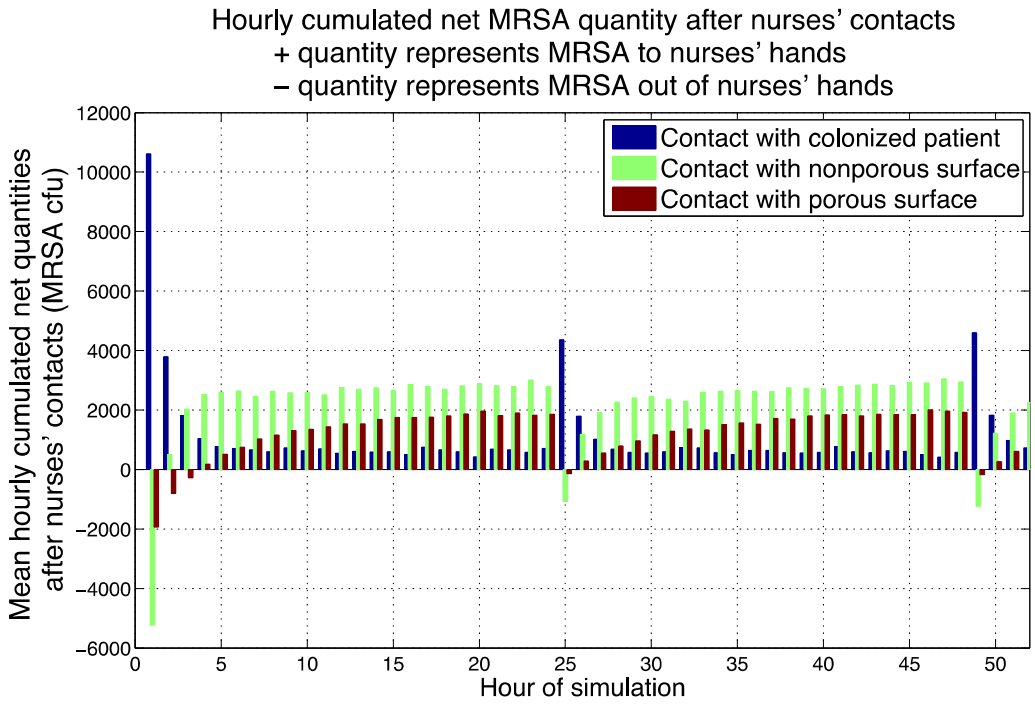


Figure 6.5: Comparison of mean hourly-cumulated net quantities after nurses' contacts with the colonized patient, the nonporous and porous surfaces in the colonized patient's room. The + quantity in Y-axis represent the MRSA quantity to nurses' hands, and the – quantity in Y-axis represent the MRSA quantity out of nurses' hands. The simulation setting is where the colonized patient sheds with a high shedding rate (0.04 cfu/cm^2/min) and nurses perform 100% hand hygiene compliance at the entry and exit of a patient's room.

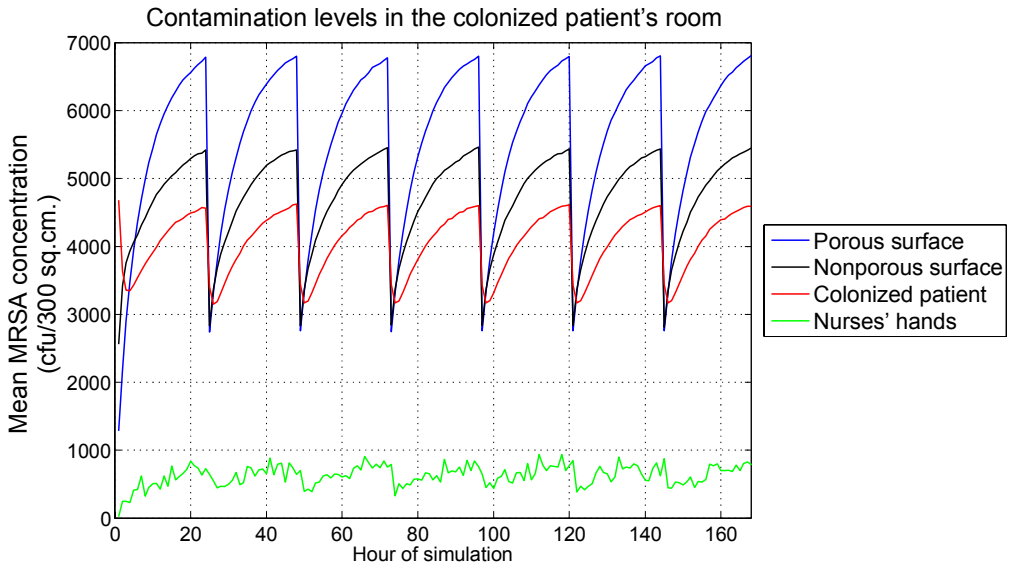


Figure 6.6: Comparison of contamination levels of the colonized patient, nurses' hands and the room surfaces in the colonized patient's room. The simulation setting is where the colonized patient sheds with a high shedding rate (0.04 cfu/cm^2/min) and nurses perform 100% hand hygiene compliance with 70% efficacy at the entry and exit of a patient's room. The concentrations are the averages of 100 simulations.

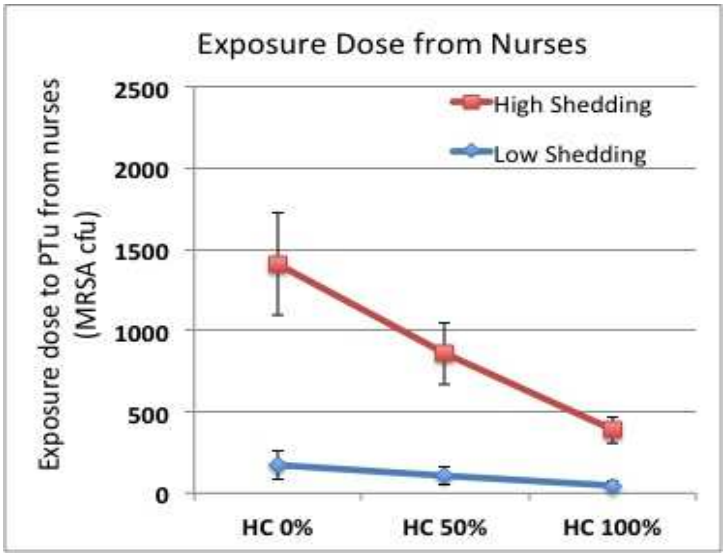
6.3.3 Effect of hand hygiene compliance in suppressing exposure doses to the uncolonized patient in low (0.004 cfu/cm^2/min) and high (0.04 cfu/cm^2/min) shedding scenarios.

In this simple structure of two hypothetical hospital rooms where nurses are the only source of MRSA into the uncolonized patient's room and nurses exhibit varying hand hygiene compliance with 70% efficacy at the entry and exit of a patient's room, figure 6.7 shows the exposure doses transfer to the uncolonized patient. Of note, the uncolonized patient receives MRSA predominantly from nurses. The nonporous surface in the uncolonized patient's room, which becomes contaminated from nurses' touching, also contributes to contamination of the uncolonized patient when the patient touches the nonporous surface.

Figure 6.7a shows that the exposure doses from nurses decrease substantially when compliance increases from 0% to 100%, but not completely due to the imperfect hygiene efficacy of 70%. It is noted that in settings where the colonized patient sheds with a high shedding rate where nurses perform 100% hand hygiene compliance, exposure to the uncolonized patient from nurses is higher than in settings with low shedding rate where nurses' compliance is 0%.

Figure 6.7b shows similar patterns with 6.7a. As hand hygiene compliance increases, the nonporous surface becomes less contaminated and transfers less exposure doses to the uncolonized patient. With the assumption of no MRSA dispersal and depositions on surfaces (air-to-surface contamination), the origin of room surface contamination is all from nurses' hands (hand-to-surface contamination). This figure shows that the effect of nurses' hand hygiene not only decreased exposure doses to the uncolonized patient from nurses' hands (hand mediated route), but also decreased exposure doses from contaminated surfaces (hand-to-surface contamination). A large variability of the exposure is related to the fluctuation of contamination after the daily surface clean.

a.



b.

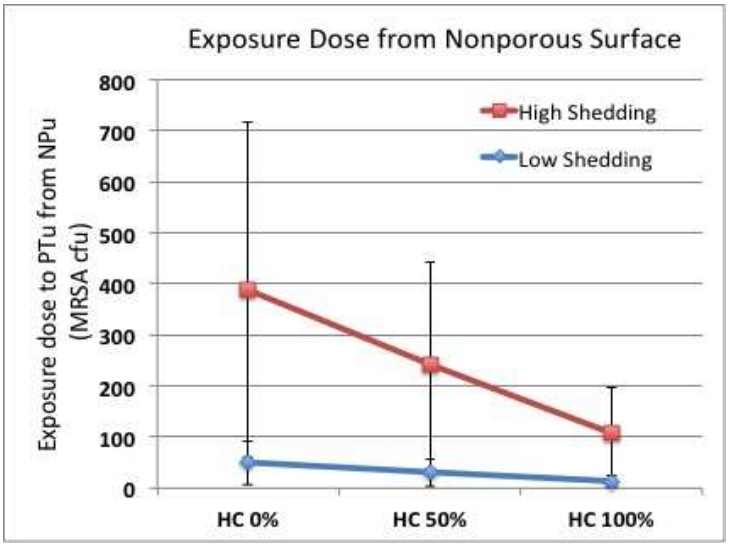


Figure 6.7a and 6.7b: A comparison of the exposure dose to the uncolonized patient (PT_u) from nurses (hand-mediated route) in 6.7a and from the nonporous surface (NP_u) in the uncolonized patient's room (hand-to-surface route) in 6.7b. Simulation scenarios are with low (0.004 cfu/cm^2/min) and high (0.04 cfu/cm^2/min) shedding rates and three hand hygiene compliance (HC) levels at 0%, 50% and 100%. Hand hygiene opportunities are at the entry and exit of a patient's room. The exposure dose is the hourly-cumulated net MRSA quantities resulting from the uncolonized patient contacts with nurses and nonporous surfaces.

6.3.4 Sensitivity analysis

6.3.4.1 Effect of 100% hand hygiene compliance when relaxing the assumption of no MRSA dispersal in the uncolonized patient's room

To examine the impact of the no dispersal across rooms assumption, we allow 1% and 10% MRSA aerial dispersal to deposit on the uncolonized patient's room surfaces. The reference scenario is where the colonized patient sheds in high shedding rate (0.04 cfu/cm^2/min) and nurses perform 100% hand hygiene compliance with 70% hygiene efficacy at the entry and exit of a patient's room. A scenario with 1% dispersal means there is 0.0004 cfu/cm^2/min MRSA deposits on the uncolonized patient's room surfaces, and 0.0396 cfu/cm^2/min MRSA on the colonized patient's room surfaces.

Figure 6.8 shows the impact of dispersal to contaminations in the uncolonized patient's room surfaces, the uncolonized patient and nurses. Small increases are seen with 1% dispersal. As dispersal and direct MRSA deposition on surfaces increases, the porous surface appears to show similar characteristics as in the colonized patient's room porous surface. That is, it collects and accumulates higher MRSA concentrations than the nonporous surface. The sum of the direct deposition of MRSA and the contamination from nurses touches exceeds the smaller MRSA quantity that is transferred from the porous surface to hands, due to the smaller transfer efficiency of porous surfaces. This net quantity leads to higher MRSA concentrations on the porous surface compared to the nonporous surface in a setting with 10% dispersal. Nevertheless, despite a higher contamination of the porous surface, the exposure dose to the uncolonized patient from the porous surface is much smaller than from the nonporous surface as seen in Figure 6.9.

Although the total MRSA loads to both rooms in the three scenarios are the same, we note that nurses have higher contamination in 10% dispersal scenario compared to both a 1% and a no dispersal scenario, as seen in Figure 6.8. This finding may be explained by the contact process where there are bidirectional flows of pathogens for every touch. As surfaces and the uncolonized patient have higher MRSA contaminations in a 10% dispersal scenario, the net flows from nurses to surfaces and to the uncolonized patient decrease. This allows nurses to retain higher MRSA, when compared to 1% and no dispersal. Nevertheless, Figure 6.9 shows that this increase in nurses' hands contamination does not lead to an increase in exposure doses to to the uncolonized patient, when compared to 1% and no dispersal scenarios.

Allowing aerial MRSA dispersal and deposition onto the uncolonized patient's surfaces means increasing air-to-surface contamination. Figure 6.9 shows the variation in the effect of 100% hand hygiene compliance in settings with high air-to-surface contamination (10% dispersal), low air-to-surface contamination (1% dispersal) and only hand-to-surface contamination (no dispersal). As air-to-surface contamination increases in a 10% dispersal scenario, the effect of hand hygiene in suppressing exposure dose decreases. The exposure doses from the nonporous surface and the porous surface to the uncolonized patient are higher with 10% dispersal, compared to 1% and no dispersal scenarios.

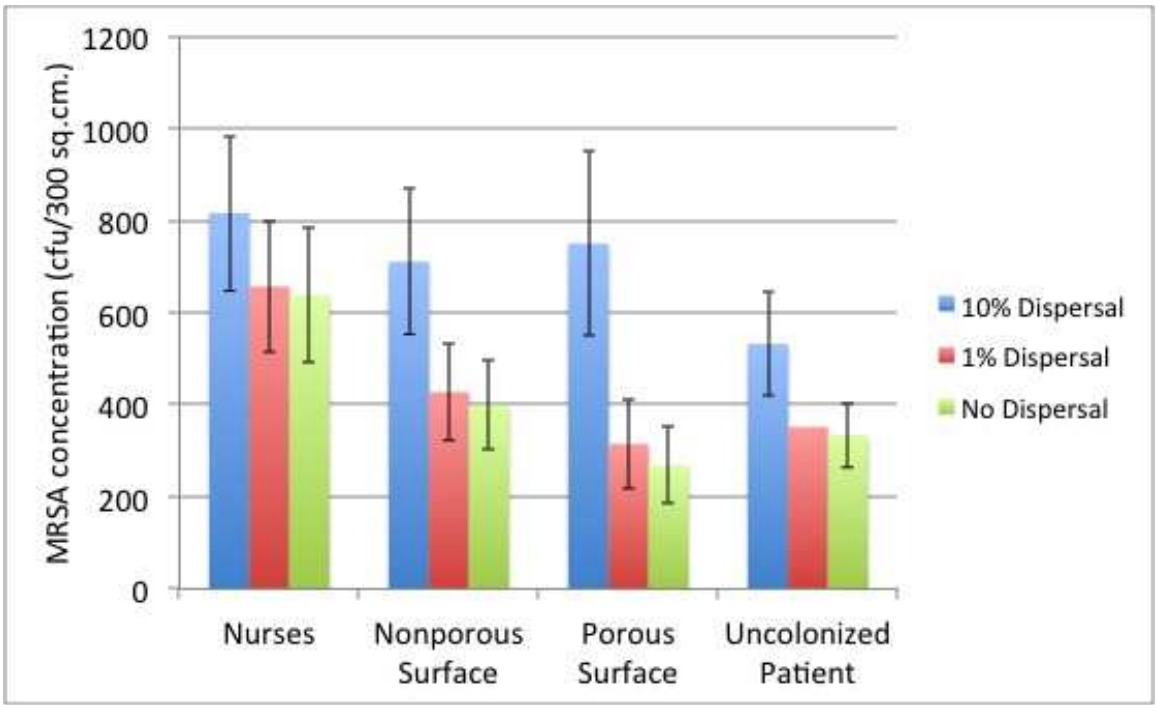


Figure 6.8: Comparison of MRSA concentrations on nurses and in the uncolonized patient's room in settings where there are no dispersal, 1% dispersal and 10% dispersal to the uncolonized patient's room. In these simulations, nurses perform 100% hand hygiene compliance with 70% efficacy at the entry and exit of a patient's room. In a scenario with no dispersal, the colonized patient sheds at the rate of 0.04 cfu/cm^2/min on the colonized patient's room surfaces. A scenario with 1% dispersal means there is 0.0004 cfu/cm^2/min MRSA dispersal and deposition on the uncolonized patient's room surfaces, and 0.0396 cfu/cm^2/min MRSA deposition on the colonized patient's room surfaces.

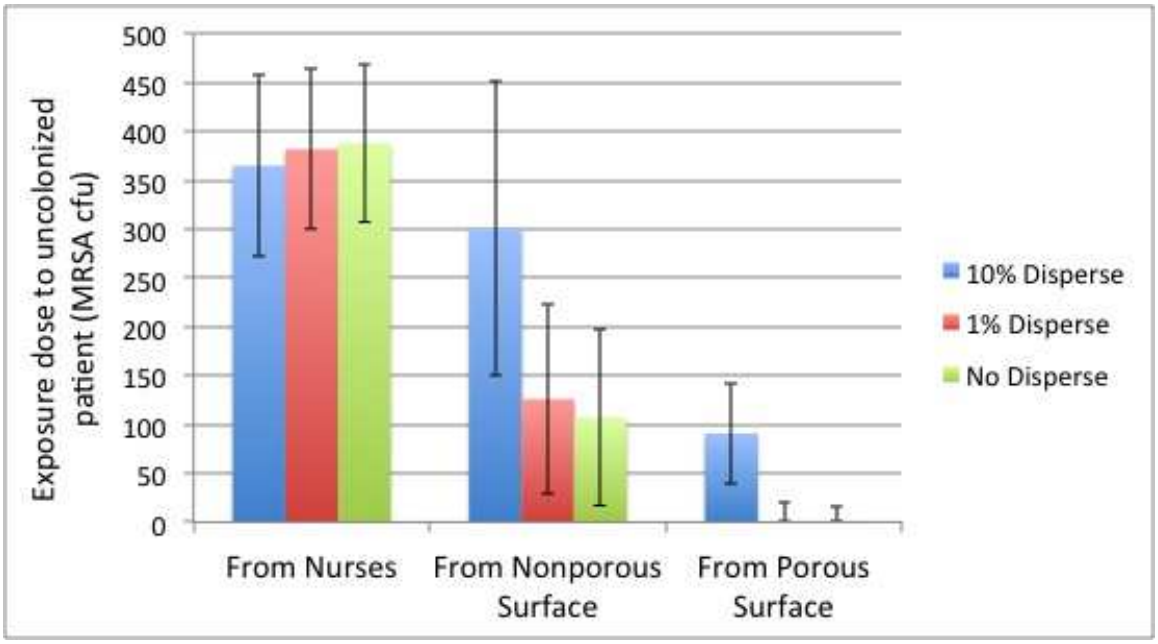


Figure 6.9: Comparison of MRSA exposure doses to the uncolonized patient from nurses and the room surfaces in the uncolonized patient's room in settings where there is no dispersal, 1% dispersal and 10% dispersal to the uncolonized patient's room. In these simulations, nurses perform 100% hand hygiene compliance with 70% efficacy at the entry and exit of a patient's room. In a scenario with no dispersal, the colonized patient sheds at the rate of 0.04 cfu/cm^2/min on the colonized patient's room surfaces. A scenario with 10% dispersal means there is 0.0004 cfu/cm^2/min MRSA dispersal and deposition on the uncolonized patient's room surfaces, and 0.0396 cfu/cm^2/min MRSA deposition on the colonized patient's room surfaces.

6.3.4.2 Effect of 100% hand hygiene compliance when relaxing the assumption of symmetrical transfer efficiency

The model assumes symmetrical transfer efficiency between hands and surfaces, as well as between hands and skin. To relax this assumption, we set the transfer efficiency from hands to surfaces to be 1% and 10% of hand hygiene efficacy (i.e. 0.007 and 0.07). Transfer efficiency between nurses' hands and the patients in both directions remains the same, which is 0.35. The reference scenario is where the colonized patient sheds with a high shedding rate (0.04 cfu/cm^2/min) and nurses perform 100% hand hygiene compliance with 70% hygiene efficacy at the entry and exit of a patient's room.

In the reference scenario, transfer efficiency from nurses' hands or patients to the nonporous surface is 0.4, and transfer efficiency from nurses' hands or patients to the porous surface is 0.1.

With the assumption of no dispersal and deposition in the uncolonized patient's room, the only source of surface contamination is through contaminated nurses' hands. Thus, as transfer efficiency from hand to surface decreases, contamination levels on surfaces decreases as in Figure 6.10 and the exposure doses from the nonporous surface decreases as in Figure 6.11.

On the contrary, the decrease in transfer efficiency from hands to surfaces allows for higher accumulation of MRSA on nurse's hands, which later transfer to the uncolonized patient. As noted in Figure 6.11, there is an increase in hand-mediated route exposure to the uncolonized patient as transfer efficiency from hands-to-surface decreases.

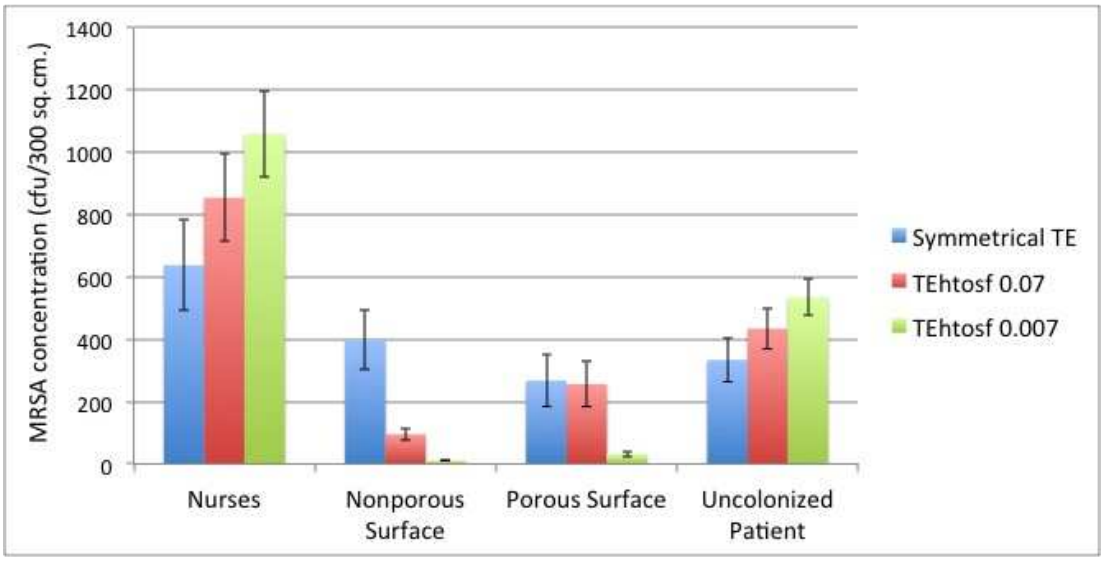


Figure 6.10: A comparison of MRSA concentrations on nurses and in the uncolonized patient's room in a reference scenario with symmetrical transfer efficiency (TE), and scenarios where transfer efficiency from hands to surfaces (TEhtosf) is set at 0.07 and 0.007. In these simulations, nurses perform 100% hand hygiene compliance with 70%

efficacy at the entry and exit of a patient's room. The colonized patient sheds at the rate of 0.04 cfu/cm^2/min on the colonized patient's room surfaces, with no dispersal to the uncolonized patient's room.

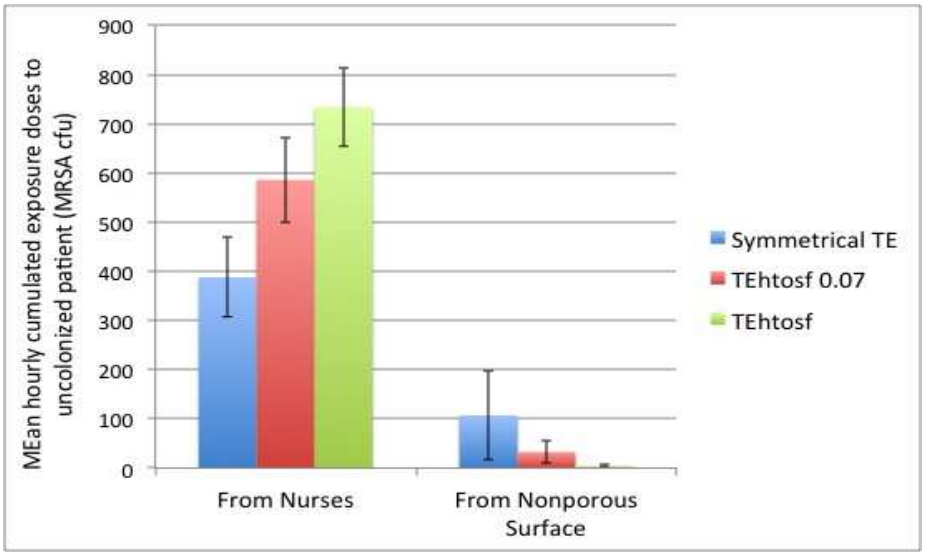


Figure 6.11: A comparison of MRSA exposure doses to the uncolonized patient from nurses and the nonporous surface in the uncolonized patient's room in reference scenario with symmetrical transfer efficiency (TE), and scenarios where transfer efficiency from hands to surfaces (TEhtosf) is set at 0.07 and 0.007. In these simulations, nurses perform 100% hand hygiene compliance with 70% efficacy at the entry and exit of a patient's room. The colonized patient sheds at a rate of 0.04 cfu/cm^2/min on the colonized patient's room surfaces, with no dispersal to the uncolonized patient's room.

6.4 Discussion

Using an EITS framework in two hypothetical hospital rooms, we demonstrate that the healthcare workers' compliance is essential in determining the effectiveness of hand hygiene, although the time when it is performed and its efficacy are also important. The model emphasizes the significance of the hand hygiene opportunity before and after touching the patient's surrounding environment, in addition to at the entry and exit of a patient's room. Despite 100% compliance at the entry and exit of a patient's room, we show that the contaminated environmental surfaces are the dominant contamination

sources to nurses' hands in the colonized patient's room. Additionally, this model shows the value of hand hygiene efficacy. With 100% compliance and 70% efficacy, in the uncolonized patient's room, nurses' hands remain contaminated enough to subsequently contaminate the patient's environment, which later become another exposure route to the uncolonized patient. Our model revealed that when surfaces become contaminated from aerially dispersed MRSA in addition to contamination from nurses' hands touching, the total environmental mediated route can be exaggerated. For hand hygiene to be effective in suppressing exposure to the uncolonized patient, healthcare workers' compliance, high efficacious techniques, and hand hygiene opportunities need to be considered.

While several aspects of hand hygiene can alter its benefit, this model also shows that the surrounding environmental contaminations can impact the effect of hand hygiene. Even with 100% hand hygiene compliance, the exposure dose to the uncolonized patient is higher in a scenario where the colonized patient sheds with a high shedding rate resulting in high surface contamination, compared to a low shedding scenario. A high shedding magnitude has been shown to be associated with carriers who are colonized at certain body sites such as at the perineum or in the gastrointestinal tract, or in patients with burns or wound infections [24-28]. A study to investigate the relationship between patients' MRSA colonization body sites and the frequency of environmental contamination suggested that MRSA colonization of the groin area correlates most strongly with environment contamination [26]. An earlier study in 1964 screened 3,508 patients admitted to a medical ward and showed that perineal carriers were not uncommon, found in 13% of screened patients [29]. A dissertation in 1965 performed bacterial quantification of *S. aureus* carriers at various body sites including nose, throat,

different skin areas (hand, finger, ear, lip, axilla, perineum and wound), vagina, feces and surrounding air. One of the main conclusions from this study was that the heaviest dispersers were among the perineal carriers who were able to disperse far greater numbers of staphylococci into the air than the nasal carriers [30]. Although early detection of these high shedder individuals may allow early isolation, active surveillance programs in the U.S. typically only include nasal swabs [31]. Not all nasal carriers are perineal carriers. In screened populations who had negative nasal swabs, 4-25% may be perineal carriers [29, 32]; among perineal carriers, 50-70% are also nasal carriers [30]. A cost-effectiveness analysis study suggested the use of chromogenic agar screening of multiple body sites to maximize the identification of MRSA carriers [33]. The successful search and destroy strategy of the Netherlands also used multiple body sites screening including nose, throat, perineum, feces, sputum (if present), urine (in the event of a bladder catheter), skin lesions, and wounds [34].

In our sensitivity analysis, relaxing the MRSA dispersal assumption allows us to further evaluate the difference between hand-to-surface contamination and air-to-surface contamination. With no MRSA dispersal in the uncolonized patient's room, hand hygiene at the entry and exit of a patient's visit can significantly decrease hand-to-surface contamination depending on hand hygiene efficacy. Whereas in settings with MRSA dispersal and deposition on the uncolonized patient's room surfaces, hand hygiene at the entry and exit of a patient's visit has a limited effect in decreasing exposure to the uncolonized patient from this air-to-surface contamination.

Aerial dispersal across rooms is likely not a rare event. Prospective studies that performed surveillance of both patients and environments found indistinguishable MRSA

strains between patients and their environment. These strains were not all confined to the immediate vicinity of the colonized patient [35, 36]. Contamination of the same strains was seen in different patients' rooms. A study that monitored *S. aureus* colony count on surfaces in surgical wards found that counts of *S.aureus* varied according to bed occupancy, with the highest counts (over 2.5 cfu/cm^2) associated with bed occupancies >95% [37]. A study that collected both clinical and air sampling from 0.5, 1, and 2-3 meters from the patients showed no decreasing trend in MRSA cfu counts with increasing distances (0.5 to 3 meters) [15]. These findings confirm that dispersal could occur farther than near to patient sites. However, quantifying the dispersal may not be straightforward as it may be influenced by many factors such as the patient's activity, the airflow in the ward, and the healthcare workers' activities [38, 39]. Healthcare workers' gowns and uniforms are known to carry and disperse MRSA in the air, regardless of their colonization status [40].

All models have limitations in that they represent a simple view of a real-world complex system and require the simplifying of assumptions. Therefore, inferences from any model rely heavily on the underlying assumptions. While there is an enormous amount of available literature that can support choices of model parameters, parameterization still requires further assumption. The same parameters may be used differently depending on model forms and research questions. The transfer efficiency is a key parameter in contact-mediated exposure assessment. It is defined as a fraction of pathogens transferred from one surface to another contacting surface after each touch [21]. Within the EITS community, only a few experiments have been used and referred to [20, 21, 41]. One experiment measured the quantity of bacteria from contaminated fabrics

to a clean fingertip [20]. The other measured the quantity of bacteria transferred from contaminated surfaces to a clean hand, as well as the quantity of bacteria transferred from a contaminated fingertip to a clean lip [21]. These data are informative and specific to various surfaces. Nevertheless, they require additional assumptions in order to use in the EITS model.

In our model there are bidirectional MRSA flows between two contacting surfaces. Thus, we assume that the transfer efficiency between clean and contaminated surfaces as calculated from the experiments is the same transfer efficiency between two contaminated surfaces used in the model. We assume that the unidirectional transfer efficiency from surfaces to hands from the experiments is the same as those from hands to surfaces. Similarly, we assume that the unidirectional transfer efficiency from fingertip to lip is the same as those from lip to fingertip, from hand to skin and from skin to hand.

The original EITS model was a simpler model of a more complex system, compared to this model [42]. The 2009 model used the EITS framework to analyze influenza transmission using 5 parameters. The average transfer efficiency of porous and nonporous surfaces, combined with 10 other factors were used to estimate a composite parameter called the 'pick up rate', which is the number of pathogens picked up by a single person per day based on the breathing and touching rates. Another EITS model also analyzed influenza transmission using the average transfer efficiency of porous and nonporous surfaces (0.1)[43]. This latter 2010 model assumed equal transfer efficiencies from hand to surface and from surface to hand, similar to this model. More recent EITS work in influenza examined hand-to-fomite and droplet-to-fomite contact mediated transmission [44]. This study used a transfer efficiency in the mid-range between porous

and nonporous surfaces (0.2). This work briefly included sensitivity analysis of asymmetrical transfer efficiency. It showed that varying transfer efficiency from hand to surface from 0 to 0.5 does not substantially affect the fomite-mediated transmission. Nevertheless, influenza and *S. aureus* largely differ in their inherit property to survive on hands as well as in the environment. Inactivation rates of influenza in air, surfaces and on hands are 0.006, 0.01, and 0.92 min^{-1}, respectively [45, 46], which are all much quicker than those of *S. aureus*. Inference in regards to the mode of transmission is also pathogen specific. Although hand-to-surface contamination may appear to play a small role for influenza due to shorter survivability on hands, our model shows that nurses' hands play a role in spreading contamination to surfaces in the uncolonized patient's room. These surfaces subsequently become an exposure route to the uncolonized patient.

The role of hand-to-surface contamination, however, is sensitive to the underlying assumption of symmetrical transfer efficiency. While it may appear unrealistic to assume such symmetry, this symmetrical assumption allows us to understand the different behaviors of contact surfaces due to the inherit differences in transfer efficiency. However, allowing symmetrical transfer efficiency also means allowing a fraction (0.4) of MRSA transfer from hands to nonporous surfaces. This fraction is relatively high when compared to the hand hygiene efficacy of 0.7, an average between soap and water and alcohol-based hand solution [22, 23]. We then performed sensitivity analysis to relax this symmetrical assumption by assigning transfer efficiency from hand to surface as 1% and 10% of hand hygiene efficacy (i.e. 0.007, 0.07). Assuming no MRSA dispersal across rooms, our finding shows that as transfer efficiency from hand to surface

decreases, the hand-to-surface contamination decreases and environmental mediated exposure to the uncolonized patient decrease.

From our analyses we demonstrated that the effect of hand hygiene at the entry and exit of a patient's room can decrease exposure from both the hand-mediated and hand-to-surface contamination routes. However, exposure from the air-to-surface contamination route could still be a threat to the uncolonized patient.

To further develop and gain insights from the EITS model, more experimental data is needed to support model parameters as well as environmental surveillance studies to quantitatively measure aerial dispersal in hospital settings. Compared to the previous EITS models, this model has more realistic features for hypothetical hospital rooms with patients, nurses and room surfaces. However, for the purposes of simplification, we made an important but unrealistic assumption that will need further exploration: we assume healthcare workers are only vectors of transmission with no capability to shed MRSA. Having nurses who can shed MRSA may increase exposure to the uncolonized patient both by direct hand mediated route, and by environmental mediated routes, either hand-to-surface contamination or air-to-surface contamination. In endemic settings, 2% to 15% of healthcare workers are known to carry MRSA [47]. Moreover, a survey showed 22% of male medical students to be perineal carriers [48]. Excluding nurses and other healthcare providers as potential shedders simplified the model and allowed us to improve our insight of patient-to-patient transmission. However, it may underestimate the exposure assessment overall.

In summary, this study has demonstrated that hand hygiene compliance is important in reducing MRSA exposure to the susceptible patient. Moreover, the

opportunities to perform hand hygiene before and after touching a patient's surrounding environment, as well as its efficacy, are also essential. Contaminated nurses' hands may transfer MRSA from direct contact with the uncolonized patient or transfer MRSA to surrounding surfaces, which subsequently become an exposure source to the patient. Also, we showed the impact of having MRSA aerial dispersal in the uncolonized patient's room. The effect of this air-to-surface contamination route can significantly increase total exposure to the uncolonized patient. MRSA infection control planning will need to emphasize both hand-based and environmental-based interventions.

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CHAPTER VII

Conclusions and Future Directions

7.1 Summary

The original motivation of this dissertation was to pursue a risk analysis model of MRSA transmission in a 20-bed intensive care unit. The model was intended to help provide infection control inferences and to help define what further data was needed to make these inferences. However, along the path of model development, it became apparent that there is a need to first understand the fate and transport processes. Thus, we elected to perform an exposure assessment in Chapters V and VI as a first step towards a future risk analysis model that can generate the data as in Chapter IV.

Our exposure assessment contributed further insight into the implication of the contaminated environment in the transmission of MRSA. This was achieved by the use of the Environmental Infection Transmission System (EITS) principle, which allows incorporation of three essential elements in infection transmission, which are the host, pathogen and environment. This dissertation also demonstrated that insight could be improved by keeping the model simple, as we have used two hypothetical hospital rooms instead of a full 20-bed unit.

7.1.1 Chapter III

From the literature review there is substantial evidence supporting environmental mediation of *S. aureus* transmission. *S. aureus* is a greatly adaptable commensal organism, as well as a major human pathogen. Colonized or infected individuals can shed *S. aureus* into air through contaminated skin scales. Several important features in regards to *S. aureus* shedding should be noted. First, there is a great variability of the shedding magnitude both within and between individuals. Some individuals appear to have a heavy dispersing ability. These individuals may include perineal carriers, gastrointestinal carriers, or nasal carriers with a high bacterial load. Second, shedding occurs continuously. Third, aerially dispersed skin scales can deposit and contaminate environments such as surfaces, floors, and clothing of healthcare workers as well as patients. Given that *S. aureus* can be shed into the environment, can survive outside of a host, and can be transferred from the environment back to a host, there is a need to further understand the fate and transport of this organism and the exposure to hosts in order to better address and understand the risk of acquisition.

7.1.2 Chapter IV

We conducted a 20-month prospective study using a nasal swab surveillance and clinical data from a 20-bed surgical intensive care unit (SICU). We examined the relationship between MRSA acquisition risk and the contextual environmental exposure to patients. These environmental exposures include the daily preacquisition colonization pressure in the SICU, the prior room occupant MRSA status, and the vacant room time between patient admissions.

Although our inferences were limited by the small sample size, our findings supported environmental factors as MRSA acquisition risks. We demonstrated the two

exposure aspects of SICU colonization pressure: exposure time and exposure magnitude. We found that a higher hazard of SICU exposure to MRSA-positive patients was seen in more recent exposures, which were in the prior day, compared to longer exposures. Also, we showed that the greater numbers of MRSA-positive patient present in the SICU led to a greater hazard of acquisition among other patients. When there were more than 3 MRSA-positive patients in the SICU, the acquisition hazard significantly increased by 6-8 fold. Additionally, patients who acquired MRSA were more likely to be admitted to rooms that were vacant for shorter durations between admissions.

7.1.3 Chapter V

We used mathematical modeling to help understand the complex mechanistic processes about which inferences are not intuitively obvious. We developed an MRSA fate and transport model based on the EITS framework to determine the effects of MRSA continually shedding i) on the direct and indirect exposure patterns of nurses and the uncolonized patient, and ii) on surface contamination levels following decontamination interventions. We also examined the effect of hand hygiene and its joint effects with two surface decontamination methods. These surface decontamination interventions were daily surface decontamination, and decontamination by wiping after each nurse touching of the nonporous surfaces.

With our deterministic differential equation based model, we described changes of MRSA contamination levels over time and the exposure patterns of the nurses and the uncolonized patient. Given the model assumptions and the parameters that were used, the model's main findings were as follows:

(a) Nurses became contaminated from indirect contact with the contaminated room surfaces more than from direct contact with patients, given the same direct and indirect touching frequency. Interestingly, this finding agrees with a recent study to examine whether healthcare workers' fingertips were contaminated with MRSA in a clinical hospital setting [1]. The study took place in 8 wards in a tertiary care hospital, and included 822 fingertip imprint cultures on MRSA chromogenic ager plates from 523 healthcare workers. The study showed that overall, 38/822 (5%) fingertips were MRSA-positive; 10/138 (10%) after contact with the patient's environment, 12/196 (6%) after clinical contact, and 15/346 (4%) of after no specific contact [1]. The implication of this finding is closely related to the importance of hand hygiene opportunities before and after contact with a patient's environment. It also highlighted potential problems with the misconception that hand hygiene is unnecessary if one does not touch the patient [2]. These selective missed opportunities may indeed pose a higher risk of MRSA transfer to the uncolonized patient than random missed opportunities.

(b) The surface decontamination frequency is as important as the surface-decontamination efficacy. With continuing MRSA shedding of the colonized patient and the ability to survive out of a host, room surfaces become re-contaminated quickly. Our model finding is supported by a study that examined the effectiveness of hydrogen peroxide vapor (HPV) decontamination in a 9-bed open-plan intensive care unit (ICU). This study showed that prior to the use of HPV, circulating MRSA strains in the environment were similar to those in colonized patients. Immediately before HPV use, all patients were removed from the ICU. After the use, HPV successfully eradicated MRSA from all environmentally sampled sites. However, within 24 hours after readmitting

patients, including two colonized patients, room surfaces were re-contaminated with MRSA. Within one week contamination was back to the level before the cleaning [3]. The authors concluded that HPV is effective in eradicating bacteria from the environment, but it is an ineffective means of maintaining low levels of contamination by itself, due to the rapid rate of recontamination. Our model findings supported their conclusion drawn from a clinical setting. We would suggest further that means to maintain low levels of contamination could be achieved by increasing the frequency of cleaning.

(c) Wiping nonporous surfaces after touching them was an efficacious decontamination method. This type of decontamination allows for more frequent cleaning of a smaller surface area. As a result, cleaning by wiping of surfaces is able to cover larger surface areas over time than daily decontamination. In order for routine surface decontamination to have the same effect, the frequency needs to be increased from once daily to every eight hours. Aside from the ease of use, wiping offers an environmentally friendly option with weak or limited microbicidal activities [4]. Nonetheless, some concerns have been raised that wipes may further spread contamination from one location to others if not used properly [4, 5].

(d) Transfer efficiency is a key parameter that differentiates surface behavior, and may be important when selecting hospital upholstery. In general, porous surfaces are harder to clean and disinfect, compared to nonporous surfaces [6]. However, in our model we demonstrated that while the porous surfaces retain higher levels of contamination, they do not contribute to exposure to nurses and the uncolonized patient as much as the

nonporous surfaces, which were less contaminated. This phenomenon is largely driven by the differences in their transfer efficiency.

(e) There is a joint effect between the surface-decontamination efficacy and hand-hygiene efficacy to the MRSA exposure dose to the uncolonized patient. While hand hygiene is intentionally modeled unrealistically with 100% compliance, we showed that the effect of hand hygiene to the uncolonized patient's exposure dose is less when surface contamination is high compared to when the surface contamination is low.

Overall, the model has proved to be informative and provided much insight into the role of the environment in the MRSA fate and transport process. We have shown that a simpler 2-bed model could make a clearer understanding, when compared to the attempt of a 20-bed model. Nevertheless, the deterministic nature did not fit well to address hand hygiene compliance, an important measure of MRSA infection control. We then took a step forward to examine hand hygiene compliance in a more realistic stochastic model as in chapter VI. The differences between the models in chapter V and VI are summarized in Table 7.1.

Table 7.1: Summary of differences between the deterministic ordinary differential equation based model in chapter V and the stochastic agent based model in chapter VI.

Features	Chapter V	Chapter VI
Structure: two hypothetical hospital rooms	Same	Same
Basic assumptions: i) The homogenously mixing assumption ii) The colonized patient as a single source of MRSA iii) Nurses as vectors of the	Same	Same

transmission iv) Symmetrical transfer efficiency		
MRSA dispersal assumption	In all analysis MRSA dispersal and deposition only occurs in the colonized patient's room.	In baseline scenario MRSA dispersal and deposition only occurs in the colonized patient's room. However, we included the sensitivity analysis when MRSA dispersal and deposition occurs in both patients' rooms.
Total surface area (cm^2) - Patients - Nurses - Surfaces	 2000 2000 2000	 2000 300 2000
Contact surface area (cm^2)	150	300
Surface area affected by each hand hygiene event (cm^2)	300	300
Hand hygiene compliance before and after a patient's room visit	0% or 100%	Variable
Surface area affected by each surface decontamination event per one surface (cm^2)	2000	2000
Surface area affected by wiping per one nonporous surface (cm^2)	150	N/A
Nurse visit duration (minutes)	20	10-30
Nurse contacts with the patient and the two room surfaces during the visit	Contacts are defined as rates. Each contact averages 4 times per visit.	Contacts are defined as risk; probabilistic event per one unit time step. Each contact occurs approximately 4 times per visit in a random order.
Patient contacts with the surfaces	Contacts are defined as rates. Each contact averages 4 times per hour.	Contacts are defined as risk; probabilistic event per one unit time step. Each contact occurs approximately 4 times per hour in a random order.

7.1.4 Chapter VI

In this chapter, we examined the effect of hand hygiene compliance by using a stochastic agent based model with more realistic features. Hand hygiene opportunities were at the entry and exit of a patient room, while in the room nurses could touch the patients and surfaces in a random order. Also, patients touched the two room surfaces in a random order. We examined the role of environmental contamination by varying the shedding magnitude that the colonized patient shed MRSA to the room surfaces. We evaluated the effect of the assumption that there was no dispersal across rooms by allowing MRSA dispersal and deposition on both patients' room surfaces. We also examined the symmetrical transfer efficiency assumption.

We demonstrated that healthcare workers' compliance is essential in determining the effectiveness of hand hygiene, although the time when it is performed and its efficacy are also important. Our model emphasizes the significance of the hand hygiene opportunity before and after touching patients' surrounding environment, in addition to at the entry and exit of a patient's room. Despite 100% compliance at the entry and exit of a patient's room, we show that the contaminated environmental surfaces can serve as pathogen reservoirs for recontamination of nurses' hands in the colonized patient's room. Additionally, this model shows the value of hand hygiene efficacy. With 100% compliance and 70% efficacy, nurses' hands remain contaminated enough to subsequently contaminate the uncolonized patient's environment, which later become another exposure route to the uncolonized patient. For hand hygiene to be effective in suppressing exposure to the uncolonized patient, healthcare workers' compliance, high efficacious techniques, and hand hygiene opportunities all need to be considered.

Given the model assumptions and parameters, the main exposure route to the uncolonized patient is through contaminated nurse's hands (hand-mediated route). Less exposure occurs from the contaminated environmental surfaces, which are contaminated from nurses' hands (hand-to-surface contamination). In our sensitivity analysis, where we allowed MRSA dispersal and deposition in the uncolonized patient's room, our model revealed that when surfaces become contaminated from MRSA dispersal and deposition (air-to-surface contamination), in addition to contamination from nurses' hands touching (hand-to-surface contamination), the total environmental mediated exposure to the uncolonized patient can be greatly exaggerated.

Once again in this model, transfer efficiency is highlighted as a key parameter that needs more supporting data. In both models we assume symmetrical transfer efficiency. However, in this model setting, we only include both nurses' hands surfaces area to represent the pathogen vectors in the system. Transfer efficiency of the nonporous surface, which is 0.4, appears to be unrealistically high, when compared to the average hand hygiene efficacy of 0.7 [7-9]. We then examined asymmetrical transfer efficiency by allowing transfer efficiency from hand to surface to be only 1% and 10% of hand hygiene efficacy. We found a drastic drop of hand-to-surface contamination. This further emphasizes the need to understand the extent of air-to-surface contamination, which might be the dominant source of environmental contamination in hospitals.

These two chapters are complimentary. While the deterministic model produces a single pattern of output, given the same initial conditions, it is not a flexible platform to evaluate probabilistic events and possible variations of output. Nevertheless, the deterministic model is helpful in the initial attempt to understand the system. It was also

useful in docking the stochastic counterpart. The advantage of the stochastic model is the ability to assess hand hygiene compliance and the flexibility to relax certain unrealistic assumptions. We elected to use an agent-based model for the flexibility to include different behaviors of individuals. Even though there are only small numbers of individuals in this model, our platform is already set up for elaboration into a 20-bed unit model.

7.2 Suggestions for future work

Our suggestions for future direction include three aspects. First, we need studies that help verify our model parameters as well as studies that collect enough relevant data, such as contact pattern data, patient colonization, and environmental contamination data. Second, we need to extend this fate and transport model into a full infection transmission model. This could be done with proper dose response data. Finally, future work should utilize molecular typing tools in combination with our models. This method would use real-world data to help improve our theory-based modeling work [10, 11]].

7.2.1 Studies to improve the model parameterization

Model parameter values are based on existing literature. Those that are not available require additional assumptions. One of the key parameters is transfer efficiency. Thus far, we assume that transfer efficiency from a contaminated to a clean surface as measured in the experiments are the same as transfer efficiency between two contaminated surfaces in the model. We also made a likely unrealistic assumption of symmetry. At this point, we need studies that examine transfer efficiency between two contaminated surfaces measured in both directions.

While experimental data is needed, generalization to clinical settings will need to be examined as well. Another method to estimate parameters could be done by using observational data in real-world settings. Since transfer efficiency varies greatly depending on many local factors related to the hosts, the surfaces, and the surrounding environment, transfer efficiency parameters obtained by this latter method may likely be more accurate. This parameter estimation could be achieved in a model that allows the parameter to vary in a prior set distribution. By using different sets of observational data from various possible contact scenarios in the model, we can determine what factors affect transfer efficiency.

Observational studies of healthcare workers' and patients' contact patterns are also needed. Studies of hand-hygiene compliance frequently counted a number of missed opportunities for each patient's visit. This compliance data is relevant. However, it will be more helpful to obtain more detailed contact pattern information, such as the description of the contacts, where in the rooms patients and nurses touch, how frequently the touches occur, and how frequently hand hygiene is performed following each types of touch.

7.2.2 Developing risk analysis models

This dissertation focused on the fate and transport processes of the transmission system. The next step would be to extend the model to a full transmission model, so we can use the model to reproduce real-world data, either that is already collected or that is to be collected. To develop such a model, a dose response function is needed to determine the colonization or infection outcome. At the present time, the only *S. aureus* dose response data available was from bacterial interference studies in newborns from

1963 [12]. More recent nasal artificial inoculation was performed in adult volunteers using high concentrations of organism [13, 14]. These studies are not easily applicable to the EITS model. An informative source of data may need to be from time dependent cumulated dose response experiments [15]. Additionally, we need an exposure pathway specific dose response experiment such as dose given to the skin or hands, as well as by the airborne route. While it is possible that susceptible patients may inhale these contaminated particles, the two models in this dissertation assume complete air deposition. Further exposure assessments may need to consider this airborne route.

7.2.3 Prospective genotyping surveillance study of patients, environment and healthcare workers

The purpose of the surveillance is to use this genotyping database in the EITS model. In this model there will be different MRSA genotypes that can be updated with surveillance data.

Thus, the surveillance will include healthcare workers, patients, and the environment. Selecting bacterial typing techniques depends on the intended epidemiological application [16]. Given that our objective is to trace person-to-person transmission through healthcare workers' hands and the hospital environment, we would need a technique with high discriminatory power, speed, and ability to handle large numbers of samples at the same time at a reasonable cost. A recent study of MRSA hospital transmission and intercontinental spread used a sequencing technology by mapping genome wide single nucleotide polymorphisms (SNPs) and insertions or deletions compared to a reference sequence [17]. This technique bridged the gap of an impractical full-genome sequence and the low discriminatory power of the multilocus sequence typing (MLST). This technique would be ideal to use in this type of study.

As demonstrated in this dissertation, contextual environmental exposure can affect acquisition risks, which is a risk at both the individual and population levels. Although we have not addressed issues at a microbial population level, studies have shown that there are bacterial interferences among common commensal organisms in the nose [18, 19]. Also, we have not addressed specific host factors which may contribute to transmission. Using a human nasal artificial inoculation model, a study demonstrated that the human factor is an important determinant of *S. aureus* nasal carriage. After being treated with intranasal antibiotic to eradicate the nasal carriage status, the majority of persistent carriers tested positive for their original resident strains after artificial inoculation with a mixture of *S.aureus* strains including their original strains [13]. To incorporate bacterial interference and host factors, a more elaborate complex system model will be needed.

While much can be pursued to further our understanding in MRSA transmission, at this point we have established an exposure assessment framework that can also be applied to other nosocomial pathogens. Several nosocomial pathogens are shed by patients and contaminate hospital surfaces, survive for extended periods, persist despite attempts to disinfect, and can be transferred to the hands of healthcare workers. Some of these pathogens include *Clostridium difficile*, vancomycin-resistant enterococci, *Acinetobacter baumannii*, and norovirus [20]. A comparative exposure assessment of these pathogens can certainly be informative for hospital infection control communities.

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APPENDICES

APPENDIX A

PREAMBLE

In this dissertation, the deterministic model was written in Berkeley Madonna version 8.3.22. The agent-based model was written in MATLAB version 7.8.0.347 (R2009a). Simulations were run on personal computers as well as on computers at the Center for Study of Complex System (CSCS) at the University of Michigan, Ann Arbor.

APPENDIX B

TO CHAPTER IV

This appendix contains additional discussion of the proposed environmental and hand-mediated MRSA acquisition diagram with the purpose to improve our understanding of the system and to provide information in the multivariate analysis plan. Even though this diagram is not the Directed Acyclic Graph (DAG) due to the unavoidable loops: 1) between 'HCW contamination' and 'ICU surface contamination', and 2) between 'HCW contamination' and 'Susceptible patient's room contamination', we assume that rules for causal diagrams are still applicable here.

This diagram is based on prior knowledge and the relationship found in our data analysis. The exposure of interest is the presence of MRSA-positive patients in the SICU. The outcome is MRSA acquisition during SICU admission. The two potential sources of bias that are discussed here are the room factors and the host factors.

B.1 Room factors

Susceptible patients' room contamination can be either a confounder or an intermediate variable depending on the time and sources of contamination, i.e. 1) from the current MRSA-positive patient through air dispersal, or 2) from the previous occupant's room contamination of the prior admission.

Considering Figure B.1, where an MRSA-positive patient dispersed and contaminated a susceptible patient's room, which led to contamination and acquisition of

the patient. For this scenario, room contamination is an intermediate variable and should not be adjusted for.

However, in another scenario, as in Figure B.2, there was no air dispersal across rooms, and the susceptible patient's room contamination was the result from a previous admission. Thus, room contamination becomes a confounder and should be controlled for.

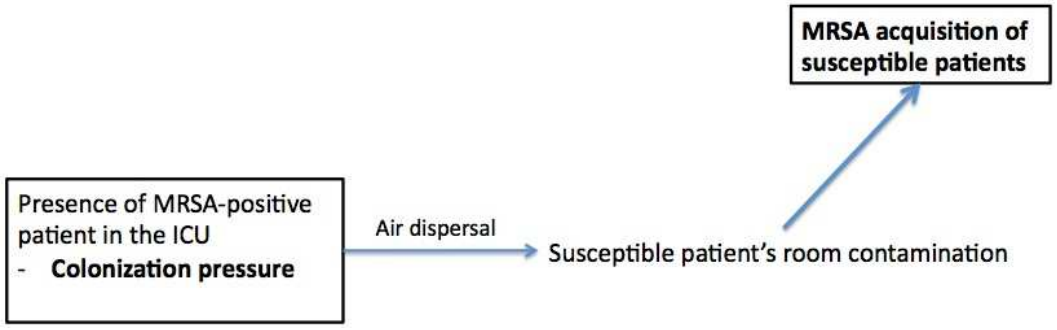

Figure B.1: Diagram showing the effect of room contamination that resulted from air dispersal from an MRSA-positive patient. This susceptible patient's room contamination leads to acquisition of the susceptible patient.

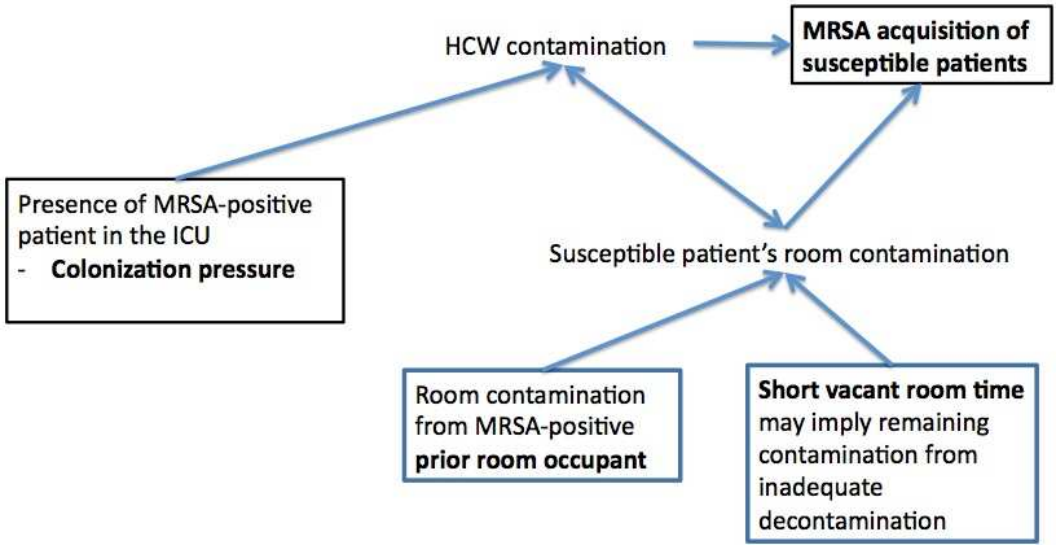

Figure B.2: Diagram showing the effect of room contamination that resulted from either the previous room occupant who was MRSA-positive or from prior inadequate room decontamination that may have been related to short vacant room time between admissions.

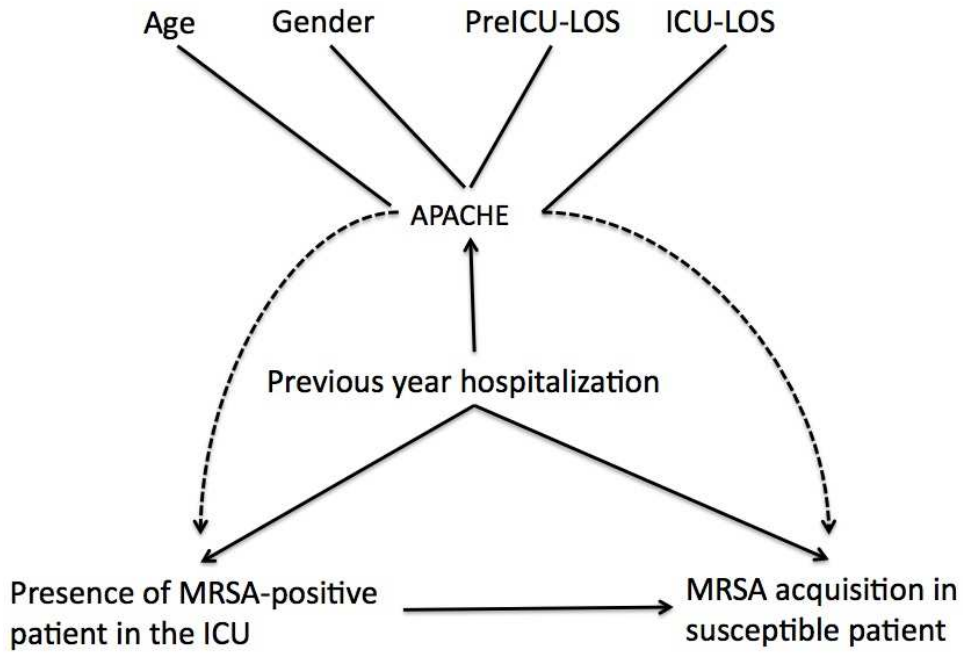

Figure B.3: Diagram of the relationships among host factors, the presence of MRSA-positive patient in the ICU and the MRSA acquisition in the susceptible patient. Solid lines depict associations (straight lines) or causal prediction (arrows) from the study. Dashed lines are from prior knowledge. PreICU-LOS means the length of stay in the hospital prior to the intensive care unit admission. ICU-LOS means the length of stay while in the intensive care unit. APACHE score stands for the Acute Physiology and Chronic Health Evaluation scoring system.

B.2 Host factors

The temporality of the time of the event is important and relevant to identifying confounders and colliders. In general, confounders should be adjusted for to minimize bias, while adjusting for colliders may create bias. In other words, an uncontrolled common cause of exposure and outcome causes bias, which is referred to as confounding. Whereas controlling for a common effect of exposure and outcome may cause bias. This

is referred to as collider-stratification-bias, selection bias or bias due to conditioning on a collider [1]. In view of time, common cause must be 'temporally prior' to both exposure and outcome. However, a measured confounder may be temporally posterior to exposure if it is on a causal pathway from the common cause to the outcome, or temporally posterior to both exposure and outcome if it is a descendent of the common cause. In contrast, a common effect must be temporally posterior to both exposure and outcome [1].

In this study there are four groups of host factors in relation to the time of exposure, which is the time that the susceptible patient was present in the SICU with other MRSA-positive patients. The first group is the factor that is unaffected by time of exposure, which is age and gender. The second group includes the host factor that is temporally prior to exposure, which are a history of previous year hospitalizations, pre-ICU length of stay (preICU_LOS), and APACHE score from an assessment upon ICU admission. The third group is ICU length of stay, which is the time when exposure and outcome occur. The last group is post-ICU length of stay, which is the host factor temporally posterior to both the exposure and outcome.

We assessed relationships among the host factors, between host factors and exposure, and between host factors and outcome. We performed linear regression to assess the relationship of host factors and the exposure (Table B.1); *t-test* and χ^2 test to compare continuous and categorical variables between 2 patient groups with and without a history of previous hospitalization (Table B.2); correlation analysis between the continuous factors (Table B.3); and Cox-proportional hazard regression to assess predictors of the outcome (Table 4.2).

Relationships of host factors, exposure and outcome are displayed in Figure B.3. The figure is based on prior knowledge and on the data analysis. Prior knowledge suggested that host risk factors for MRSA acquisition may include older age, prolonged hospitalization, use of a feeding tube, presence of skin lesions, wounds, ulcers, and previous hospitalization or surgery [2]. In our analysis, we noted that a history of hospitalization was a better predictor of changes of the exposure, compared to other factors (Table B.1). Patients with previous hospitalizations also had a higher hazard of MRSA acquisition (Table 4.2). This suggested that a history of previous hospitalization is a confounder of the relationship between the colonization pressure and the MRSA acquisition and should be controlled for.

Patients with previous hospitalizations had higher APACHE scores than those without (Table B.2). In the correlation analysis in Table B.3, the APACHE score was significantly correlated with age, pre-SICU, SICU and post-SICU length of stay, although the correlation coefficients were rather small ($r = 0.10 – 0.30$). Intuitively, patients that are more severely ill likely have multiple invasive medical devices that disrupt their normal host defense mechanism, which leads to a higher risk of MRSA acquisition. However, a study of over 10,000 ICU patients in Europe showed that the relationship of the APACHE II score and prevalence of MRSA infection was not linear. In the comparison to MRSA prevalence among ICU patients with various categories of APACHE scores, the prevalence increased as the score increased. The prevalence was highest among patients with a score of 16-20, and subsequently decreased as the score rose higher. The authors suggested that because of high mortality these patients expire before they can acquire MRSA [3]. While the APACHE score has a potential to be a

confounder, in this analysis the APACHE score was not found to be associated with either the exposure or outcome.

The APACHE score is also not a collider, since it was temporally prior to both exposure and outcome, therefore it could not be a common effect. Post-ICU length of stay is a potential collider because it is likely a consequence of MRSA acquisition that prolongs hospitalization and there may be an unmeasured variable that was associated with the exposure and also lead to a prolonged hospitalization. We did not control for post-ICU length of stay.

To conclude, due to the limited number of outcomes, we only chose one room factor and one host factor that were likely confounders in the multivariate analysis. These were the vacant room time and the history of previous hospitalization.

Table B.1: Analysis of host factors as predictors of the exposure. This exposure was the fraction of MRSA-positive patients prior to the day of acquisition. The analysis was performed using a linear regression with exposure as the dependent variable. These 471 patients were admitted to 20-bed Surgical Intensive Care Unit between October 1, 2006 and June 15, 2008, and participated in an MRSA Nasal Colonization Active Surveillance Program. They were patients at-risk for MRSA. They had more than one culture taken and their first cultures were negative.

Variables	**Parameter estimates**
Age	-0.001
Gender [a]	0.055
APACHE score	-0.001
History of hospitalization in previous year [b]	0.216
Pre-ICU length of stay (days)	0.001
ICU length of stay (days)	-0.000
Post-ICU length of stay (days)	0.004

[a] using female as the reference group.
[b] $p < 0.05$

Table B.2: Comparison of host factors and the exposure, i.e. the fraction of MRSA-positive patients present in the day prior to the acquisition or the swab (CP_1) between patients with history of hospitalization in the past year and those without. These 471 patients were admitted to 20-bed Surgical Intensive Care Unit between October 1, 2006 and June 15, 2008, and participated in an MRSA Nasal Colonization Active Surveillance Program. They were patients at-risk for MRSA. They had more than one culture taken and their first cultures were negative.

Variables	**Patients without history of previous year hospitalization**	**Patients with history of previous year hospitalization**	**P value**
Mean (95%CI)[a]	(n = 313)	(n=158)	
Age	56.87 (54.93 – 58.82)	57.22 (54.81– 59.65)	0.83
Gender (% male)[b]	55.59	55.06	0.91

APACHE score	59.34 (56.57 – 62.10)	66.81 (63.08– 70.54)	<0.01
Pre-ICU length of stay (days)	7.43 (5.98 – 8.88)	7.99 (6.26 – 9.71)	0.64
ICU length of stay (days)	10.46 (9.32 – 11.59)	9.43 (8.19 -10.67)	0.28
Post-ICU length of stay (days)	10.11 (8.22 – 11.99)	8.54 (7.15 – 9.93)	0.19
CP_1[c]	5.21 (4.57 – 5.85)	6.41 (5.50 – 7.32)	0.03

[a] These are upper and lower 95% confidence limits of the means using *t*-test.
[b] Comparing proportions using χ^2 test.
[c] Colonization pressure or a fraction of MRSA-positive patient in the surgical intensive care unit on the day prior to acquisition.

Table B.3: Correlation between host factors. These patients' characteristics were from 471 patients enrolled in a prospective cohort study in a 20-bed Surgical Intensive Care Unit between October 1, 2006 and June 15, 2008, and participated in an MRSA Nasal Colonization Active Surveillance Program. These were patients at-risk for MRSA acquisition. They had more than one culture taken and their first cultures were negative for MRSA.

Correlation coefficient, p value	**Age**	**APACHE[a]**	**PreICU_LOS[b]**	**ICU_LOS[c]**	**postICU_LOS[d]**	**CP_1[d]**
Age	-	0.13 <0.01	0.006 0.90	-0.03 0.55	-0.03 0.57	-0.02 0.70
APACHE[a]		-	0.22 <0.01	0.30 <0.01	0.10 0.03	-0.02 0.61
preICU_LOS[b]			-	0.17 <0.01	0.22 <0.01	0.01 0.89
ICU_LOS[c]				-	0.15 <0.01	-0.00 0.95
postICU_LOS[d]					-	0.05 0.23
CP_1[d]						-

[a] APACHE score stands for the Acute Physiology and Chronic Health Evaluation scores. It is an estimate of intensive care unit mortality based on a number of laboratory values and patient signs taking both acute and chronic disease into account.
[b] preICU_LOS is the number of days that the patient stayed in the hospital prior to the SICU admission.
[c] ICU_LOS is the number of days that the patient stayed in the SICU.
[d] postICU_LOS is the number of days that the patient stayed in the hospital following the SICU discharge.

APPENDIX B REFERENCES

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APPENDIX C

TO CHAPTER V

Table C.1: Mathematical descriptions of model events and their compartmental flows

Events	Descriptions
1. Shedding onto environmental surfaces	
a. PTc to the porous surface (Pc)	αA_p
b. PTc to the nonporous surface (NPc)	αA_{np}
2. Input to the colonized patient	αA_{pt}
3. The colonized patient touches surfaces	
i. The colonized patient (PTc) touches the porous surface (Pc)	
a. Pathogens flow from PTc to Pc	$PT_c \frac{A_c}{A_{pt}} \rho_p \tau_{pt-p}$
b. Pathogens flow from Pc to PTc	$P_c \frac{A_c}{A_p} \rho_p \tau_{pt-p}$
ii. The colonized patient (PTc) touches the nonporous surface (NPc)	
a. Pathogens flow from PTc to NPc	$PT_c \frac{A_c}{A_{pt}} \rho_{np} \tau_{pt-np}$
b. Pathogens flow from NPc to PTc	$NP_c \frac{A_c}{A_{np}} \rho_{np} \tau_{pt-np}$
4. The uncolonized patient touches surfaces	
i. The uncolonized patient (PTu) touches the porous surface (Pu)	
a. Pathogens flow from PTu to Pu	$PT_u \frac{A_c}{A_{pt}} \rho_p \tau_{pt-p}$

b. Pathogens flow from Pu to PTu	$P_u \frac{A_c}{A_p} \rho_p \tau_{pt-p}$
ii. The uncolonized patient (PTu) touches the nonporous surface (NPu)	
a. Pathogens flow from PTu to NPu	$PT_u \frac{A_c}{A_{pt}} \rho_{np} \tau_{pt-np}$
b. Pathogens flow from NPu to PTu	$NP_u \frac{A_c}{A_{np}} \rho_{np} \tau_{pt-np}$
5. Nurses touch surfaces	
i. Nurse (NS) touches the porous surface (Pc) in the colonized patient's room	
a. Pathogens flow from NS to Pc	$NS \frac{A_c}{A_{ns}} \rho_p \tau_{ns-p}$
b. Pathogens flow from Pc to NS	$P_c \frac{A_c}{A_p} \rho_p \tau_{ns-p}$
ii. Nurse (NS) touches the nonporous surface (NPc) in the colonized patient's room	
a. Pathogens flow from NS to NPc	$NS \frac{A_c}{A_{ns}} \rho_{np} \tau_{ns-np}$
b. Pathogens flow from NPc to NS	$NP_c \frac{A_c}{A_{np}} \rho_{np} \tau_{ns-np}$
iii. Nurse (NS) touches the porous surface (Pu) in the uncolonized patient's room	
a. Pathogens flow from NS to Pu	$NS \frac{A_c}{A_{ns}} \rho_p \tau_{ns-p}$
b. Pathogens flow from Pu to NS	$P_u \frac{A_c}{A_p} \rho_p \tau_{ns-p}$
iv. Nurse (NS) touches the nonporous surface (NPu) in the uncolonized patient's room	
a. Pathogens flow from NS to NPu	$NS \frac{A_c}{A_{ns}} \rho_{np} \tau_{ns-np}$
b. Pathogens flow from NPu to NS	$NP_u \frac{A_c}{A_{np}} \rho_{np} \tau_{ns-np}$
6. Nurse touches patients	
i. Nurse (NS) touches the colonized patient (PTc)	
a. Pathogens flow from NS to PTc	$NS \frac{A_c}{A_{ns}} \rho_{sk} \tau_{ns-pt}$
b. Pathogens flow from PTc to NS	$PT_c \frac{A_c}{A_{pt}} \rho_{sk} \tau_{ns-pt}$
ii. Nurse (NS) touches the uncolonized patient	

(PTu)	
a. Pathogens flow from NS to PTu	$NS\frac{A_c}{A_{ns}}\rho_{sk}\tau_{ns-pt}$
b. Pathogens flow from PTu to NS	$PT_u\frac{A_c}{A_{pt}}\rho_{sk}\tau_{ns-pt}$
7. Self inoculation	
i. The colonized patient (PTc) touches nose (PTcn)	
a. Pathogens flow from PTc to PTcn	$PT_c\frac{A_f}{A_{pt}}\rho_n\tau_n$
b. Pathogens flow from PTcn to PTc	$PT_{cn}\frac{A_f}{A_n}\rho_n\tau_n$
ii. The uncolonized patient (PTu) touches nose (PTun)	
a. Pathogens flow from PTu to PTun	$PT_u\frac{A_f}{A_{pt}}\rho_n\tau_n$
b. Pathogens flow from PTun to PTu	$PT_{un}\frac{A_f}{A_n}\rho_n\tau_n$
iii. Nurse (NS) touches nose (NSn)	
a. Pathogens flow from NS to NSn	$NS\frac{A_f}{A_{ns}}\rho_n\tau_n$
b. Pathogens flow from NSn to NS	$NS_n\frac{A_f}{A_n}\rho_n\tau_n$
8. Natural die off	
i. On the colonized patient (PTc)	$PT_c\mu_{sk}$
ii. On the porous surface (Pc) in the colonized patient's room	$P_c\mu_p$
iii. On the nonporous surface (NPc) in the colonized patient's room	$NP_c\mu_{np}$
iv. On the uncolonized patient (PTu)	$PT_u\mu_{sk}$
v. On the porous surface (Pu) in the uncolonized patient's room	$P_u\mu_p$
vi. On the nonporous surface (NPu) in the uncolonized patient's room	$NP_u\mu_{np}$
vii. On nurses	$NS\mu_{sk}$

9. Start of nursing shift	$NSs(t),$ $where\ s(t) = 1$ $every\ 8\ hours,$ $otherwise\ s(t) = 0$
10. Surface decontamination	
i. Daily surface decontamination	
a. The porous surface in the colonized patient's room (Pc)	$P_c\varepsilon_d h(t),$ $where\ h(t) = 1$ $every\ 24\ hours,$ $otherwise\ h(t) = 0$
b. The nonporous surface in the colonized patient's room (NPc)	$NP_c\varepsilon_d h(t)$
c. The porous surface in the uncolonized patient's room (Pu)	$P_u\varepsilon_d h(t)$
d. The nonporous surface in the uncolonized patient's room (NPu)	$NP_u\varepsilon_d h(t)$
ii. Surface decontamination by wiping	
a. The nonporous surface in the colonized patient's room (NPc)	$NP_c\frac{A_c}{A_{np}}\varepsilon_w\omega_{ns-np}f(t),$ $where\ f(t) = 1,$ $t \in \left[n-1, n-\frac{2}{3}\right), n \in Z+$

b. The nonporous surface in the uncolonized patient's room (NPu)	$NP_u \frac{A_c}{A_{np}} \varepsilon_w \omega_{ns-np} g(t),$ $where\ g(t) = 1,$ $t \in \left[n - \frac{2}{3}, n - \frac{1}{3}\right), n \in Z +$
11. Hand hygiene	
a. Before the colonized patient's room visit	$NS \frac{A_h}{A_{ns}} \varepsilon_h u(t),$ $where\ u(t) = 1,$ $t = n - 1, n \in Z +$
b. After the colonized patient's room visit	$NS \frac{A_h}{A_{ns}} \epsilon_h v(t),$ $where\ v(t) = 1,$ $t = n - 2/3$
c. Before the uncolonized patient's room visit	$NS \frac{A_h}{A_{ns}} \varepsilon_h x(t),$ $where\ x(t) = 1$ $t = n - \frac{2}{3} + dt$
d. After the uncolonized patient's room visit	$NS \frac{A_h}{A_{ns}} \epsilon_h y(t),$ $where\ y(t) = 1$ $t = n - 1/3$