

Quantitative Microbial Risk Assessment of Antimicrobial Resistant and Susceptible *Staphylococcus aureus* in Reclaimed Wastewaters

Mary E. Schoen,* Michael A. Jahne, Jay Garland, Lucia Ramirez, Allison J. Lopatkin, and Kerry A. Hamilton



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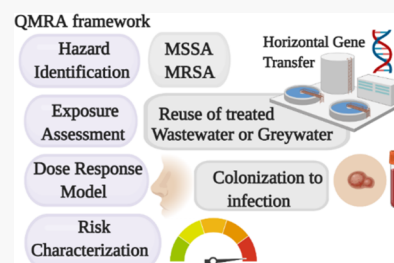
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ABSTRACT: The annual risks of colonization, skin infection, bloodstream infection (BSI), and disease burden from exposures to antibiotic-resistant and susceptible *Staphylococcus aureus* (*S. aureus*) were estimated using quantitative microbial risk assessment (QMRA). We estimated the probability of nasal colonization after immersion in wastewater (WW) or greywater (GW) treated across a range of treatment alternatives and subsequent infection. Horizontal gene transfer was incorporated into the treatment model but had little effect on the predicted risk. The cumulative annual probability of infection (resulting from self-inoculation) was most sensitive to the treatment log₁₀ reduction value (LRV), *S. aureus* concentration, and the newly calculated morbidity ratios and was below the health benchmark of 10^{−4} infections per person per year (ppy) given a treatment LRV of roughly 3.0. The predicted annual disability-adjusted life years (DALYs), which were dominated by BSI, were below the health benchmark of 10^{−6} DALYs ppy for resistant and susceptible *S. aureus*, given LRVs of 4.5 and 3.5, respectively. Thus, the estimated infection risks and disease burdens resulting from nasal colonization are below the relevant health benchmarks for risk-based, nonpotable, or potable reuse systems but possibly above for immersion in minimally treated GW or WW. Strain-specific data to characterize dose–response and concentration in WW are needed to substantiate the QMRA.

KEYWORDS: QMRA, MRSA, *Staphylococcus aureus*, horizontal gene transfer, reclaimed, wastewater, greywater, antimicrobial resistance



1. INTRODUCTION

To withstand the growing pressures on water supplies (such as increased demand and climate change), water managers from municipalities up through international agencies have promoted water reuse to use energy efficiently and increase the sustainability and resiliency of water resources.¹ Wastewater (WW) and greywater (GW) are increasingly reclaimed for potable and nonpotable purposes but require treatment to control for a range of human infectious pathogens.¹ Risk-based pathogen treatment requirements for reuse are calculated to achieve a specific level of health protection for consumers and are typically based on the control of enteric pathogens;^{1,2} however, the microbiota of GW is dominated by skin-associated bacteria,³ some of which may be pathogenic, for example, *Staphylococcus aureus* (*S. aureus*).

S. aureus can be a human commensal bacterium but is also responsible for a range of infections from minor skin infections to urinary tract infections, conjunctivitis, as well as potentially fatal necrotizing pneumonia and bloodstream infections (BSIs).^{4,5} Methicillin-resistant *S. aureus* (MRSA) is resistant to penicillin and other β -lactam antimicrobial drugs (e.g., methicillin and oxacillin),⁶ while methicillin-susceptible *S. aureus* (MSSA) responds to methicillin and oxacillin treatment. MRSA infections are associated with greater morbidity and mortality than those caused by MSSA.^{7,8}

The skin is an effective barrier against *S. aureus* infection.^{9–11} Thus, MRSA infections obtained through community exposures [i.e., community-acquired MRSA (CA-MRSA)] are generally associated with risk factors like poor hygiene and crowded living environments; colonization (i.e., asymptomatic carriage without infection) has also been identified as a risk factor for infection.¹² Approximately 25–50% of the population is colonized with *S. aureus*, with the anterior nares being the most important colonization site.¹³ After colonization, skin infection can result from bacterial transfer (e.g., hand contact) from the colonization site to open microlesions and wounds on the skin elsewhere on the body.¹⁴ BSI can result from skin, respiratory, or urinary tract infection caused by *S. aureus*.¹⁵ There is evidence of an increased risk of infection after colonization with MRSA (e.g., USA300) compared with MSSA.¹⁶

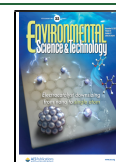
Despite the occurrence of MRSA and MSSA in WWs,^{17–20} few epidemiological studies have evaluated if reclaimed water (or WW-impacted water) poses a *S. aureus* risk to those

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Table 1. Input Assumptions

input	description	unit	value ^a	N ^b	references
$C_{\text{raw},S.\text{aureus},\text{WW}}$	<i>S. aureus</i> concentration in raw WW	cfu/mL	$\log_{10}$ uniform (min = $10^{0.5}$, max = $10^{3.8}$)	18	17, 18
$C_{\text{raw},S.\text{aureus},\text{GW}}$	<i>S. aureus</i> concentration in raw GW	cfu/mL	log normal ($\mu = 8.217$, $\sigma = 1.98$)/100 ^c	40	45
F_r	resistant fraction of <i>S. aureus</i> in raw WW	NA	uniform (min = 0.15, max = 0.2)	15	17,46
HGT	HGT rate expressed as transconjugant cell concentration/recipient cell concentration	NA	$\log_{10}$ uniform (min = −9, max = −5.6)	NA	28
$\text{LRV}_{\text{treat}}$	treatment LRV for bacteria	NA	deterministic	NA	selected
$\text{LRV}_{\text{HGT,MSSA}}$	treatment HGT LRV for MSSA	NA	$10^{-7.65}$ CI ($10^{-9.27}$, $10^{-6.04}$)	NA	calculated using ²⁸
$\text{LIV}_{\text{HGT,MRSA}}$	treatment HGT LIV for MRSA	NA	$10^{-7.0}$ CI($10^{-8.60}$, $10^{-5.37}$)	NA	calculated
vol	exposure volume	mL	uniform (min = 7.5×10^{-2} , max = 9.9×10^{-2})	NA	calculated
NA	dose–response relationship of probability of colonization (P_{col})	NA	$P_{\text{col}} = 1 - (1 + \frac{\text{dose}}{N_{50}} \times (2^{1/a} - 1))\alpha = 0.3999$; $N_{50} = 26660$	NA	44
$P_{\text{skin,inf}}^{\text{col}}_{\text{MRSA}}$	probability of skin infection given colonization MRSA	NA	0.38	56	50
$P_{\text{skin,inf}}^{\text{col}}_{\text{MSSA}}$	probability of skin infection given colonization MSSA	NA	0.03	229	50
$P_{\text{bs,inf}}^{\text{col}}_{\text{MRSA}}$	probability of BSI given colonization MRSA	NA	1.5×10^{-2}	NA	calculated
$P_{\text{bs,inf}}^{\text{col}}_{\text{MSSA}}$	probability of BSI given colonization MSSA	NA	1.2×10^{-3}	NA	calculated
$\text{DALY}^{\text{bs,inf}}_{\text{MRSA}}$	DALY per case of BSI MRSA	DALY	4.26	NA	53
$\text{DALY}^{\text{bs,inf}}_{\text{MSSA}}$	DALY per case of BSI MSSA	DALY	2.02	NA	calculated
$\text{DALY}^{\text{skin,inf}}_{\text{MRSA/MSSA}}$	DALY per case of skin infection MRSA/MSSA	DALY	3.26×10^{-3}	NA	53

^a μ and σ are the mean and standard deviation of the lognormal distribution on the (natural) log scale; LIV and LRV are summarized by median and 95th percentile confidence interval (CI); NA is not applicable. ^bN is the number of samples. ^cThe lognormal distribution is divided by 100 to adjust for units.

exposed.^{21–23} Quantitative microbial risk assessment (QMRA) has been used to evaluate bacterial risks from reclaimed water¹ and proposed as a tool to evaluate potential risks resulting from antimicrobial exposures in reclaimed waters; however, QMRA has not yet been used to estimate the potential risk from exposure to MRSA in reclaimed waters. The lack of analysis is due to implementation challenges.²⁴ Mainly, there is a paucity of human dose–response data for antimicrobial resistant bacteria, including MRSA. The most prevalent MRSA strain in the United States, USA300, is highly virulent compared to the most common susceptible strain, USA100.¹⁴

A second knowledge gap relevant to MRSA is the level of acquisition of antibiotic resistance by horizontal gene transfer (HGT) in the WW setting.²⁵ HGT refers to the non-genological transmission of genetic materials between organisms and is the predominant mechanism responsible for the worldwide spread of antibiotic resistance.^{26,27} Methicillin-resistance gene cassettes are known to be transferrable via HGT, transforming a methicillin-sensitive strain into one that is methicillin-resistant (e.g., MRSA).²⁸ However, whether the rate at which HGT occurs in the natural or built environment is sufficiently high to impact health outcomes is unknown. Quantitative studies that measure MRSA enrichment in situ in the WW treatment process are not available, but information about potential rates is available from bench-scale estimates of HGT in experimental matrices that mimic relevant processes.²⁸

QMRA has been used to predict acute MSSA skin infection.^{29–32} In contrast, this work evaluated the repercussions of microbiome colonization of MSSA and MRSA. The objective was to estimate the probability of nasal colonization and subsequent delayed infection and disease burden resulting from exposures to MRSA and MSSA in reclaimed WW and

GW, considering a range of treatment levels. In doing so, we evaluated whether risk-based reuse treatment requirements based on enteric pathogens for nonpotable reuse^{33,34} and potable use³⁵ are also protective against *S. aureus*. A secondary objective was to identify the dominant mechanisms that influence MRSA exposure in a reuse scenario with HGT occurring in treatment.

2. MATERIALS AND METHODS

We used QMRA to model the annual probability of colonization and subsequent infection risk and disease burden for healthy adults from exposures to MRSA or MSSA resulting from residential and municipal nonpotable and potable uses of reclaimed water. We considered a range of *S. aureus* treatment $\log_{10}$ reduction values (LRVs) that included the bacteria $\log_{10}$ reduction targets (LRTs) for nonpotable indoor GW and WW reuse of 3.5 and 6.0, respectively,^{33,34} for reuse of GW for showering of 5.7,³¹ as well as an estimated LRV for potable reuse of 15.³⁵ We adopted the conservative exposure assumption of daily immersion for all residential and municipal nonpotable and potable uses (described further in Section 2.1.1). We used familiar health outcomes expressed as infection risk per person per year (ppy) or as disease burden [i.e., disability-adjusted life years (DALYs)], which is defined as the sum of years of life lost by premature mortality and years lived with a disability.³⁶

First, we estimated the daily MRSA and MSSA nasal doses for the selected *S. aureus* treatment removal (Section 2.1). Using the doses, we calculated the daily MRSA and MSSA probabilities of colonization of the nose using dose–response data from an epidemic strain (Section 2.2). Following nasal

colonization, we estimated the daily MRSA and MSSA probabilities of BSI or skin infection (elsewhere on the body) using newly estimated morbidity ratios for healthy adults (Section 2.3). Finally, we calculated the annual probabilities of skin infection, BSI, or cumulative BSI and skin infection and the resulting disease burden (Section 2.4).

We conducted a Monte Carlo simulation with 10,000 iterations (which resulted in stable results across runs) to capture the variability in the factors that influence exposure (Table 1). In each iteration, representing a year, probability distributions of the exposure factors were sampled 365 times. Thus, the independent, daily MSSA and MRSA doses varied by day and across years. Sensitivity analysis was performed to identify dominant mechanisms of MRSA exposure (Section 2.5). The inputs used to characterize the estimated risks (Sections 2.3 and 2.4) were more uncertain than variable and characterized as point estimates, which were further explored through uncertainty analysis (Section 2.6). The 95th percentile annual probabilities of infection and annual DALYs (the percentile utilized to calculate the nonpotable LRTs¹) were compared to selected annual health benchmarks. We adopted the WHO tolerable burden of disease of 10^{-6} DALYs ppy,³⁷ which was previously used for nonpotable reuse,^{38–40} and an infection risk of 10^{-4} ppy, which was previously used for potable reuse applications⁴¹ and recent guidance for nonpotable reuse.¹

2.1. Daily MRSA and MSSA Doses. We calculated the daily dose of MRSA or MSSA applied to the anterior nares of the nose resulting from immersion in treated GW or WW. The doses of MRSA or MSSA from exposure to source water s ($Dose_{MRSA,s}$ or $Dose_{MSSA,s}$) are estimated from the raw source water concentrations ($C_{raw,MSSA,s}$ and $C_{raw,MRSA,s}$) by applying $\log_{10}$ reduction/increase values resulting from HGT and treatment removal.

$$dose_{MRSA,s} = C_{raw,MRSA,s} \times 10^{-LRV_{treat}} \times 10^{-LIV_{HGT,MRSA}} \times vol \quad (1)$$

$$dose_{MSSA,s} = C_{raw,MSSA,s} \times 10^{-LRV_{treat}} \times 10^{-LRV_{HGT,MSSA}} \times vol \quad (2)$$

where $C_{raw,MSSA,s}$ and $C_{raw,MRSA,s}$ are the concentrations in the raw source water (cfu/mL), LRV_{treat} is the bacteria reduction from treatment, $LRV_{HGT,MSSA}$ is the MSSA LRV from HGT in treatment, $LIV_{HGT,MRSA}$ is the MRSA $\log_{10}$ increase value (LIV) from HGT in treatment, and Vol is the exposure volume (mL). Both treatment removal and HGT are expressed as $\log_{10}$ reduction/decrease for comparison. The data used to characterize the exposure factors are described in the following sections.

2.1.1. Exposure Volume from Immersion. We calculated Vol (Table 1) using the thickness of the film of water that remains on the skin after immersion⁴² of 4.99×10^{-3} cm multiplied by the surface area of the anterior nares of the nose,⁴³ which we characterized as uniformly distributed with minimum and maximum values of 15.0 and 19.0 cm² using data from adults only. The selected exposure volume resulting from immersion is most representative of potable reuse (e.g., bathing) and conservative for nonpotable uses such as toilet flushing, for which an exposure model based on hand-to-face contact⁴⁴ or aerosol inhalation may be more relevant.

2.1.2. MRSA and MSSA Concentrations. Due to limited data, we characterized the concentration of total *S. aureus* in raw WW or GW and estimated a possible resistant fraction

(Table 1). We used a $\log_{10}$ uniform distribution to characterize the concentration of total *S. aureus* in WW using data from three sampling events at six urban WW treatment plants (WWTPs) in northeast Spain, which ranged between $10^{0.5}$ and $10^{3.8}$ cfu/mL.¹⁸ Falling in this range, the average *S. aureus* concentration of 15 influent samples from an urban WWTP in Barcelona was $10^{2.29}$ cfu/mL.¹⁷ From the GW concentration data recently reviewed,³⁰ we fit a lognormal distribution using the reported mean and standard deviation from 40 samples of mixed GW (including baths, showers, and washbasins) from a collection system of 14 apartments.⁴⁵

We characterized the fraction of the total *S. aureus* that is resistant in GW or WW as uniformly distributed using the fraction estimated from WW samples¹⁷ and bathing water samples,⁴⁶ based on selective enrichment with and without antibiotics and subsequent *S. aureus* and *mecA* gene confirmation. We assume that the *S. aureus* in bathing waters is shed by bathers similarly to users contributing to WW by shedding. Separate studies have identified USA300 as the predominant MRSA lineage in U.S. WWs,^{47,51} whereas USA100 is the predominant MSSA.⁴⁷

2.1.3. Horizontal Gene Transfer. Our HGT modeling approach was tailored to accommodate the available conjugate frequency experimental data. In bench-scale experiments, the HGT rate is often expressed as a frequency of transconjugant cell concentrations/recipient cell concentrations at the endpoint of the experiment. To implement the experimentally derived HGT rate in the treatment context, the transconjugant is the newly formed MRSA and the recipient is the MSSA. The reverse process (i.e., the natural segregation error rate associated with losing a mobile element) was assumed to be negligible.

Because the retention time of the treatment compartments of interest [e.g., membrane bioreactor (MBR)²⁵] is long compared to the HGT timescale (e.g., MBR hydraulic retention time of 2 to 6 h vs presumably minutes), we assumed that HGT occurs before the treatment removal. The MSSA concentration in water s after HGT ($C_{HGT,MSSA,s}$) is

$$C_{HGT,MSSA,s} = \frac{C_{raw,MSSA,s}}{1 + HGT} \quad (3)$$

or expressed in the familiar log reduction value form

$$\log_{10}(C_{HGT,MSSA,s}) = \log_{10}(C_{raw,MSSA,s}) - LRV_{HGT,MSSA} \quad (4)$$

where $C_{raw,MSSA,s}$ is the initial MSSA concentration in water s , HGT is the HGT rate expressed as transconjugant/recipient cells, and $LRV_{HGT,MSSA} = \log_{10}(1 + HGT)$.

The MRSA concentration after HGT in water s ($C_{HGT,MRSA,s}$) expressed in the LRV format is

$$\log_{10}(C_{HGT,MRSA,s}) = \log_{10}(C_{raw,MRSA,s}) - LIV_{HGT,MRSA} \quad (5)$$

and

$$LIV_{HGT,MRSA} = \log_{10} \left(1 + \frac{HGT \times (1 - F_r)}{F_r \times (1 + HGT)} \right) \quad (6)$$

where $C_{raw,MRSA,s}$ is the initial MRSA concentration in water s , HGT is the HGT rate expressed as transconjugant/recipient cells, and F_r is the fraction of *S. aureus* that is resistant in raw water at the start of the process [$F_r = C_{raw,MRSA,s} / (C_{raw,MRSA,s} +$

$C_{\text{raw,MRSA},s}$]. Equation 6 is derived in the Supporting Information.

We characterized $\text{LRV}_{\text{HGT,MSSA}}$ as uniformly distributed (Table 1) using the maximum and minimum reported HGT rates of $10^{-9.0}$ and $10^{-5.6}$ for single species (*S. aureus*) co-culture in the absence of antibiotics over a 24 h experiment (calculated as transconjugants/recipients).²⁸ Using the same maximum and minimum HGT rates, we calculated the $\text{LIV}_{\text{HGT,MRSA}}$.

2.2. Daily MRSA and MSSA Probabilities of Colonization of the Nose. The daily doses of MRSA and MSSA (eqs 1 and 2) were used to estimate the daily probabilities of nasal colonization from immersion in treated source water s ($P_{\text{col,MSSA},s}$ and $P_{\text{col,MRSA},s}$) using a beta-Poisson dose–response model (Table 1) previously applied in the QMRA of MRSA.⁴⁴ The model was fit to data from two studies of human volunteers in the absence of antibiotics,^{48,49} in which susceptible *S. aureus* strain 52/52A/80/81, later classified as clonal complex 30 (CC30), was delivered via solution. The human studies defined a positive colonization response as the presence of MRSA in the nose five days after inoculation. We use the *S. aureus* strain 52/52A/80/81 relationship as a surrogate for MRSA and MSSA. See Sections 4.3.1 and 4.4.1 for discussion of this assumption. We assumed that the entire population is susceptible to colonization.

2.3. Daily MRSA and MSSA Probabilities of BSI or Skin Infection. We estimated the daily probabilities of infection for two health outcomes, skin infection and BSI. The daily probabilities of BSI or skin infection from exposure to hazard h (i.e., MRSA or MSSA) subsequent to immersion in treated source water s ($P_{\text{bs,inf},h,s}$ and $P_{\text{skin,inf},h,s}$, respectively) are

$$P_{\text{skin,inf},h,s} = P_{\text{col},h,s} \times P_{\text{skin,inf}} \Big|_{\text{col}_h} \quad (7)$$

$$P_{\text{bs,inf},h,s} = P_{\text{col},h,s} \times P_{\text{bs,inf}} \Big|_{\text{col}_h} \quad (8)$$

where $P_{\text{bs,inf},h,s}$ and $P_{\text{skin,inf},h,s}$ are morbidity ratios or the conditional probabilities of BSI and skin infection, given colonization.

The daily cumulative probability of infection for hazard h from treated source water s ($dP_{\text{inf},h,s}$) is

$$dP_{\text{inf},h,s} = 1 - (1 - P_{\text{skin,inf},h,s}) \times (1 - P_{\text{bs,inf},h,s}) \quad (9)$$

Due to limited data, we estimated new morbidity ratios for delayed skin infection following colonization ($P_{\text{skin,inf},h,s}$) using observations from a study of military recruits. Ellis et al.⁵⁰ reported that 24 of 812 (3%) military recruits carried MRSA in the nares, and 9 of the 24 soldiers (38%) developed a skin infection elsewhere on the body within 10 weeks. Abscesses that were sampled for culture and analyzed were indistinguishable from the strain present at the initial sampling. Only 8 of 229 (3%) soldiers colonized with an MSSA strain developed a skin infection in the same period (and none developed BSI). A study of pediatric patients presenting for an outpatient physician's office visit had similar estimates. Roughly 32% of pediatric patients colonized with MRSA self-reported a skin infection during a 1 year follow-up, while 10% of those colonized with MSSA reported a skin infection in the same time period.⁵¹

We estimated a delayed morbidity ratio for BSI ($P_{\text{bs,inf},h,s}$) utilizing data from the Active Bacterial Core Surveillance

program, a population-based surveillance component of the Emerging Infections Program Network (EIPN) in the United States.⁵² Of the recorded CA-MRSA infections from 2001 to 2002, the majority were skin infections (77%) and a smaller percentage were BSIs (3%). Because the colonization state of those infected was not recorded, $P_{\text{bs,inf},h,s}$ was estimated as $P_{\text{skin,inf},h,s}$ multiplied by the ratio of BSI/skin infection cases, that is, $P_{\text{bs,inf},h,s} = P_{\text{skin,inf},h,s} \times 3/77$. We applied the MRSA ratio of BSI/skin infection to the MSSA calculation due to the lack of data, that is, $P_{\text{bs,inf},h,s} = P_{\text{skin,inf},h,s} \times 3/77$. This estimate is likely conservative because the EIPN only recorded reported skin infections.

2.4. Annual Probabilities of Infection and DALYs. The annual probabilities of infection of skin or BSI for hazard h from treated source water s ($A_{\text{Pskin,inf},h,s}$ and $A_{\text{Pbs,inf},h,s}$) are

$$A_{\text{Pskin,inf},h,s} = 1 - \prod_{\text{day}=1}^{365} (1 - P_{\text{skin,inf},h,s,\text{day}}) \quad (10)$$

$$A_{\text{Pbs,inf},h,s} = 1 - \prod_{\text{day}=1}^{365} (1 - P_{\text{bs,inf},h,s,\text{day}}) \quad (11)$$

The cumulative annual probability of infection for hazard h from treated source water s ($P_{\text{inf},h,s}$) is

$$P_{\text{inf},h,s} = 1 - (1 - A_{\text{Pskin,inf},h,s}) \times (1 - A_{\text{Pbs,inf},h,s}) \quad (12)$$

To capture the severe health outcomes resulting from BSI, the annual DALYs of skin infection and BSI for hazard h from treated source water s ($A_{\text{DALY,skin},h,s}$ and $A_{\text{DALY,bs},h,s}$, respectively) are

$$A_{\text{DALY,skin},h,s} = A_{\text{Pskin,inf},h,s} \times \text{DALY/skin,inf}_h \quad (13)$$

$$A_{\text{DALY,bs},h,s} = A_{\text{Pbs,inf},h,s} \times \text{DALY/bs,inf}_h \quad (14)$$

where DALY/skin,inf_h and DALY/bs,inf_h are the DALYs per case of skin infection and BSI for hazard h , respectively.

The cumulative annual DALY for hazard h from treated source water s ($\text{DALY}_{h,s}$) is

$$\text{DALY}_{h,s} = A_{\text{DALY,skin},h,s} \times A_{\text{DALY,bs},h,s} \quad (15)$$

The DALYs attributed to MRSA and MSSA infections vary geographically⁵³ because *S. aureus* strains vary geographically⁵⁴ as well as the ability to prevent and control infections. $\text{DALY/skin,inf}_{\text{MRSA}}$ and $\text{DALY/bs,inf}_{\text{MRSA}}$ were set to the median estimated across the European Union.⁵³ We adopted the MRSA value for $\text{DALY/skin,inf}_{\text{MSSA}}$ due to the lack of data. MRSA BSI resulted in 108 more deaths per 1000 cases and increased the length of hospital stay by 3 days compared to MSSA BSI.⁵³ To estimate the $\text{DALY/bs,inf}_{\text{MSSA}}$, we modified the $\text{DALY/bs,inf}_{\text{MRSA}}$ to capture this difference in mortality and length of stay (see Supporting Information).

2.5. Sensitivity Analysis. To identify the dominant mechanisms of MRSA exposure and risk, we performed multivariate sensitivity analysis of $\text{Dose}_{\text{MRSA,GW}}$ and $dP_{\text{inf,MRSA,GW}}$ to all inputs (Table 1) based on principal component (PC) analysis using the R function `multisensi`.⁵⁵ For the sensitivity analysis bounds, $P_{\text{skin,inf},h,s}$ was assigned a range of 0.01–1.0. $P_{\text{bs,inf},h,s}$ was assigned a possible range of 10^{-4} – 10^{-2} . The generalized sensitivity indices (GSIs) are reported, which are the averages of the PC indices weighted by the PC percentages of inertia.

2.6. Uncertainty Analysis of Risk and Disease Burden. We examined the change in the predicted 95th percentiles of

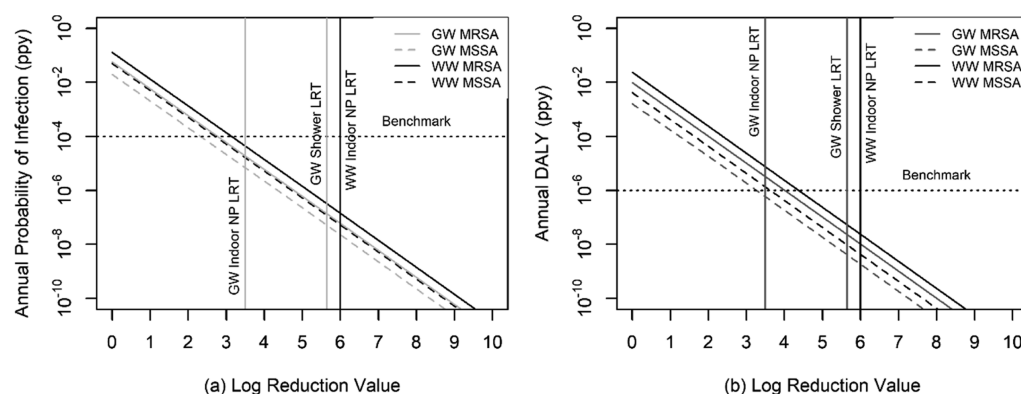


Figure 1. Predicted 95th percentile annual cumulative probability of infection (a) or DALY (b) by MRSA or MSSA from immersion in treated GW or WW. The vertical lines show risk-based LRTs for bacteria.^{33,34} The indoor nonpotable LRT is for the domestic indoor nonpotable uses of clothes washing and toilet flushing. The horizontal lines show benchmark levels.

$P_{inf,h,GW}$ and $DALY_{h,GW}$ to plausible changes in the characterization of the most influential inputs identified by the multivariate sensitivity analysis, including $C_{raw,S.aureus,GW}$. To conduct the uncertainty analysis, we reran the full Monte Carlo QMRA model for each combination of the selected uncertain inputs. The second input that was varied was $P_{skin,inf|col_h}$ for $P_{inf,h,GW}$ and the uncertain $P_{bs,inf|col_h}$ for $DALY_{h,GW}$. The results present the LRV at which the predicted annual risk or DALYs equal the health benchmark (i.e., the LRT) for the selected input ranges.

3. RESULTS

3.1. Annual Probabilities of Colonization and Infection. The predicted annual probabilities of colonization of the nose for healthy adults from daily immersion in treated GW or WW decreased as the level of treatment (i.e., LRV) applied to GW and WW increased (Figure S1). The annual probabilities of colonization were greater for MSSA than MRSA (assuming the absence of antibiotics) by less than 1 $\log_{10}$ for both GW and WW (Tables S1 and S2), given a selected LRV. This resulted from the higher fraction of total *S. aureus* in the GW and WW that was MSSA than MRSA and the assumption that MSSA and MRSA shared the same colonization dose–response relationship.

Conversely, the predicted annual cumulative probabilities of infection (including both skin infection and BSI) were higher for MRSA than MSSA for both GW and WW, given a selected LRV (Figure 1 and Tables S5 and S8), due to the higher probability of skin infection, given colonization ($P_{skin,inf|col_{MRSA}}$) for MRSA compared to MSSA (Table 1). The predicted annual cumulative probabilities of infection for both MRSA and MSSA were dominated by the skin infections rather than the BSIs (Tables S3 and S4 and S6 and S7). The predicted 95th percentile annual cumulative probabilities of infection for MRSA and MSSA were less than 10^{-4} ppy for WW and GW immersion, given bacteria LRVs of roughly 3 (or more) and for the selected LRTs for nonpotable reuse^{33,34} (Figure 1) as well as an estimated LRV of 15 for potable reuse.³⁵

3.2. Disability-Adjusted Life Years. The predicted annual cumulative DALYs for MRSA and MSSA were dominated by the BSI burden (Tables S9 and S10 and Tables S12 and S13), even though the predicted annual cumulative probabilities of infection were dominated by the probability of skin infection. This is due to the relatively high severity of BSI

(Table 1). For both WW and GW reuse, the predicted 95th percentile annual cumulative DALYs resulting from MRSA infection were greater than those from MSSA, a difference of less than 1 $\log_{10}$ (Figure 1 and Tables S11 and S14) given a selected LRV. The predicted 95th percentile annual cumulative DALYs from MRSA or MSSA infection were less than the benchmark of 10^{-6} DALY ppy when the treatment LRV was greater than roughly 4.5 or 3.5, respectively, for WW or GW reuse. This includes the selected LRTs for nonpotable reuse^{33,34} (Figure 1) as well as an estimated LRV of 15 for potable reuse,³⁵ with one exception; the predicted 95th percentile annual cumulative DALY from MRSA was greater than the benchmark assuming immersion in GW treated to the level prescribed by the nonpotable indoor use LRT for bacteria.¹ However, applicable indoor uses (i.e., toilet flushing and clothes washing) do not require immersion.¹

3.3. Sensitivity Analysis. Multivariate sensitivity analyses of the MRSA dose and daily cumulative probability of infection were performed for all input parameters. The dose was most sensitive to the treatment LRV and the *S. aureus* concentration in the raw water (GSIs of 7.4×10^{-1} and 7.3×10^{-1} , respectively) compared to the inputs of volume, the fraction that is resistant, and the HGT rate (GSIs of 1.3×10^{-2} , 3.8×10^{-4} , and 7.2×10^{-13} , respectively). Sensitivity analysis of the cumulative probability of MRSA infection also identified the treatment LRV and the *S. aureus* concentration in the raw water as important inputs along with the probability of skin infection given colonization (GSIs of 3.2×10^{-1} , 3.2×10^{-1} , and 2.6×10^{-1} , respectively). The exposure volume, the fraction that is resistant, probability of BSI given colonization, and the HGT rate were less important (GSIs of 3.6×10^{-3} , 2.4×10^{-3} , 2.4×10^{-5} , and 2.0×10^{-13} , respectively).

3.4. Uncertainty Analysis. For GW reuse, LRVs less than roughly 3.0 resulted in 95th percentile MSSA and MRSA annual cumulative probabilities of infection that exceeded the health benchmark, given the input characterizations in Table 1 (Figures 1 and 2). An increase in the raw GW MSSA or MRSA concentration of 3 $\log_{10}$ or more resulted in an LRT (based on infection or DALY benchmarks) that was less than the level of treatment expected by risk-based reuse systems (i.e., bacteria LRV of 6.0 or greater),^{18,20} assuming the best estimate values of $P_{skin,inf|col_h}$ and $P_{bs,inf|col_h}$ (Figures 2, S2, and S3). If $P_{bs,inf|col_{MRSA}}$ and $P_{skin,inf|col_{MRSA}}$ decreased to those of MSSA, then even greater increases in concentration (i.e., >3

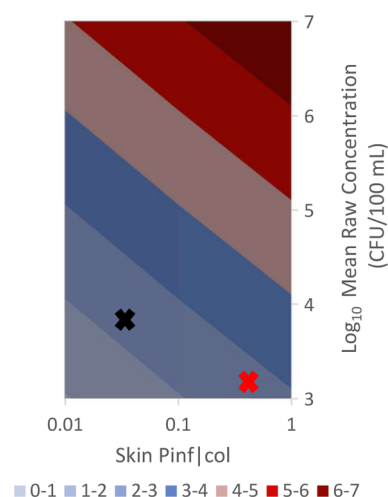


Figure 2. LRV required to achieve an annual infection risk of 10^{-4} ppy from immersion in treated GW across a range of possible raw GW MRSA/MSSA concentrations and morbidity ratios. The best estimate probability of skin infection given colonization (x-axis) and mean MRSA or MSSA concentration in raw GW (y-axis) are represented for MRSA (red x) and MSSA (black x).

$\log_{10}$ increase) could occur while maintaining an LRT of 6.0 or less.

4. DISCUSSION

Mean concentrations of *S. aureus* in GW and WW, including difficult to treat antimicrobial resistant strains,^{20,56} are higher than those of enteric bacteria typically used to evaluate the risk of water reuse in the QMRA (e.g.,^{33,35}), based on the limited available data. Yet, few QMRAs have evaluated the risks from exposure to *S. aureus* in reclaimed water (e.g., refs 29 and 30) and none consider the potential risk or disease burden from exposure to MRSA.

We evaluated the probability of nasal colonization by MRSA or MSSA for adults from daily immersion in treated GW or WW and subsequent skin infection elsewhere on the body and BSI in the absence of antibiotics. This is a newly proposed approach to modeling delayed infection risk of *S. aureus* because the previous QMRA (e.g., refs 32 and 57) used a dose–response relationship of acute skin infection, which we believe is less representative of potential infections from reclaimed water (further discussed in Section 4.3.1). We evaluated GW and WW treatment systems across a range of WW treatments with a focus on the LRVs corresponding to risk-based LRTs for nonpotable and potable reuse.

4.1. Risk from MSSA and MRSA in Reclaimed Water. The predicted annual infection risks for healthy adults from exposure to MRSA or MSSA resulting from immersion in treated GW or WW in the absence of antibiotics were relatively low ($<10^{-4}$ infections ppy), assuming a treatment LRV of at least 3.0 based on the limited available exposure and risk-characterization data. Thus, GW and WW treatment systems that comply with risk-based LRTs for bacteria that are 3.0 or greater (for example, indoor nonpotable reuse,^{2,33} showering,³⁴ and direct potable reuse³⁵) may result in relatively low infection risks from MSSA or MRSA for healthy adults. Still, daily immersion in minimally treated (or raw) GW or WW (with LRVs near zero) may result in relatively high infection risks for both MSSA and MRSA. Again, daily immersion is a conservative exposure estimate for nonpotable uses (Section

2.1.1), for which only a fraction of the anterior nares may be exposed and with less frequency. The predicted infection risk for MRSA and MSSA in raw WW fell below the benchmark when roughly 0.1% of the surface area of the anterior nares of the nose was exposed daily (results not shown).

4.2. Disease Burden from MSSA and MRSA in Reclaimed Water. The predicted annual DALYs capture the severity associated with health outcomes resulting from invasive infections.⁶ Whereas the annual cumulative probability of infection was dominated by the predicted skin infection risk, the predicted annual cumulative DALY was dominated by the BSI burden. The predicted 95th percentile DALYs were below the health benchmark of 10^{-6} ppy³⁷ for MSSA or MRSA, given LRVs of roughly 3.5 or 4.5, respectively. Thus, GW and WW treatment systems that comply with risk-based LRTs for bacteria may also result in low disease burden from MSSA, given the limited available input data.

Although the LRT for bacteria for nonpotable uses can be less than 4.5, the actual bacteria LRV for these systems is often greater in practice, given the corresponding virus LRT. For example, a selected indoor use LRT of 3.5 for bacteria may underestimate the actual LRV, given the corresponding LRT of 6.0 for viruses, which results in a much larger LRV for bacteria as a consequence.^{33,35} The uncertainty analysis showed that the LRT (based on the risk or DALY benchmarks) remained below 6.0 even for large increases in MRSA raw water concentration. Thus, the MRSA disease burden may also be below the health benchmark for risk-based, nonpotable, or potable reuse systems. Still, minimally treated GW or WW may result in MSSA or MRSA immersion exposures with predicted annual DALYs that exceed the benchmark (Figure 1). The predicted MRSA disease burden from raw WW fell below the benchmark when roughly 0.01% of the surface area of the anterior nares of the nose was exposed daily (results not shown).

4.3. Limitations and Improvements of the MSSA/MRSA QMRA. **4.3.1. Dose–Response.** CA-MRSA infections occur with and without prior nasal colonization.⁶ This QMRA modeled colonization and delayed infection, rather than acute skin infection, given that risk factors generally accompany acute infection.¹² An “acute” skin infection human dose–response relationship for *S. aureus*^{31,58} was previously used in QMRA^{29,30} and was based on dose–response data from a nontypeable strain.⁵⁹ The dose was incubated on the skin for 6 days using moist wrappings after previous human infection studies showed that the skin is an effective barrier against *S. aureus* infection.^{9–11} The resulting lesion resembled “contact dermatitis” and lacked the “classical signs of infection.”⁵⁹ The predicted probability of infection from MSSA or MRSA using the acute skin infection model⁵⁸ was less than 10^{-4} ppy across the selected LRV range (results not shown).

The greatest limitation of this QMRA is the lack of strain-specific data to inform the dose–response relationships. We used nasal colonization dose–response data of epidemic strain 52/52A/80/81 (CC30). Strain 52/52A/80/81 caused outbreaks of severe disease in the mid-20th century.⁶⁰ Current strains belonging to the same clonal complex 30 gained a mutation that made them less virulent.⁶¹ Due to the limited dose–response data, this epidemic strain was used to characterize nasal colonization for the (epidemic) MRSA strain USA300 and MSSA. Given the many host and strain factors that affect colonization, including the ability to adhere to the nasal epithelial cells and evade host immune defenses,⁶²

we were unable to differentiate between the ability of the reference hazard to colonize and the strains of interest. Additionally, we assumed that the entire exposed population was susceptible to colonization, whereas bacterial interference by established strains may prevent colonization by newly introduced strains.⁶²

The QMRA did not explicitly account for risk factors that may contribute toward infection, such as crowded conditions, poor hygiene, previous antibiotic exposure, damaged skin, or underlying disease.^{12,63} Rather, we developed morbidity ratios to estimate the delayed skin infection and BSI outcomes, which implicitly account for risk factors within the studied population of soldiers.^{50,51} It is unclear if the studied population of soldiers is representative of the general population due to the lack of data. The studies used to characterize the probability of skin infection given colonization reported no BSI.^{25,26} Based on the total number of subjects in each study, we would expect that *Pbs.inflcol*_{MRSA} and *Pbs.inflcol*_{MSSA} (characterized in Table 1 as 1.5×10^{-2} and 1.2×10^{-3} , respectively) would be less than 4.0×10^{-2} and 4.0×10^{-3} , respectively.

4.3.2. Data Collection Needs. To improve the QMRA of MSSA and MRSA, data collection efforts should prioritize the collection of dose–response data and conditional probabilities of infection for the predominant strains of interest (i.e., USA300 and USA100 in the United States). Although a variety of animal models of infection have been proposed for *S. aureus*,⁶⁴ the experiments typically administer a single, high dose to ensure infection. Dose–response data for USA300 and USA100 across a range of realistic doses with colonization and skin infection as the endpoints are needed to substantiate this QMRA model.

The sensitivity analysis identified concentration as the most important factor in exposure, and data on *S. aureus* concentrations in GW or WW remain limited. Likewise, the treatment performance of GW or WW treatment units for *S. aureus* is also sparse.^{65,66} Further evidence regarding the prevalence of various *S. aureus* lineages in WW, their concentrations, and their differential treatment responses would improve the risk estimates for reuse as well as possible recreational exposures.

4.4. Implications for QMRA. This work provides insights into areas of QMRA that are in development for resistant organisms. A widely recognized knowledge gap is the paucity of human dose–response data for resistant organisms. Section 4.3.1 describes the surrogate approach we used given the lack of MRSA dose–response data.

4.4.1. Resistance Treatment Removal or Enrichment. To estimate the MRSA and MSSA concentrations in treated WW and GW, we considered the removal and enrichment of MRSA and MSSA in treatment. We expressed the removal of MSSA and enrichment of MRSA from HGT in the form of LRVs and LIVs to directly compare with the removal expected by treatment. The predicted log₁₀ increase in MRSA from HGT was small compared to the treatment LRVs of interest for nonpotable and potable risk-based treatment systems. The global sensitivity analysis also indicated that HGT in the treatment system had little influence on exposure.

For HGT rates to be relevant for the dose of MRSA in a reuse treatment system, the transconjugant/recipient rate must be on the order of 10^{-1} or more to be more comparable with an LRV of 3 or greater expected by risk-based reuse treatment systems. The rates found in the experimental literature were on the order of $10^{-5.6}$ – 10^{-9} for single species (*S. aureus*) co-

culture, for which the SSmec gene is known to play a role in the transference between MSSA and MRSA.²⁸ The HGT rates were relatively negligible (from an exposure perspective), despite the experimental duration of 24 h and a ratio of donor/recipient of 1:1, whereas the WW treatment compartments of interest have shorter retention time and fewer (*S. aureus*) donors. These rates were not determined in a WW matrix and therefore may not be representative of in-field conditions. Other scenarios, such as the exposure to antibiotic contamination, may drastically alter these numbers resulting in an observed effect.⁶⁷ The complex environment of biological WW treatment may also positively affect *in situ* HGT.²⁰

Although HGT in WW treatment was not identified as an important factor in MRSA exposure in this QMRA of water reuse, HGT may still be an important factor in the water reuse context. Other species are more likely to share genes (e.g., *E. coli*).^{68,69} There is also a concern of gene transfer among different species/genera, particularly when the recipient is a new pathogen.⁷⁰ Additionally, HGT may occur within the biofilm of premise plumbing or possibly within the host, given the colonization of the gut.^{71–73}

4.4.3. Broader Public Health Impacts. Because HGT is known to be the dominant mechanism responsible for the global dissemination of antibiotic resistance in pathogens,^{74,75} incorporating HGT may be important for estimating the broader health impacts of the loss of antibiotic efficacy, contribution to antibiotic resistance reservoirs, and the emergence of new resistance. In these cases, “acute” measurements of infection and disease burden will likely not be sufficient metrics; rather, a different set of metrics that account for the compounding effect of multiple HGT events through multiple cycles of water use/reuse are likely needed to assess the broader health impacts of emergent resistant pathogens.

Although this QMRA was limited to MRSA, we suspect that other resistant bacteria will also be sufficiently treated in risk-based reuse systems to result in low acute infection risks given the high treatment levels. However, that does not mean that management of antimicrobial resistance is not required in reuse applications. WW or GW treatment systems with more lenient treatment requirements, for example, conventional municipal treatment systems, may result in exposures with high predicted risks and disease burdens and have been identified as environmental sources of resistant bacteria and genes, impacting surface water quality and potential recreational exposures.²² Regardless of the level of treatment, the broader health impacts related to the spread and dissemination of resistant genes through water reuse warrant future research and may require management in a form different from the LRTs aimed to achieve benchmark levels of acute or delayed risk.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.1c04038>.

Predicted annual probabilities of colonization, infection, and DALYs, uncertainty analysis of the annual DALY, and calculation of the MSSA BSI DALY per case of infection (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Mary E. Schoen – Soller Environmental, LLC, Berkeley, California 94703, United States; orcid.org/0000-0002-2837-3517; Email: mschoen@sollerenvironmental.com

Authors

Michael A. Jahne – U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, Ohio 45268, United States; orcid.org/0000-0002-4741-9859

Jay Garland – U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, Ohio 45268, United States

Lucia Ramirez – School of Mathematical and Statistical Sciences, Arizona State University, Tempe, Arizona 85281, United States

Allison J. Lopatkin – Department of Biology, Barnard College, New York, New York 10027, United States; Department Ecology, Evolution, and Environmental Biology, Data Science Institute, and Department of Systems Biology, Columbia University, New York, New York 10027, United States; orcid.org/0000-0003-0018-9205

Kerry A. Hamilton – School of Sustainable Engineering and the Built Environment, Tempe, Arizona 85281, United States; The Biodesign Center for Environmental Health Engineering, Tempe, Arizona 85287, United States; orcid.org/0000-0003-2991-7325

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acs.est.1c04038>

Author Contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript. All authors contributed equally. M.E.S., M.A.J., J.G., A.J.L., L.R., and K.A.H.

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Notes

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