Science of the Total Environment 595 (2017) 35–40

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Higher prevalence of coagulase-negative staphylococci carriage among reclaimed water spray irrigators

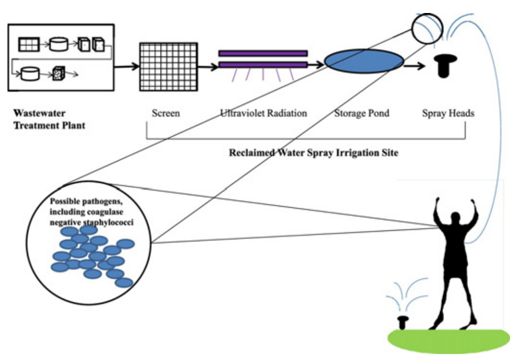
Rachel Rosenberg Goldstein [a,*,1], Lara Kleinfelter [a,b,1], Xin He [c], Shirley A. Micallef [d,e], Ashish George [a], Shawn G. Gibbs [f], Amy R. Sapkota [a]

[a] Maryland Institute for Applied Environmental Health, University of Maryland School of Public Health, College Park, MD, USA
[b] Department of Microbiology & Immunology, Albert Einstein College of Medicine, Bronx, NY, USA
[c] Department of Epidemiology and Biostatistics, University of Maryland School of Public Health, College Park, MD, USA
[d] Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742, USA
[e] Center for Food Safety and Security Systems University of Maryland, College Park, Maryland, USA
[f] Department of Environmental Health, Indiana University School of Public Health, Bloomington, Indiana, USA,

HIGHLIGHTS

- Prevalence and odds of CoNS carriage evaluated in reclaimed water spray irrigators.
- CoNS prevalence higher in reclaimed water spray irrigators compared to controls.
- MRCoNS carriage higher among reclaimed water spray irrigators compared to controls.
- Odds of CoNS carriage significantly increased with exposure to reclaimed water.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 December 2016
Received in revised form 14 March 2017
Accepted 19 March 2017
Available online 1 April 2017

Editor: Simon Pollard

Keywords:
Antibiotic-resistant bacteria
CoNS

ABSTRACT

Coagulase negative staphylococci (CoNS) are leading causes of nosocomial infections and community-acquired methicillin-resistant CoNS (MRCoNS) infections are increasing. CoNS have been previously detected in reclaimed water. To date, no studies have evaluated the prevalence of CoNS carriage among humans exposed to reclaimed water in the U.S. We examined the prevalence and odds of CoNS and antibiotic-resistant CoNS carriage in spray irrigators exposed to reclaimed water compared to controls. We collected nasal and dermal swab samples from 19 reclaimed water spray irrigation workers ($n = 96$ total samples) and 24 controls ($n = 92$ total samples). Samples were analyzed for CoNS using culture-based assays. Isolates were confirmed using biochemical tests and PCR. Antimicrobial susceptibility testing was performed using disk diffusion. Data were analyzed by two-sample proportion tests, logistic regression, and generalized linear mixed effects models.

Abbreviations: CoNS, coagulase-negative staphylococci; BHI, Brain Heart Infusion; GLMM, generalized linear mixed effects model; MRCoNS, methicillin-resistant CoNS; WWTP, wastewater treatment plant.

* Corresponding author at: University of Maryland School of Public Health, Maryland Institute for Applied Environmental Health, School of Public Health Building (255), 4200 Valley Drive, College Park, MD 20742, USA.

E-mail addresses: rerosenb@umd.edu (R.R. Goldstein), lara.kleinfelter@phd.einstein.yu.edu (L. Kleinfelter), xinhe@umd.edu (X. He), smicall@umd.edu (S.A. Micallef), ars@umd.edu (A. George), gibbss@indiana.edu (S.G. Gibbs).

[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.scitotenv.2017.03.174

Occupational exposure
Alternative water sources
Spray irrigation

The prevalence of CoNS, antibiotic-resistant CoNS, and MRCoNS carriage among spray irrigation workers was 79% (15/19), 32% (6/19), and 16% (3/19), compared to 13% (3/24), 4% (1/24), and 0% (0/24) of controls. Spray irrigators were more likely to be carriers of CoNS ($p < 0.01$), antibiotic-resistant CoNS ($p < 0.01$), and MRCoNS ($p = 0.02$) compared to controls. The odds of CoNS carriage significantly increased with exposure to reclaimed water ($p = 0.04$) even accounting for changes over time ($p = 0.05$). Our data highlight the need to further examine the potential dissemination of CoNS and antibiotic-resistant CoNS from reclaimed water into the environment and human communities and related public health implications.

1. Introduction

Coagulase-negative staphylococci (CoNS) are ubiquitous bacteria in the environment, found in dust, soil, air, water, mammals, and some food products (May et al., 2014). Their wide distribution and environmental persistence allow them to act as effective opportunistic pathogens, and they are responsible for a significant proportion of nosocomial infections including bacteremias, catheter-related infections, endocarditis, surgical site infections, urinary tract infections, and exposed wound infections (May et al., 2014; Rogers et al., 2009). Nosocomial CoNS infections have increased in recent years due to the greater use of prosthetic devices, intravascular catheters, and invasive technologies in patients who are immunocompromised or in critical condition, and cause 20–30% of bloodstream infections (May et al., 2014; Rogers et al., 2009).

CoNS also are characterized by increasing rates of antibiotic-resistance which lead to decreased treatment options among infected individuals (May et al., 2014). Of particular concern are methicillin-resistant coagulase-negative staphylococci (MRCoNS). Resistance to oxacillin (a commonly-used analog of methicillin) among CoNS was about 80% as determined by a longitudinal survey of hospital-acquired isolates between 1999 and 2012 (May et al., 2014). It is also common to observe resistance among CoNS to other clinically-relevant antibiotic classes including aminoglycosides, tetracyclines, macrolides, and fluoroquinolones (Rogers et al., 2009). Moreover, there has been a recent rise in community-acquired MRCoNS infections—illnesses among individuals who are not exposed to healthcare settings—raising the question as to whether there may be increasing (and yet unknown) environmental sources of these microorganisms (Lebeaux et al., 2012).

One potential environmental source of CoNS within agricultural and non-agricultural communities may be reclaimed water (treated municipal wastewater) that is applied in reuse settings. Across the United States, municipalities faced with increasing populations and critical water shortages are utilizing reclaimed water as a resource for many applications including recharging groundwater reservoirs, irrigation, and industrial use (Levine and Asano, 2004; Tonkovic and Jeffcoat, 2002). Although the use of reclaimed water is gaining popularity as water shortages become more frequent and wastewater treatment processes continue to improve, there are limited data regarding the extent to which any pathogens persisting in reclaimed water can be disseminated in the environment and impact human populations. *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) have been isolated from wastewater effluent intended for reuse by our research group and from greywater intended for reuse in Israel (Maimon et al., 2014; Rosenberg Goldstein et al., 2012). In addition, a recent Spanish study detected *Aeromonas* and *Arcobacter* in tertiary-treated water intended for reuse (Fernandez-Cassi et al., 2016). CoNS also can survive wastewater treatment and have been isolated from treated effluent (Faria et al., 2009). However, to our knowledge no previous studies have evaluated whether occupational exposures to reclaimed water could affect the prevalence of CoNS carriage among reclaimed water spray irrigation workers.

The goal of this study was to examine the prevalence and odds of CoNS and antibiotic-resistant CoNS carriage among spray irrigators exposed to reclaimed water compared to office worker controls. Our findings address an important knowledge gap relating to potential human health effects associated with the use of this alternative freshwater source.

2. Materials and methods

2.1. Study site

Spray irrigation workers employed at a reclaimed water spray irrigation site in the Mid-Atlantic region of the U.S. were included in this study. The site was chosen based on the willingness of the site operator and workers to participate in the study. The reclaimed water spray irrigation site receives treated wastewater from a tertiary wastewater treatment plant.

Detailed schematics and descriptions of the wastewater treatment plant (WWTP) and spray irrigation site are published in Rosenberg Goldstein et al. (2012, 2014a, 2014b) and Carey et al. (2016) (Carey et al., 2016; Rosenberg Goldstein et al., 2012; Rosenberg Goldstein et al., 2014a). Briefly, Mid-Atlantic WWTP1 is a tertiary WWTP in an urban area (Fig. 1). Tertiary wastewater treatment includes primary treatment (physical removal of solids), secondary treatment (biological treatment), and additional treatment that can include, but is not limited to, chlorination, ultraviolet (UV) radiation, filtration or lagooning. The raw wastewater influent (681,390 m^3/day) at Mid-Atlantic WWTP1 includes domestic and hospital wastewater and the plant uses the following treatment steps: screens, primary clarifier, primary aeration tank, secondary aeration tank, secondary clarifier, multimedia filter, chlorination, dechlorination and discharge. The chlorination dose at this plant was 2–3 mg/L, followed by dechlorination with sodium bisulfite such that the chlorine residual in effluent is <0.1 mg/L. This treated effluent is then piped to the spray irrigation site where it passes through a double-walled aluminum screen and is then treated with 254 nm ultraviolet (UV) radiation bulbs that produce a minimum of 30,000 μW-s/cm^2. After UV treatment, the water is pumped into an open-air storage pond at a rate of 230,000 gal per day with a peak capacity of 4 million gallons. Based on turf irrigation needs, the reclaimed water is then pumped from the storage pond to spray heads (Fig. 1). Spray irrigation workers also carry backpack spray systems to irrigate additional areas. The spray irrigation site employs eight full-time employees and approximately 22 seasonal employees each year.

2.2. Subject selection

This study was approved by the University of Maryland College Park, Institutional Review Board, IRB Protocol 09-0211. Subject selection was previously described in detail in Rosenberg Goldstein et al. (Rosenberg Goldstein et al., 2014b). A total of 43 subjects were enrolled in the study: 19 spray irrigation workers who were occupationally exposed to reclaimed water and 24 office worker controls from an academic work setting who were not exposed to reclaimed water or healthcare settings on the job. Study subjects were selected through a convenience sample based on employment status. Office worker controls were matched by sex and race and were within a similar age range (±5 years) to the spray irrigation workers. Controls were recruited into the study in person and over email. Individuals were excluded

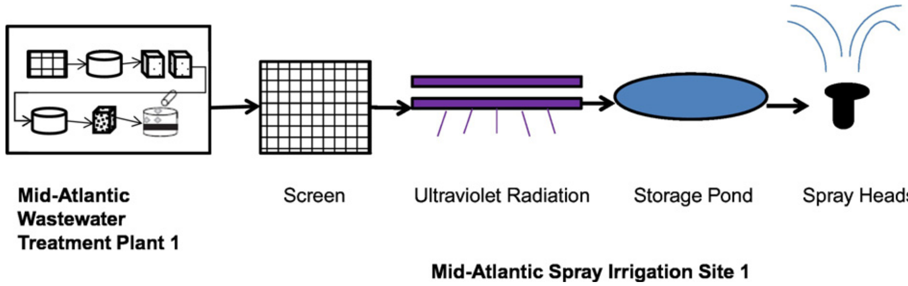


Fig. 1. Schematic of treatment and distribution processes at the reclaimed water spray irrigation site.

from participation if they reported a nosebleed three days prior to sample collection to avoid dislodging blood clots during sample collection.

2.3. Survey

Participants were asked to complete a short survey containing questions on demographics as well as potential risk factors associated with CoNS carriage including age, duration of job, yearly income, household income, exposure to healthcare settings, and current smoking status. The survey was completed by participants on site at each sampling event. A detailed description of the survey and its results are published in Rosenberg Goldstein et al. (2014b).

2.4. Sample collection

A total of 94 nasal swab samples (48 from spray irrigation workers and 46 from office worker controls) and 94 dermal swab samples (48 from spray irrigation workers and 46 from office worker controls) were collected. Samples from spray irrigation workers were recovered when the irrigation spray heads were in use during summer. Samples from controls were collected during the summer, fall, or winter of the same year as the spray irrigator samples. Spray irrigator and office worker control participants were sampled at multiple time points when possible. Nasal swabs were collected using Liquid Stuart Medium Transport swabs (Copan, Italy). The swab was inserted approximately 1.25 cm into the participant's right nostril and gently rotated five times on the inside wall of the nostril (Bassetti et al., 2005; Ochei and Kolhatkar, 2000). Dermal swabs were also collected using the same type of swabs. An approximately five-by-five cm area of the participant's right forearm was swabbed by rolling the swab back and forth 15 times (Kullander et al., 2009). All samples were transported to the laboratory at 4 °C and processed within 24 h.

2.5. Isolation and purification

All media was obtained from Becton, Dickinson and Company (Franklin Lakes, NJ).

Nasal and dermal swabs were streaked onto Baird Parker plates within 24 h of collection for the selection of *Staphylococcus* spp. Presumptive CoNS colonies were those that were brown or dark grey to black in color and lacked clear zones around the colony, which are characteristic of *S. aureus*. Presumptive CoNS were purified on Brain Heart Infusion (BHI) agar. After purification, all isolates were stored at −80 °C in Brucella broth with 15% glycerol.

2.6. Biochemical testing

Presumptive CoNS were gram stained, tested for catalase activity, and tested for coagulase activity (Becton, Dickinson and Company). Gram positive cocci exhibiting catalase activity and lacking coagulase activity were confirmed as CoNS as described below.

2.7. Confirmation of CoNS

Conventional multiplex PCR was used to confirm the identity of presumptive CoNS by amplification of the 16S rRNA gene as a *Staphylococcus*-specific internal control and the *nuc* gene, which is specific to *S. aureus*. Amplification of the *mecA* gene was also performed as a predictor of methicillin-resistance. The PCR conditions and primers used were described in Maes et al. (Maes et al., 2002) with the following modifications. One colony purified on BHI agar was suspended in 100 μL sterile water. Of each suspension, 3 μL provided the DNA template for the reaction. *S. aureus* ATCC 43300 was used as a positive control for PCR amplification of *nuc* and *mecA* genes, while water was used as a negative control. The 50 μL PCR reaction mix contained 1X PCR buffer (New England BioLabs), 2.5 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphates (Bio-Rad), 0.4 μM nuc-specific primers (Invitrogen), 1 μM *mec*A-specific primers (Invitrogen), 0.8 μM 16S rDNA-specific primers (Invitrogen), and 1.25 units/μL standard Taq DNA polymerase (New England BioLabs). PCR amplification consisted of 95 °C for 3 min, then 34 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and finally 72 °C for 5 min. Resulting amplicons were separated on a 1% agarose gel. Isolates showing amplification of the 16S rDNA gene, but not the *nuc* gene were confirmed as CoNS. In addition, isolates showing amplification of the *mecA* gene were considered presumptive MRCoNS. These isolates were confirmed by another multiplex PCR assay using a second set of primers against the same genes. These primers are described in Edwards et al. (Edwards et al., 1989) and Fang and Hedin (Fang and Hedin, 2003). The 30 μL PCR master mix contained 1X PCR buffer (New England BioLabs), 2 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphates (Bio-Rad), 0.25 μM nuc-specific primers (Invitrogen), 1 μM *mec*A-specific primers (Invitrogen), 0.5 μM 16S rDNA primers (Invitrogen), and 0.6 units/μL standard Taq DNA polymerase (New England BioLabs).

2.8. Antibiotic susceptibility testing

Standard disk diffusion methods (CLSI, 2013) on Muller Hinton agar (Becton, Dickinson and Company) were used to evaluate phenotypic antimicrobial resistance of confirmed CoNS. Specifically, disk diffusion testing was performed using 30 μg cefoxitin disks, 15 μg erythromycin disks and 30 μg tetracycline disks (Becton, Dickinson and Company), representing three classes of antibiotics: penicillins, macrolides, and tetracyclines. The following zone of inhibition diameters were used to define resistance: cefoxitin, ≤24 mm; erythromycin, ≤13 mm; tetracycline, ≤14 mm (CLSI, 2013). *S. aureus* ATCC 29523 was used for quality control. Cefoxitin was used as a surrogate for oxacillin resistance in CoNS as recommended by the CLSI performance standards (CLSI, 2013). Multi-drug resistance (MDR) was defined as resistance to three or more classes of antibiotics (Magiorakos et al., 2012). Isolates were considered clones if they were obtained from the same sampling location on the same sampling trip, showed the same amplification bands by PCR, and exhibited zone of inhibition diameters within 2 mm of each other for all three antibiotics. Only one isolate from each set of clones was included in subsequent analyses.

2.9. Statistical analyses

Descriptive statistics included the percentages of human biological samples that were positive for CoNS, antibiotic-resistant CoNS, and MRCoNS. Two-sample tests of binomial proportions were used to compare the percentages of spray irrigation workers and controls that had a nasal or dermal swab test positive for CoNS, antibiotic-resistant CoNS, or MRCoNS at any sampling event. Logistic regression models were used to determine how occupational exposure to reclaimed water affected the odds of carriage with CoNS or antibiotic-resistant CoNS at any sampling date. Confounders were identified from the survey data described above and defined as a change in odds ratio (OR) of ≥10%. We controlled for age, duration of job, yearly income (dichotomized as above or below the 2009 poverty guideline), household member exposure to healthcare settings, personal exposure to healthcare settings, and current smoking status in the model that included CoNS carriage as the outcome, and we controlled for duration of job, yearly income, and current smoking status in the model that included antibiotic-resistant CoNS carriage as the outcome. A generalized linear mixed effects model (GLMM) was used to evaluate the odds of CoNS or antibiotic-resistant CoNS carriage over time by occupational status. In all cases, p-values of ≤0.05 were defined as statistically significant. All statistical analyses were performed using Stata/IC 10 (StataCorp LP, College Station, TX).

3. Results

The participation rate for the study was 88% (43/49). The detailed results from the survey are published in Rosenberg Goldstein et al. (2014b). Briefly, the majority of participants were Caucasian males with a mean age of 34 and 33 years for spray irrigation workers and office worker controls, respectively. Controls had higher levels of education ($p < 0.01$), higher yearly incomes ($p = 0.01$), and were more likely to currently smoke ($p < 0.01$) and have smoked >100 cigarettes in the past six months ($p < 0.01$).

3.1. Prevalence of CoNS, antibiotic-resistant CoNS, and MR-CoNS

CoNS were detected in either nasal or dermal swabs among 79% (15/19) of spray irrigation workers and 13% (3/24) of the office worker controls ($p < 0.01$). The prevalence of antibiotic-resistant CoNS (resistant to at least one of the three antibiotics tested) carriage among spray irrigation workers and office worker controls was 32% (6/19) and 4% (1/24), respectively ($p < 0.01$). When comparing only participants with at least one positive nasal swab sample, the increased prevalence among spray irrigation workers was still evident (CoNS: 74% spray irrigation workers, 13% controls, $p < 0.01$; antibiotic-resistant CoNS: 32% spray irrigation workers, 4% controls, $p < 0.01$).

MRCoNS was recovered from 16% (3/19) of spray irrigation workers. All MRCoNS were detected in nasal swabs. No MRCoNS were detected in any of the nasal or dermal swabs collected from the office worker controls. The probability of ever carrying MRCoNS in the nares was significantly higher among spray irrigation workers compared to controls ($p = 0.02$).

3.2. Antibiotic resistance patterns

A total of 43 CoNS isolates, confirmed as unique through phenotypic analyses, were recovered throughout the study from nasal ($n = 38$ isolates) and dermal swabs ($n = 5$ isolates) and used for statistical analysis: 40 isolates from spray irrigation workers (35 nasal, 5 dermal) and 3 isolates from office worker controls (3 nasal, 0 dermal). Only one isolate from a spray irrigation worker was multidrug resistant. Looking at nasal and dermal swabs combined, a greater percentage of CoNS isolates from spray irrigation worker swabs were resistant to cefoxitin ($p = 0.71$) and tetracycline ($p = 0.74$) compared to that of office worker controls. A greater percentage of CoNS isolates from office workers were resistant to erythromycin ($p = 0.88$) compared to that of spray irrigation workers (both nasal and dermal swabs). However, these differences were not statistically significant.

Looking at the nasal swabs only, the patterns were the same, but the associations were stronger. The greater percentage of erythromycin-resistant CoNS isolates from office workers compared to spray irrigation workers was statistically significant ($p = 0.05$) (Fig. 2). A greater percentage of CoNS isolates from spray irrigation worker nasal swabs were resistant to cefoxitin ($p = 0.27$) and tetracycline ($p = 0.25$) (Fig. 2); however, these differences were not statistically significant.

When assessing the dermal swabs only, five dermal swabs were positive for CoNS from spray irrigation workers and no dermal swabs were positive from controls. Among the five unique isolates from the five positive spray irrigation worker dermal swabs, 40% ($n = 2$ isolates) were resistant to erythromycin.

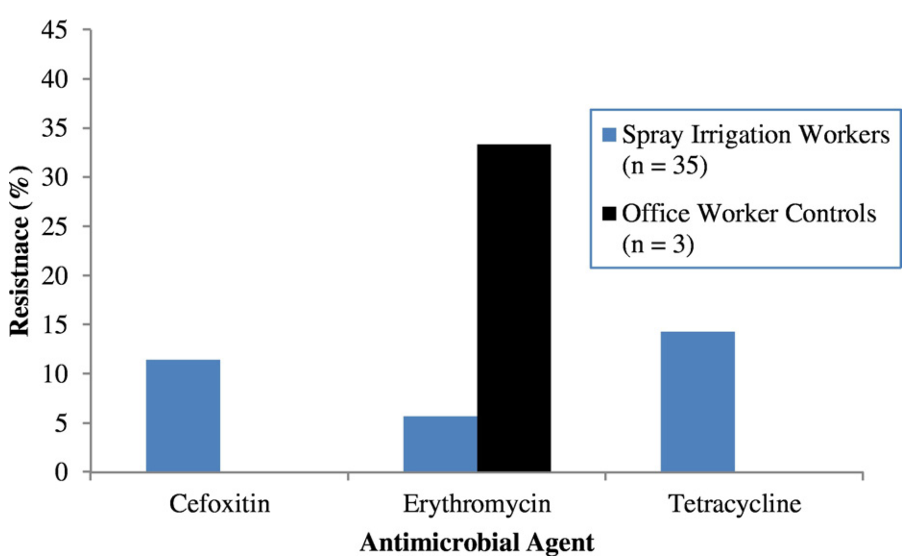


Fig. 2. Percent resistance to antimicrobial agents observed among CoNS isolates recovered from spray irrigation worker and office worker control A) nasal ($n = 38$ isolates) and B) dermal swabs ($n = 5$ isolates).

Table 1
Estimated odds of CoNS carriage or antibiotic-resistant CoNS carriage by occupational status.

Outcome	Exposure	Unadjusted OR 95% CI *p*-value	Adjusted OR 95% CI *p*-value
CoNS carriage	Spray irrigation worker	26.25 (5.11, 134.92) <0.001	50.20[a] (1.10, 2282.09) 0.044
	Office worker	Ref	Ref
Antibiotic-resistant CoNS carriage	Spray irrigation worker	10.62 (1.15, 98.09) 0.037	4.17[b] (0.28, 62.73) 0.302
	Office worker	Ref	Ref

[a] Controlled for age, duration of job, yearly income, household member exposure to healthcare settings, personal exposure to healthcare settings, and current smoking status.
[b] Controlled for duration of job, yearly income, and current smoking status.

3.3. Impact of occupational exposure on CoNS carriage

The odds of CoNS carriage was greater among spray irrigation workers compared to controls ($p = 0.04$) (Table 1), even when changes over time were taken into account using the GLMM model ($p = 0.05$). The odds of antibiotic-resistant CoNS carriage were also greater among spray irrigation workers compared to controls, however the difference was not statistically significant (Table 1). There were no statistically significant differences in the odds of carrying antibiotic-resistant CoNS ($p = 0.37$) after adjusting for changes over time using the GLMM in addition to adjusting for duration of job, yearly income, and current smoking status (data not shown). The impact of occupational status on MRCoNS carriage could not be evaluated because of data sparsity.

4. Discussion

4.1. Community-acquired MRCoNS

In the current study, the prevalence of MRCoNS carriage among spray irrigation workers and office worker controls was 16% and 0% respectively. MRCoNS have historically been regarded as hospital-associated bacteria; however, there has been a recent rise in community-associated MRCoNS (Lebeaux et al., 2012). A study by Ruppé et al. (2009) analyzing CoNS and MRCoNS among outpatients in four geographically diverse countries found an average MRCoNS prevalence of 22% (Ruppé et al., 2009). Among outpatients in Moldova, the only developed country included in the Ruppé et al. (2009) study, the prevalence of MRCoNS was 11% (Ruppé et al., 2009). MRCoNS prevalence among individuals being admitted to a French hospital who had not been previously exposed to a healthcare setting was 16.5% in a study by Barbier et al. (2010) (Barbier et al., 2010). A study among Amerindians in French New Guinea by Lebeaux et al. (2012) found an unusually high prevalence of MRCoNS in the community of 48.7% averaged over two years of sampling (Lebeaux et al., 2012).

CoNS tend to express higher rates of antibiotic resistance compared to *S. aureus*, but community-associated CoNS usually exhibit lower rates of antibiotic resistance compared to hospital-associated CoNS (Archer and Armstrong, 1983; Rogers et al., 2009; Ruppé et al., 2009). In an analysis of drug resistance among community-acquired MRCoNS isolates, erythromycin and tetracycline resistance was found in 68.6% and 27.4% of isolates in the Barbier et al. (2010) study, and 48% and 55% of isolates recovered in the Ruppé et al. (2009) study when isolates from all countries were combined (Algeria, Moldova, Mali, and Cambodia). In the current study, the percentage of MRCoNS isolates from spray irrigation worker samples that were resistant to erythromycin and tetracycline was 25% (1/4) and 25% (1/4). There were no MRCoNS isolates from the office worker controls, but the one office worker CoNS isolate displaying any antibiotic resistance was resistant to erythromycin.

It is unclear where methicillin-resistance among CoNS in the human community is acquired. It has been suggested that wastewater could be a source of antibiotic-resistant bacteria and genes in the environment for a number of bacteria, including CoNS (Faria et al., 2009; Rosenberg Goldstein et al., 2012).

4.2. CoNS and antibiotic-resistant CoNS in wastewater and reclaimed water

Only one published study by Faria et al. (2009) has evaluated wastewater for the presence of antibiotic-resistant CoNS (Faria et al., 2009). Faria et al. (2009) calculated that 10^5 CFU/100 mL staphylococci were present in raw influent and 10^3 CFU/100 mL staphylococci were present in effluent that had undergone treatment with an activated sludge process. Of 48 staphylococci isolates from the WWTP identified to the species level, 47 were determined to be CoNS (Faria et al., 2009). No MRCoNS were isolated from WWTP samples collected in Portugal by this group, however, 10% and 20% of all CoNS isolates recovered in that study were resistant to tetracycline and erythromycin, respectively (Faria et al., 2009).

Our research group has detected vancomycin-resistant enterococci in reclaimed water (Carey et al., 2016) and MRSA in secondary-treated wastewater intended for reuse (Rosenberg Goldstein et al., 2012). The increased odds of CoNS carriage among reclaimed water spray irrigation workers in the current study coupled with the detection of CoNS in reclaimed water and treated wastewater from other studies suggest that the spray irrigation workers could be occupationally exposed to CoNS through reclaimed water.

4.3. Transfer of resistance genes between MRCoNS and MRSA

The relationship and possible transfer of genetic material between MRCoNS and MRSA in the environment is a public health concern. It has been shown that MRCoNS can share methicillin resistance genes with *S. aureus* through horizontal gene transfer, resulting in MRSA (Hanssen and Ericson Sollid, 2006; Wielders et al., 2001). One study found >99% similarity in a type of *SCCmec*, a mobile genetic element that carries the *mecA* gene complex, between a community-acquired methicillin resistant *S. epidermidis* strain (the most common type of CoNS among humans) and a community-acquired MRSA strain (Barbier et al., 2010; Lebeaux et al., 2012). MRSA poses a threat to human health due to the severity of infections it causes and the reduced treatment options available to infected individuals (Klevens et al., 2007). Therefore, the potential release of MRCoNS into the environment and community through reclaimed water, occupational exposure, and its possible association with the development and spread of MRSA in the community warrants further investigation.

4.4. Limitations

Because it was not the goal of this study to analyze reclaimed water for CoNS or perform microbial source tracking, we cannot confirm that CoNS, antibiotic-resistant CoNS, and MRCoNS found in nasal and dermal samples originated from reclaimed water. Moreover, since we did not track the study subjects for a long period of time, we do not know whether the CoNS carriage noted in this study influenced the risk of CoNS infection among the study participants. To our knowledge, the relationship between CoNS carriage and the risk of CoNS infection has not been fully explored; therefore, the public health implications of CoNS carriage observed in this study are unclear. The small number of human subjects is also a limitation that could not be overcome due to the very few spray irrigators that are employed at water reuse sites. This is a continual challenge in occupational health research where the specific occupation under study is characterized by very small numbers. Finally, the generalizability of our results is limited because spray irrigation workers employed at only one spray irrigation site from one U.S. region were included in this study.

5. Conclusions

Our study showed that the prevalence and odds of CoNS, antibiotic-resistant CoNS and MRCoNS carriage was higher among individuals who were occupationally exposed to reclaimed water compared to office worker controls. Although we cannot confirm that the source of these microorganisms was the reclaimed water that the spray irrigators handled, our data emphasize the need for further research into the potential dissemination of MRCoNS, antibiotic-resistant CoNS, and other bacterial pathogens from these water types into the environment and human communities.

Acknowledgements

We thank the manager and workers at the spray irrigation site for their participation and assistance. This work was supported by R03 Small Grants Program, Grant # 1-R03-OH009598-01 from the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention (CDC). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of CDC. The Maryland Water Resources Research Center also provided a summer fellowship to Rachel Rosenberg Goldstein that supported this work.

References

Archer, G.L., Armstrong, B.C., 1983. Alteration of staphylococcal flora in cardiac surgery patients receiving antibiotic prophylaxis. J. Infect. Dis. 147, 642–649.

Barbier, F., Ruppé, E., Hernandez, D., Lebeaux, D., Francois, P., Felix, B., et al., 2010. Methicillin-resistant coagulase-negative staphylococci in the community: high homology of sccmec iva between *Staphylococcus epidermidis* and major clones of methicillin-resistant *Staphylococcus aureus*. J. Infect. Dis. 202, 270–281.

Bassetti, S., Bischoff, W.E., Walter, M., Bassetti-Wyss, B.A., Mason, L., Reboussin, B.A., et al., 2005. Dispersal of *Staphylococcus aureus* into the air associated with a rhinovirus infection. Infect. Control Hosp. Epidemiol. 26, 196–203.

Carey, S.A., Goldstein, R.E.R., Gibbs, S.G., Claye, E., He, X., Sapkota, A.R., 2016. Occurrence of vancomycin-resistant and -susceptible *Enterococcus* spp. in reclaimed water used for spray irrigation. Environ. Res. 147, 350–355.

CLSI, 2013. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-third Informational Supplement. vol. M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA.

Edwards, U., Rogall, T., Blocker, H., Emde, M., Bottger, E., 1989. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16s ribosomal rna. Nucleic Acids Res. 17, 7843–7853.

Fang, H., Hedin, G., 2003. Rapid screening and identification of methicillin-resistant *Staphylococcus aureus* from clinical samples by selective-broth and real-time pcr assay. J. Clin. Microbiol. 41, 2894–2899.

Faria, C., Vaz-Moreira, I., Serapicos, E., Nunes, O.C., Manaia, C.M., 2009. Antibiotic resistance in coagulase negative staphylococci isolated from wastewater and drinking water. Sci. Total Environ. 407, 3876–3882.

Fernandez-Cassi, X., Silvera, C., Cervero-Aragó, S., Rusiñol, M., Latif-Eugeni, F., Bruguera-Casamada, C., et al., 2016. Evaluation of the microbiological quality of reclaimed water produced from a lagooning system. Environ. Sci. Pollut. Res. 1–18.

Hanssen, A., Ericson Sollid, J., 2006. Scc*mec* in staphylococci: genes on the move. FEMS Immunol. Med. Microbiol. 46, 8–20.

Klevens, R.M., Morrison, M.A., Nadle, J., Petit, S., Gershman, K., Ray, S., et al., 2007. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA 298, 1763–1771.

Kullander, J., Forslund, O., Dillner, J., 2009. *Staphylococcus aureus* and squamous cell carcinoma of the skin. Cancer Epidemiol. Biomark. Prev. 18, 472–478.

Lebeaux, D., Barbier, F., Angebault, C., Benmahdi, L., Ruppé, E., Felix, B., et al., 2012. Evolution of nasal carriage of methicillin-resistant coagulase-negative staphylococci in a remote population. Antimicrob. Agents Chemother. 56, 315–323.

Levine, A.D., Asano, T., 2004. Peer reviewed: recovering sustainable water from wastewater. Environ. Sci. Technol. 38, 201A–208A.

Maes, N., Magdalena, J., Rottiers, S., De Gheldre, Y., Struelens, M.J., 2002. Evaluation of a triplex pcr assay to discriminate *Staphylococcus aureus* from coagulase-negative staphylococci and determine methicillin resistance from blood cultures. J. Clin. Microbiol. 40, 1514–1517.

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., et al., 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18, 268–281.

Maimon, A., Friedler, E., Gross, A., 2014. Parameters affecting greywater quality and its safety for reuse. Sci. Total Environ. 487, 20–25.

May, L., Klein, E.Y., Rothman, R.E., Laxminarayan, R., 2014. Trends in antibiotic resistance in coagulase-negative staphylococci in the United States, 1999 to 2012. Antimicrob. Agents Chemother. 58, 1404–1409.

Ochei, J., Kolhatkar, A., 2000. Medical Laboratory Science: Theory and Practice. 10th ed. Tata McGraw-Hill, New Delhi.

Rogers, K.L., Fey, P.D., Rupp, M.E., 2009. Coagulase-negative staphylococcal infections. America]–>Infect. Dis. Clin. N. Am. 23, 73–98.

Rosenberg Goldstein, R.E., Micallef, S.A., Gibbs, S.G., Davis, J.A., He, X., George, A., et al., 2012. Methicillin-resistant *Staphylococcus aureus* (mrsa) detected at four U.S. wastewater treatment plants. Environ. Health Perspect. 120, 1551–1558.

Rosenberg Goldstein, R.E., Micallef, S.A., Gibbs, S.G., George, A., Claye, E., Sapkota, A., et al., 2014a. Detection of vancomycin-resistant enterococci (vre) at four U.S. wastewater treatment plants that provide effluent for reuse. Sci. Total Environ. 466–467, 404–411.

Rosenberg Goldstein, R.E., Micallef, S.A., Gibbs, S.G., He, X., George, A., Sapkota, A., et al., 2014b. Occupational exposure to *Staphylococcus aureus* and *Enterococcus* spp. among spray irrigation workers using reclaimed water. Int. J. Environ. Res. Public Health 11, 4340–4355.

Ruppé, E., Barbier, F., Mesli, Y., Maiga, A., Cojocaru, R., Benkhalfat, M., et al., 2009. Diversity of staphylococcal cassette chromosome mec structures in methicillin-resistant *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* strains among outpatients from four countries. Antimicrob. Agents Chemother. 53, 442–449.

Tonkovic, Z., Jeffcoat, S., 2002. Wastewater reclamation for use in snow-making within an alpine resort in Australia - resource rather than waste. Water Sci. Technol. 46, 297–302.

Wielders, C.L.C., Vriens, M.R., Brisse, S., de Graaf-Miltenburg, L.A.M., Troelstra, A., Fleer, A., et al., 2001. Evidence for in-vivo transfer of meca DNA between strains of *Staphylococcus aureus*. Lancet 357, 1674–1675.