



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



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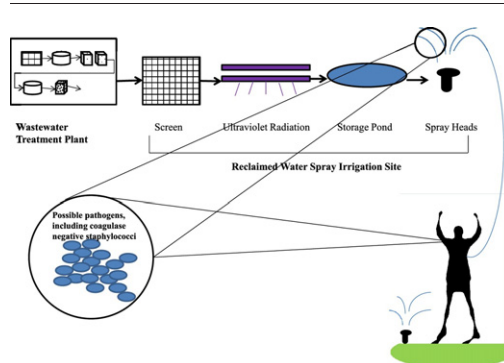
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HIGHLIGHTS

- 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



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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.

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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.

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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 m³/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/cm². 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

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