Published in final edited form as:

J Am Dent Assoc. 2015 April ; 146(4): 241–245. doi:10.1016/j.adaj.2014.12.013.

Isolation of *Raoultella planticola* from refillable antimicrobial liquid soap dispensers in a dental setting

Ms. Stephanie S. Momeni, MS, MBA,

Department of Pediatric Dentistry, School of Dentistry, University of Alabama at Birmingham, LHRB Room 238, 1720 2nd Avenue South, Birmingham, AL 35294-0007

Ms. Nancy Tomlin, RDH, and

Department of Clinical Affairs, School of Dentistry, University of Alabama at Birmingham

Dr. John D. Ruby, DMD, PhD

Department of Pediatric Dentistry, School of Dentistry, University of Alabama at Birmingham

Stephanie S. Momeni: sks@uab.edu

Abstract

Background—Liquid antimicrobial soaps are commonly used in the dental healthcare setting for hand washing to minimize the potential spread of infectious agents to healthcare workers (HCW) and patients. The purpose of the current study was to evaluate possible bacterial contamination of antimicrobial liquid soap dispensers located in two institutional comprehensive dental care clinics.

Methods—Fourteen soap dispensers and original stock containers were sampled. A 1 ml aliquot was diluted in 10 ml of phosphate buffer (Tween 80). Serial dilutions were plated in duplicate on neutralizing agar and incubated for 7 days. Molecular identification was performed using 500 bp comparisons of 16S rDNA sequencing. Taq PCR was performed with sequence specific primers for *Raoultella* species.

Results—Bacterial growth was observed at 18 hours for 57% (8/14) of soap dispenser samples. Bacterial densities ranged from 4×10^2 – 6×10^9 CFU/mL. Original commercial containers exhibited no growth. Isolates were identified as *Raoultella* (*Klebsiella*) *planticola*.

Conclusions—This is the first study indicating recovery of *R. planticola* from antimicrobial liquid soap dispensers. *R. planticola* is a recognized environmental opportunistic pathogen that potentially poses a health concern.

Practical Implications—These findings indicate compliance problems with infection prevention recommendations and support the CDC's recommendation that dispensers should not be "topped off". High bacterial loads of *R. planticola* are inconsistent with infection control

© 2014 American Dental Association. All rights reserved.

Correspondence to: Stephanie S. Momeni, sks@uab.edu.

Disclosure. None of the authors reported any disclosures.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

practices and are a concern since transmission and possible infection to the HCW or the patient may occur.

Keywords

Raoultella (*Klebsiella*) *planticola*; liquid soap; handwashing; bacterial contamination

A central issue in infection prevention and control today is the importance of hand washing as the primary defense in the efforts of healthcare workers (HCW) to prevent the spread of infectious disease in the clinical setting. To address this significant topic the Centers for Disease Control and Prevention (CDC) established the “Guideline for Hand Hygiene in Health-Care Settings” and “Guidelines for Infection Control in Dental Health-Care Settings, 2003” recommending proper hand-washing in healthcare settings.^{1,2}

Dispensed liquid soap is generally preferred in healthcare settings since bar soaps can become heavily contaminated with microorganisms.^{1–4} Liquid soaps are commercially available as non-medicated (plain) and medicated (antimicrobial). Manufacturers often provide instructions for hand washing use but typically do not provide instructions for refilling dispensers. Such instructions would be beneficial since one-gallon bottles are typically purchased to refill dispensers. In addition, when reusable pumps are provided and transferred between multiple bottles cross contamination is a concern.^{5,6} Another concern is that some liquid soaps have expiration dates. Regular observation of expiration dates is important since active agents or preservatives can degrade over time, which may reduce the efficacy of the soap against bacteria.⁶ While facilities may have documented cleaning protocols for liquid soap dispensers, non-compliance can be an issue leading to contamination.⁷

Several studies have investigated microbial contamination of plain and medicated liquid soaps in hospital and community settings. A number of bacterial opportunistic pathogens have been recovered from liquid soaps including *Klebsiella pneumoniae*, *Serratia marcescens*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas* spp. and *Enterobacter* spp.^{5–9} Therefore, the CDC has recommended that if refillable soap dispensers are used, the units must be washed and thoroughly dried before refilling since adding soap to a partially empty dispenser, or “topping off,” might promote microbial contamination.^{1,2,10}

The transmission of *Klebsiella* species has been attributed to hand washing in the healthcare setting.⁸ *Klebsiella* spp. are commonly linked with respiratory and urinary tract infections.¹¹ Advancements in molecular methods have lead to the partial reclassification of the genus *Klebsiella* to genus *Raoultella*.¹² These organisms were previously thought to be environmental only but recent studies suggest an emerging pathogenic association. Recently, *Raoultella planticola* has been shown to be present in severe pancreatitis, surgical site infections and has been described as histamine producers.^{13–15} Only one study has previously reported the recovery of *R. planticola* isolated from sealed batches of non-medicated liquid soap in a hospital setting.¹⁶

This study was designed to assess the microbial contamination of refillable medicated soap dispensers in the dental clinic setting, identify any microorganisms recovered, and suggest improvements of infection control measures to limit exposure for both HCW and patients.

METHODS

Approximately 5 mL of liquid soap was collected from 14 refillable soap dispensers throughout a dental clinic. Nine original open stock container soaps and 7 unopened direct from manufacturer soaps were also sampled. Listed active ingredients in the stock soaps were triclosan (0.2–0.25%), chlorohexidine gluconate (2–4%), and chloroxylenol (0.5%). Approximately 1 mL samples were diluted in 10 mL of phosphate buffer, pH 7.2 (Ricca Chemical Co, Arlington, TX) with 2% polysorbate-80 (Tween-80) added as a neutralizer for antibacterial agents. Samples were vortexed for 1 minute until homogenous. Serial dilutions to 10^2 – 10^6 were plated in duplicate by spread-plate method on Dey-Enger (D/E) Neutralizing Agar (Becton Dickinson, Sparks, MD) which is formulated to neutralize a variety of antimicrobial agents. Plates were incubated aerobically for 7 days at 35°C. Plates were enumerated and isolates were identified by 500 bp comparisons of 16S rDNA (Accugenix®, Newark, DE).

Taq PCR Identification

Isolates were plated on Trypticase Soy Agar and incubated aerobically for 24 hours at 35°C. A single colony was inoculated in 5 mL of Trypticase Soy Broth and incubated aerobically for at 35°C for 24 hours. DNA was extracted using UltraClean™ Microbial DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) by the manufacturer's instructions. Quantitation of DNA was performed using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA). An absorbance 260/280-nm ratio between 1.8 and 2.2 was considered acceptable. Traditional Taq PCR was performed using primers specific to the *bla* gene previously described to differentiate between *R. planticola* and *Raoultella ornithinolytica*.¹⁷ The 12.5 µL reaction mixture consisted of a final concentration of 1X Taq PCR Master Mix (Qiagen, Valencia, CA) and 0.3 µM of each primer with 2 µL of template DNA (~20 ng/µL). Amplification was performed using a Bio-Rad IQ5 (Bio-Rad Laboratories, Hercules, CA) with parameters: 1 cycle of 94°C for 3 minutes followed by 30 cycles of 1 min at 95°C, 1 min at 50°C and 1 min 10 sec at 72°C with a final cycle for 10 min at 72°C. Amplicons were subjected to electrophoresis in a 1% agarose gel for 1 hour at 150 volts.

RESULTS

Appearance of samples collected from dispensers all varied in color and viscosities (Table) inconsistent with the one brand of medicated soap clinic policy had approved for clinic use. A search of the clinics found 7 different medicated brands of liquid soaps of which 3 were expired and 4 had no expiration date listed.

Bacterial growth was observed at 18 hours on D/E Neutralizing Agar plates from 8 of the 14 refillable soap dispensers. Bacterial densities for positive soap samples at 7 days ranged from 2×10^2 to 6×10^9 CFUs/ml (Table). Control samples from the stock soaps from original containers (open and unopened) exhibited no growth at 7 days. All 8 of the colonies isolated

were identified as *R. (Klebsiella) planticola/ornithinolytica* using 16S rDNA sequencing (Figure 1). Since 16S rDNA sequencing was unable to conclusively differentiate between *R. planticola* and *R. ornithinolytica*, traditional PCR with Taq was performed. Six isolates were determined to be *R. planticola* by sequence specific primers (Figure 2). Two samples did not grow and were not available for PCR.

DISCUSSION

The purpose of this study was to evaluate refillable antimicrobial liquid soap dispensers in a dental setting for bacterial contamination. Of the 14 samples collected, 57% (n=8) were positive for *R. planticola/ornithinolytica* by 16S identification, 6 (43%) of which were further identified as *R. planticola* (two isolates could not be recovered) with subsequent testing with sequence specific primers. Six of the 8 positive samples (43%) had between 4×10^6 and 6×10^9 CFU/mL. It has been shown that soaps containing greater than 5×10^3 CFU/mL can lead to detectable levels on hands and that counts above 2.52×10^5 CFU/mL can cause surface contamination.⁹ The absence of other bacteria in this study was surprising giving that DE neutralizing agar supports the growth of a number of commonly occurring environmental isolates.

There was no growth observed in any of the original source soaps, which is consistent with another study.⁵ This suggests that the contamination of soap in the dispensers occurred after the soap was poured into the refillable dispenser. Since the collected samples exhibited multiple colors it was apparent that units were being “topped off” and thick viscosities, either from disuse or improper cleaning of dispensers, suggests that soaps were old or possibly contaminated demonstrating an infection control compliance issue. This study was unable to determine if contamination was the result of “topping off” or resulted from physical contact with the dispensers.

The discovery of *R. planticola* in 57% of the dispensers tested was unexpected, presenting compliance and possible health risk concerns. While no adverse health outbreaks have been reported to date, high bacterial loads are inconsistent with infection control practices and transmission to the HCW or the patient may be possible. *Raoultella* species are typical considered environmental organisms.¹¹ Limited data is available on the pathogenic potential on this organism due to its recent re-classification from the genus *Klebsiella* to *Raoultella* in 2001.¹² Studies in France and Germany suggest that as many as 19% of clinically identified *Klebsiella* spp. may actually be *R. planticola*. Recent case studies suggest the *R. planticola* may be an emerging pathogen associated with histamine fish poisoning and bacteremias.^{15,18–20} Other explanations are that the organism was only discovered in 1981 and identification can be problematic with traditional biochemical identification methods.^{18,21}

Considering that high microbial loads are inconsistent with healthcare infection control practices and may pose potential health risk, corrective action was taken to minimize bacterial contaminations based on the findings of this study. All built-in bulk soap dispensers were removed from service and replaced with hands free units with sealed system single use refills. In addition, clinic infection control procedures were modified to include

weekly external cleaning of units, which was assessed in monthly audits and clinic personnel were trained on types of soaps allowed, expiration dates, and proper maintenance in annual departmental training.

This study is the first to demonstrate the isolation of *R. planticola* from medicated dental clinic liquid soap dispensers. *R. planticola* is recognized as an environmental opportunistic pathogen that has been isolated from wounds, urinary tract and blood infections. This study also highlights the importance of compliance issues with recommended infection control practices. Facilities should develop written policies for the selection of hand-hygiene products and personnel should receive initial/recurring training to prevent contamination of liquid soaps. This study's findings further support the CDC's recommendation that "topping off" refillable soap dispensers may lead to bacterial contamination. Refillable soap dispensers should be thoroughly cleaned and dried before filling or replaced with sealed system dispensers with single use refills when possible.

Acknowledgments

Ms. Momeni is a Dental Academic Research Training (DART) Pre-doctoral Fellow under National Institutes of Dental and Craniofacial Research institutional grant T-90 DE022736. This study was generously supported by departmental funds. Accugenix provided 16S rDNA analysis for this study.

References

1. Boyce JM, Pittet D. Guideline for Hand Hygiene in Health-Care Settings. Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. Society for Healthcare Epidemiology of America/Association for Professionals in Infection Control/Infectious Diseases Society of America. MMWR Recomm Rep. 2002; 51(RR-16):1–45. quiz CE41–44. [PubMed: 12418624]
2. Kohn WG, Collins AS, Cleveland JL, Harte JA, Eklund KJ, Malvitz DM. Guidelines for infection control in dental health-care settings--2003. MMWR Recomm Rep. 2003; 52(RR-17):1–61. [PubMed: 14685139]
3. Kabara JJ, Brady MB. Contamination of bar soaps under "in-use" conditions. J Environ Pathol Toxicol Oncol. 1984; 5(4–5):1–14. [PubMed: 6394740]
4. McBride ME. Microbial flora of in-use soap products. Appl Environ Microbiol. 1984; 48(2):338–341. [PubMed: 6486782]
5. Sartor C, Jacomo V, Duvivier C, Tissot-Dupont H, Sambuc R, Drancourt M. Nosocomial *Serratia marcescens* infections associated with extrinsic contamination of a liquid nonmedicated soap. Infect Control Hosp Epidemiol. 2000; 21(3):196–199. [PubMed: 10738989]
6. Chattman M, Gerba SL, Maxwell CP. Occurrence of heterotrophic and coliform bacteria in liquid hand soaps from bulk refillable dispensers in public facilities. J Environ Health. 2011; 73(7):26–29. [PubMed: 21413559]
7. Brooks SE, Walczak MA, Hameed R, Coonan P. Chlorhexidine resistance in antibiotic-resistant bacteria isolated from the surfaces of dispensers of soap containing chlorhexidine. Infect Control Hosp Epidemiol. 2002; 23(11):692–695. [PubMed: 12452299]
8. Casewell M, Phillips I. Hands as route of transmission for *Klebsiella* species. Br Med J. 1977; 2(6098):1315–1317. [PubMed: 589166]
9. Zapka CA, Campbell EJ, Maxwell SL, et al. Bacterial hand contamination and transfer after use of contaminated bulk-soap-refillable dispensers. Appl Environ Microbiol. 2011; 77(9):2898–2904. [PubMed: 21421792]
10. Graf W, Kersch D, Scherzer G. Microbial contamination of liquid-soap wall dispensers with one-way bottles. Zentralbl Bakteriol Mikrobiol Hyg B. 1988; 186(2):166–179. [PubMed: 3138834]

11. Abbott, SL. *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia*, *Plesiomonas*, and Other *Enterobacteriaceae*. In: Murray, PR., editor. *Manual of Clinical Microbiology*. 9. Vol. 1. Washington, D. C: ASM Press; 2007. p. 700-711.
12. Drancourt M, Bollet C, Carta A, Rousselier P. Phylogenetic analyses of *Klebsiella* species delineate *Klebsiella* and *Raoultella* gen. nov., with description of *Raoultella ornithinolytica* comb. nov., *Raoultella terrigena* comb. nov. and *Raoultella planticola* comb. nov. *Int J Syst Evol Microbiol*. 2001; 51(Pt 3):925–932. [PubMed: 11411716]
13. Alves MS, Riley LW, Moreira BM. A case of severe pancreatitis complicated by *Raoultella planticola* infection. *J Med Microbiol*. 2007; 56(Pt 5):696–698. [PubMed: 17446297]
14. Wolcott R, Dowd S. Molecular diagnosis of *Raoultella planticola* infection of a surgical site. *J Wound Care*. 2010; 19(8):329–332. [PubMed: 20852504]
15. Kanki M, Yoda T, Tsukamoto T, Shibata T. *Klebsiella pneumoniae* produces no histamine: *Raoultella planticola* and *Raoultella ornithinolytica* strains are histamine producers. *Appl Environ Microbiol*. 2002; 68(7):3462–3466. [PubMed: 12089029]
16. Garcia-San, Miguel L.; Saez-Nieto, JA.; Medina, MJ., et al. Contamination of liquid soap for hospital use with *Raoultella planticola*. *J Hosp Infect*. 2014
17. Walckenaer E, Poirol L, Leflon-Guibout V, Nordmann P, Nicolas-Chanoine MH. Genetic and biochemical characterization of the chromosomal class A beta-lactamases of *Raoultella* (formerly *Klebsiella*) *planticola* and *Raoultella ornithinolytica*. *Antimicrobial agents and chemotherapy*. 2004; 48(1):305–312. [PubMed: 14693555]
18. Mau N, Ross LA. *Raoultella ornithinolytica* bacteremia in an infant with visceral heterotaxy. *Pediatr Infect Dis J*. 2010; 29(5):477–478. [PubMed: 20072080]
19. Morais VP, Daporta MT, Bao AF, Campello MG, Andres GQ. Enteric fever-like syndrome caused by *Raoultella ornithinolytica* (*Klebsiella ornithinolytica*). *J Clin Microbiol*. 2009; 47(3):868–869. [PubMed: 19144809]
20. Castanheira M, Deshpande LM, DiPersio JR, Kang J, Weinstein MP, Jones RN. First descriptions of blaKPC in *Raoultella* spp. (*R. planticola* and *R. ornithinolytica*): report from the SENTRY Antimicrobial Surveillance Program. *J Clin Microbiol*. 2009; 47(12):4129–4130. [PubMed: 19812278]
21. Bagley ST, Seidler RJ, Brenner DJ. *Klebsiella planticola* sp. nov.: a new species of *Enterobacteriaceae* found in nonclinical environments. *Curr Microbiol*. 1981; 6:105–109.

Figure 1.

Representative alignment of the 16S rDNA 500 base pair sequencing of 8 *Raoultella planticola* isolates from liquid soap dispensers.

Figure 2.

Taq PCR amplicons for 6 bacterial isolates using *Raoultella planticola* sequence specific primers (amplicon size 1121 bp) on 1% agarose gel. Lanes are labeled with collection locations. NTC lanes = no template controls.

Abbreviation Key.

TABLE

Average bacterial counts recovered from medicated liquid soap dispensers

LOCATION	COLOR	AVG CFUs/mL
1	nude	NGD
4	nude	200
9	pink clear	3.35×10^8
10	nude	5.10×10^9
11	pink-cream	3.76×10^9
12	cream	1.4×10^9
12-E	clear	NGD
16	pink clear	6.0×10^9
16-E	white, thick	NGD
17	pink clear	900
19	pink-cream	4.00×10^6
20	white chunky	NGD
21	rust	NGD
22	clear	NGD

NGD^a = No Growth Detected.