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Presence of *Pseudomonas aeruginosa* in coliform-free sachet drinking water in Ghana

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ABSTRACT

Sachet water is now an important source of drinking water security in West Africa, and the sachet industry continues to mature as market share increasingly shifts from cottage industry players to high-volume corporate producers. Modern sachet production lines are prone to the development of biofilms, and traditional microbiological indicators of fecal water contamination may not capture all the potential risks to human health in such a widely-consumed product. This study tested a sample of 80 sachets purchased along two commercial transects in low-income neighborhoods of Accra, Ghana, for total coliforms (TC), fecal coliforms (FC), *Escherichia coli* (EC), total heterotrophic bacteria (THB), and *Pseudomonas aeruginosa* (PA), and examined the relationships between these indicators and brand reputation. Just 5% of samples tested positive for TC, and none tested positive for FC and EC, yet 41% of samples tested positive for PA. After controlling for one popular brand, a negative brand reputation was associated with both THB presence ($P = 0.015$) and the number of samples with THB > 500 CFU/mL ($P = 0.038$), but PA was found in brands of both positive and negative reputations, and was only correlated with THB counts. The emergence of PA presents an opportunity for the re-evaluation of packaged water quality standards in a rapidly-globalizing, urban environment.

1. Introduction

West African nations continue to lag in their progress toward Millennium Development Goals Target 7C, which aimed to halve the proportion of the global population without sustainable access to safe drinking water between 1990 and 2015 (WHO/UNICEF, 2012a). As the percentage of urban residents with piped water access continues its decline in the region (WHO/UNICEF, 2011), sachet water has emerged as a significant private sector innovation to fill West African gaps in urban household drinking water security, particularly among the urban poor (Stoler, 2012). The 2010 Ghana Census underscores this trend: 28% of all residents in the Accra Metropolitan Area (AMA) reported using sachet water as their primary household drinking water source (Ghana Statistical Service, 2012), a dramatic increase from levels of 0% (1998) and 6.4% (2003) in low-income AMA clusters from the first two Ghana Demographic and Health Surveys (Stoler, Weeks, & Fink, 2012).

The quality of sachet water sold in West African nations has been a subject of inquiry for over a decade. The early literature, while often limited by small sample sizes and other design flaws (Stoler, Weeks, et al., 2012), has consistently reported poor bacteriological quality in Ghana (Addo, Mensah, Bekoe, Bonsu, & Akyeh, 2009; Ampofo, Andoh, Tetteh, & Bello, 2007; Dodoo, Quagraine, Okai-Sam, Kambo, & Headley, 2006; Kwakye-Nuako, Borketey, Mensah-Attipoe, Asmah, & Ayeh-Kumi, 2007; Obiri-Danso, Okore-Hanson, & Jones, 2003; Osei, Newman, Mingle, Ayeh-Kumi, & Kwasi, 2013; Oyelude & Ahenkorah, 2012), though the most recent of these studies reflect data collected in 2008 (Oyelude & Ahenkorah, 2012) and 2010–2011 (Osei et al., 2013). The sachet water industry has experienced rapid transformation over the last 5 years as large corporate manufacturers with economies of scale have gradually replaced small cottage industry businesses, and a survey conducted in Greater Accra in June 2013 found that sachets

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sampled from two urban slums were completely free of fecal contamination, perhaps signaling a new era in quality control (Stoler, Tutu, Ahmed, Asantewa Frimpong, & Bello, 2014). In that study, a sachet brand's reputation for quality was found to be the primary factor associated with levels of total heterotrophic bacteria in the sachet water after controlling for various vendor characteristics. Although it was the first community-scale study to reveal high-quality sachet water, notably in water-stressed slum communities that typically receive little regulatory oversight, the study was conducted during Ghana's rainy season when water demand is lower and consumers have more options. All forms of water demand surge during the dry season, and while sachet water inventory turns over at a faster pace, it is unknown if quality control standards are maintained as stringently across the industry during peak production periods. It is also unclear if traditional fecal indicators remain the best proxy for sachet water quality in a modern manufacturing environment.

Pseudomonas aeruginosa (PA) is a bacterial pathogen found in diverse environments and can cause a range of human illnesses including endocarditis, osteomyelitis, pneumonia, urinary tract infections, gastrointestinal infections, meningitis, and septicemia (Mena & Gerba, 2009). It is known to colonize biofilms in plumbing fixtures and other infrastructure related to urban water systems (Bressler, Balzer, Dannehl, Flemming, & Wingender, 2009; Mena & Gerba, 2009), can survive in chlorinated water environments such as swimming pools (Seyfried & Fraser, 1980), and has been documented as a potential water-borne illness for over fifty years (Reitler & Seligmann, 1957). It requires a high ingestion dose to cause illness in healthy individuals, as extrapolated from rodent models (George, Kohan, Claxton, & Walsh, 1989), but it adversely affects immunocompromised individuals, and is a common cause of nosocomial infection, as multi-drug resistant strains have been known to cause infections in intensive care units and burn units (Dembry, Zervos, & Hierholzer, 1998; Hota et al., 2009). Antibiotic-resistant strains have also been known to spread acquired resistance via drinking water systems (Vaz-Moreira, Nunes, & Manaia, 2012).

PA has been isolated in Ghana (Hagan, Wright, Newman, Dolin, & Johnson, 1995), including in hand-tied bagged water in Kumasi over a decade ago (Obiri-Danso et al., 2003), while more recent literature notes that drug-resistant strains continue to persist in Ghanaian hospitals (Newman, Frimpong, Donkor, Opintan, & Asamoah-Adu, 2011). The organism may pose a disproportionate risk in Accra due to the municipal rationing regime imposed on the city, a policy which attempts to create a more equitable distribution of limited piped water resources by diverting water to and from various neighborhoods on set schedules (Stoler, Fink, et al., 2012). Ongoing disruptions to water volume and pressure within the pipe network are known to cause intrusions or biofilm dislodgments that can trigger outbreaks of gastrointestinal illness (LeChevallier, Gullick, Karim, Friedman, & Funk, 2003; Nygard et al., 2007). The recent industrialization of sachet processing may also introduce new environments for the persistence of opportunistic pathogens such as PA. While Accra has historically had low rates of HIV infection (Duda et al., 2005), other pockets of immunocompromised populations at risk of such pathogens and nosocomial infections have always existed due to factors such as chemotherapy, malnutrition, recent surgery or stress (Griffin, 1986). Thus the widespread use of sachet water in vulnerable, low-income communities presents a much broader potential population at risk.

This investigation of PA in packaged water in Ghana may be the first in over a decade, is conducted in the context of a more modern sachet industry, and builds on our recent understanding of the potential dichotomy in sachet water quality between corporate and cottage industry brands. We hypothesize that the presence of PA will be more likely to appear in brands with a negative reputation, and will be positively associated with other forms of bacterial contamination.

2. Methods

2.1. Study site and sample

We collected sachet water samples during February 4–5, 2014, squarely in the middle of Ghana's primary dry season which runs from December to March. The study neighborhoods of Nima, Mamobi, Kotobabi, and New Town are comprised of predominantly low-socioeconomic status households, and probably collectively exceed 250,000 residents, as the last population estimate of the Nima-Mamobi area alone was 150,000 (Oppong, Mayer, & Oren, 2014). These communities contain a large number of people living in slum-like conditions with intermittent access to basic services such as piped water and electricity. Nima, Mamobi, and New Town were categorized in the bottom quintile of a Housing Quality Index based on 2000 Ghana Census data, with Kotobabi in the second-lowest quintile (Weeks et al., 2012). Recent studies situated in Nima have noted especially low-quality housing and high population density (Arku, Luginaah, Mkandawire, Baiden, & Asiedu, 2011), and estimated that 40% of the household budget was spent on water acquisition (Oppong et al., 2014). Fig. 1 presents the context of the study area within Accra, and the neighborhood boundaries are draped over a multispectral QuickBird image acquired in January 2010, with the image data presented using the infrared band to highlight vegetation in red tones. Note the stark differences in vegetation between the study area and contiguous upper-class neighborhoods such as Roman Ridge and Kanda Estate; the lower proportion of vegetated land cover is a hallmark feature of the urban slum landscape in Accra (Weeks, Hill, Stow, Getis, & Fugate, 2007).

Eighty sachet water samples were purchased from vendors along two transects corresponding to the primary commercial roads that serve as market centers for the contiguous communities of Nima, Mamobi, Kotobabi, and New Town; the sampling transects are highlighted in yellow in Fig. 1. The Kotobabi-New Town transect (henceforth abbreviated as "K-NT") begins at Pig Farm Junction (the intersection of Achimota Road and Kotobabi Road) and runs south down Kotobabi Road and New Town Road, ending at Mallam Atta Market. The Mamobi-Nima transect ("M-N") begins in Mamobi at the intersection of Achimota Road and Nima Road and runs south along Nima Road, through Nima Market, and ends at the Nima Roundabout.

The eighty sachets were sampled equally from these four neighborhoods and represent well over half of all point-of-sale locations for sachet water, excluding vendors (usually girls) selling from head-top baskets. This informal and ubiquitous vending style, known colloquially as an *apampam store* (literally *a store on the top of the head* in Twi), was excluded from analysis due to the unknown, and likely variable, effects of sunlight exposure on sachet water quality. In order to standardize the vending environment, all sachet samples were purchased from kiosks or stores where the sachets were stored in coolers or refrigerators (avoiding direct sunlight), and always chilled. Because sachet consumption is brisk during the dry season, the price of a single sachet was consistently 10 pesewas and without the discounts previously observed during the wet season (Stoler et al., 2014). All sachets purchased for the sample had been acquired by the vendor no more than three days prior; most (80%) were acquired that same morning. All sachet brands were categorized as having either a positive or negative reputation for quality. This categorization was informed by previous focus group discussions about drinking water quality