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Possibility of a case of pneumonia caused by green tea contaminated with *Pseudomonas aeruginosa* and countermeasures against it

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ABSTRACT

We report a patient who developed pneumonia after prolonged use of spray bottle containing green tea for hydration purposes. The cause was suspected to be a contamination of green tea because the patient's symptoms persisted and did not improve until stopping the use of the spray bottle and we also found the green tea in the spray bottle to harbor a high number of *Pseudomonas aeruginosa* (1.2×10^7 colony forming units (cfu)/mL). It is not uncommon to use green tea for hydration or gargling purposes in some patient care settings considering the antibacterial effects of catechins contained in green tea. Our findings suggest the importance of keeping vigilance on consuming green tea in spray bottles in hospital settings since it may readily be contaminated by pathogens such as *P. aeruginosa*.

Key words: contamination, green tea, *Pseudomonas aeruginosa*, spray

HIGHLIGHTS

- A cause of nosocomial pneumonia was determined to be caused by the use of spray bottle containing green tea for hydration purposes.
- Spray bottle containing green tea was determined to harbor high number of *Pseudomonas aeruginosa*, a common nosocomial pathogen.
- Microorganisms such as *P. aeruginosa* were determined to grow well in manufactured green tea products, but not in black tea or coffee products.

INTRODUCTION

Pseudomonas aeruginosa is widely distributed in moist environments and causes serious infection in compromised hosts. We report a patient (82 years, male, immunological background; cancer) who sprayed green tea contained in a spray bottle (nozzle-type container) that was contaminated with *P. aeruginosa* in their oral cavity, causing pneumonia. We discuss the risk of contamination of a spray bottle.

METHODS

When infection prevention team members entered the patient's room for environmental surveillance, a spray bottle was placed in the room. We asked the patient 'What do you use this spray bottle for?' and the patient who was recovering from surgery for esophageal cancer answered 'Since I can't help being thirsty, I spray green tea in the oral cavity using this spray bottle. I spray it about 20 times a day. Green tea contained in this spray bottle is delivered at meal time and I exchange it in the spray bottle 3 times a day'. As contamination by microorganisms was of concern due to the difficulties of consistent and thorough washing and drying of the spray bottles, contamination of green tea in the spray bottle was investigated.

Disinfection of the inside of spray bottle

Approximately 100 mL of ethanol for disinfection (76.9–81.4 vol% ethanol, Kenei Pharm. Co., Osaka) was added into the spray bottle. The inner wall was in contact with ethanol for 10 min and ethanol was sprayed 10 times to disinfect the inside of the spray bottle, including the nozzle region.

Bacterial strains and drinking water used

The *P. aeruginosa* isolated from the green tea in the spray bottle (henceforth referred to as *P. aeruginosa* GT), *P. aeruginosa* IFO3919, and other common pathogens: *Escherichia coli* NIHJ-JC2, *Serratia marcescens* IFO3936, *Staphylococcus aureus* 209P, *Candida albicans* IFO1594, and the clinical isolate methicillin-resistant *Staphylococcus aureus* (MRSA) were used.

Green tea prepared by the nutritionist in the hospital kitchen, green tea prepared by an automated machine in the hospital rest room, and seven commercially available brands of green tea in plastic bottles were tested: Oi Ocha Original (green tea A), Oi Ocha Dark (green tea B, Ito En Co., Tokyo, Japan), Ayataka (green tea C, Japan Coca-Cola Co., Tokyo), Iyemon (green tea D), Iyemon Green Espresso (green tea E, Suntory Co., Osaka, Japan), Sencha (green tea F, Yamazaki Baking Co., Tokyo), and Namacha (green tea G, Kirin Co., Tokyo). Three commercially available brands of black tea containing no sugar were tested: GoGo no Koucha unsweetened (black tea A), GoGo no Koucha straight (black tea B, Kirin Co., Tokyo), and Teo (black tea C, Asahi Soft Drinks Co., Tokyo). Three coffee beverages containing no sugar were also tested: BOSS (coffee A, Suntory Co.), UCC unsweetened (coffee B, UCC Ueshima Coffee Co., Kobe, Japan), and FIRE (coffee C, Kirin Co.). In addition, one mineral water product, I LOHAS (Japan Coca-Cola Co.), was included in the study. All of the beverages tested for microbial viability were purchased within the hospital. For the control, saline and distilled water for injection (Hikari Pharm. Co., Tokyo) were used.

Viability of microorganisms in drinking water

The samples of green tea contaminated by *P. aeruginosa* GT in the spray bottle were diluted in sterile distilled water, and diluted samples were added to the beverages, sterile distilled water, or sterile saline, yielding concentrations of approximately 10^3 colony forming units (cfu) of *P. aeruginosa* per mL. All other microbes used in the experiment were cultured on trypticase soy agar (TSA; Eiken Chemical, Tokyo) for 1–4 days at 30 °C, scraped into sterile phosphate-buffered saline (PBS), and centrifuged three times at 3,000 rpm for 10 min to remove the growth medium. Resuspension was carried out in PBS, yielding concentration of approximately 10^3 cfu. Next, 0.05 mL of the resuspension was added to 4.95 mL of each beverage tested. The test solutions were incubated at 30 °C, and plate counts were performed at 6 h, and 1, 2, and 7 days. Each experiment was repeated three times and the mean of the three repeats was calculated.

Pulsed-field gel electrophoresis

P. aeruginosa isolated from green tea in the spray bottle, *P. aeruginosa* isolated from sputum of the patient, and *P. aeruginosa* IFO3919 were subjected to pulsed-field gel electrophoresis. The high-molecular-weight chromosomal DNA was prepared according to the method of Murray *et al.* (1991), and the DNA sample in a small slice of an agarose plug in 200 μL of reaction buffer was digested with 30 U of SpeI (New England Bio Labs, USA). Pulsed-field gel electrophoresis was carried out with the Bio-Rad Gene Path system (Bio-Rad, USA) in 1% agarose gel in 0.5 × TBE buffer at 14 °C with a linear ramp time of 1–23 s over a period of 18.5 h. Thereafter, the gels were stained with ethidium bromide and photographed.

RESULTS

Possibility of a case of pneumonia caused by green tea in the spray bottle

The green tea in the spray bottle used to dispense hydrating liquid to the patient was contaminated with 1.2×10^7 colony forming units (cfu)/mL of *P. aeruginosa* (Figure 1). We had the patient discontinue the use of the bottle after suspecting bacterial infection. Pulsed-field gel electrophoresis fingerprinting confirmed the match between the *P. aeruginosa* isolated from this patient's sputum and *P. aeruginosa* from the green tea in the spray bottle (Figure 2). We confirmed improvements in C-reactive protein levels, white blood cell count, and average body temperature after discontinuing the use of the contaminated spray bottle for dispensing green tea.

Disinfection of the spray bottle

Disinfection of the inside of the spray bottle was not possible even though ethanol for disinfection was used, i.e., *P. aeruginosa* was detected even after disinfection with ethanol for disinfection.

Viability of microorganisms in different types of drinking water

P. aeruginosa isolated from green tea in the spray bottle (*P. aeruginosa* GT) was tested for growth in the three kinds of beverages (Figure 3). *P. aeruginosa* GT rapidly proliferated in all four green tea preparations, in addition to the sterile

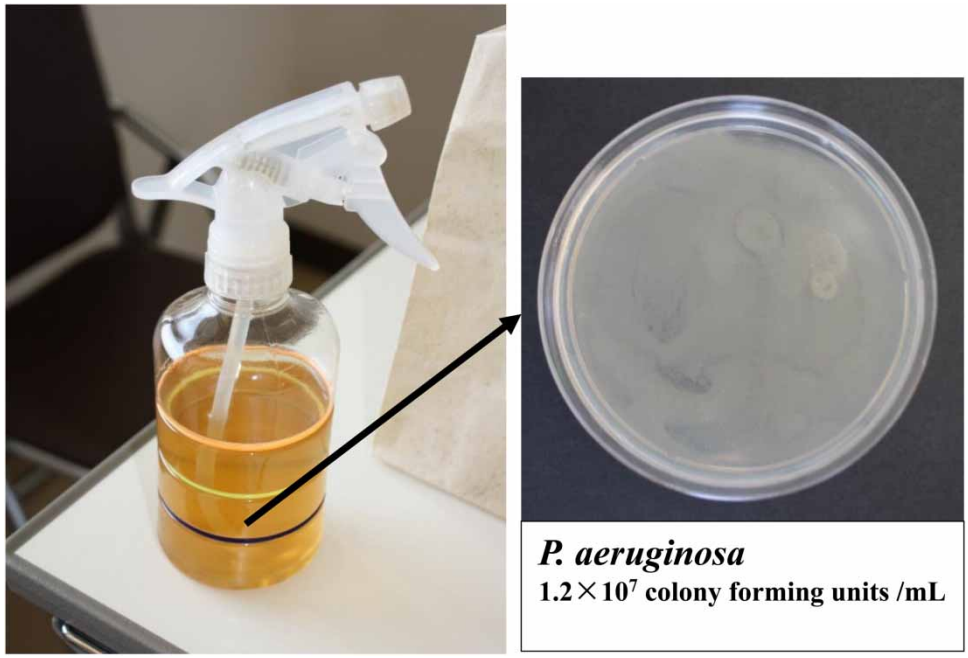

Figure 1 | Green tea in a spray bottle using by a patient was contaminated with high density of *Pseudomonas aeruginosa*.

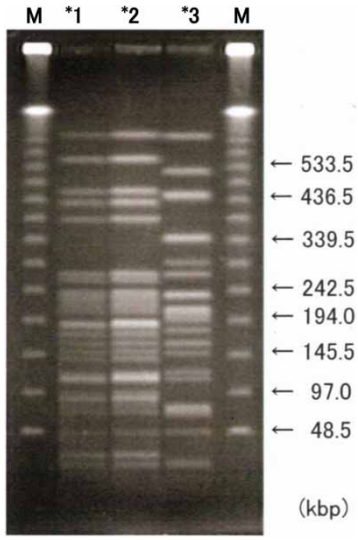

Figure 2 | Pulsed-field gel electrophoresis of three strains of *Pseudomonas aeruginosa*. M, DNA size marker. *1, a strain isolated from patient sputum. *2, a strain isolated from green tea in spray bottle. *3, a standard strain (*P. aeruginosa* IFO 3919).

saline and sterile distilled water. *P. aeruginosa* GT did not survive for more than 24 h in black tea or coffee, although it remained at high concentrations for at least 1 week in green tea.

P. aeruginosa IFO3919 was also assayed for survival in seven varieties of commercially available bottled green tea and a mineral water (Figure 4). *P. aeruginosa* IFO3919 proliferated robustly, reaching 10^7 cfu/mL in all green teas tested, although the concentration remained at 10^5 cfu/mL in mineral water. *P. aeruginosa* IFO3919 did not survive in the three varieties of black tea and coffee beverages tested (not shown).

The viability of *P. aeruginosa* IFO3919 is compared with that of five other pathogenic strains of microbes in one type of bottled green tea (green tea A) in Figure 5. Similar to *P. aeruginosa* IFO3919, *Escherichia coli* NIHJ-JC2, *Serratia marcescens*

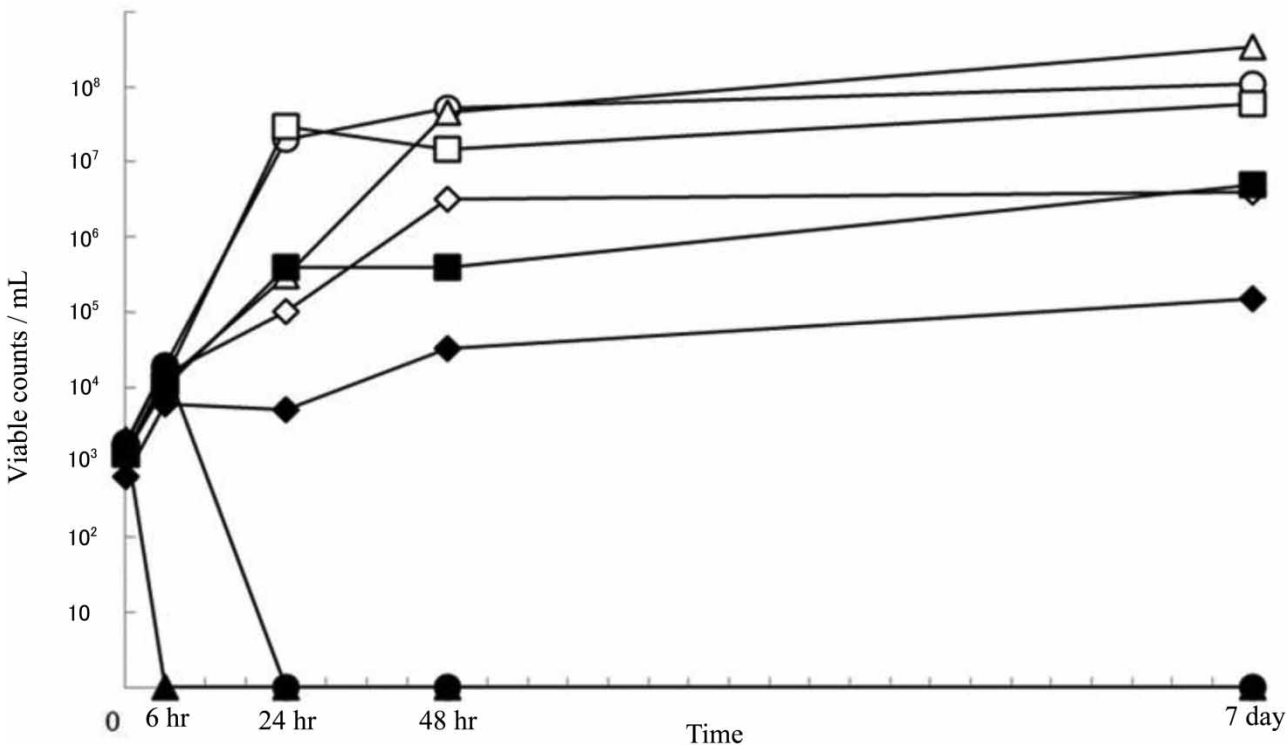

Figure 3 | Viability of *Pseudomonas aeruginosa* (*P. aeruginosa* GT) isolated from the green tea at 30 °C in green tea A (○), green tea B (△), green tea prepared by automated machine (□), green tea prepared in hospital kitchen (◇), black tea A (●), coffee A (▲), sterile saline for injection (■), and distilled water for injection (◆).

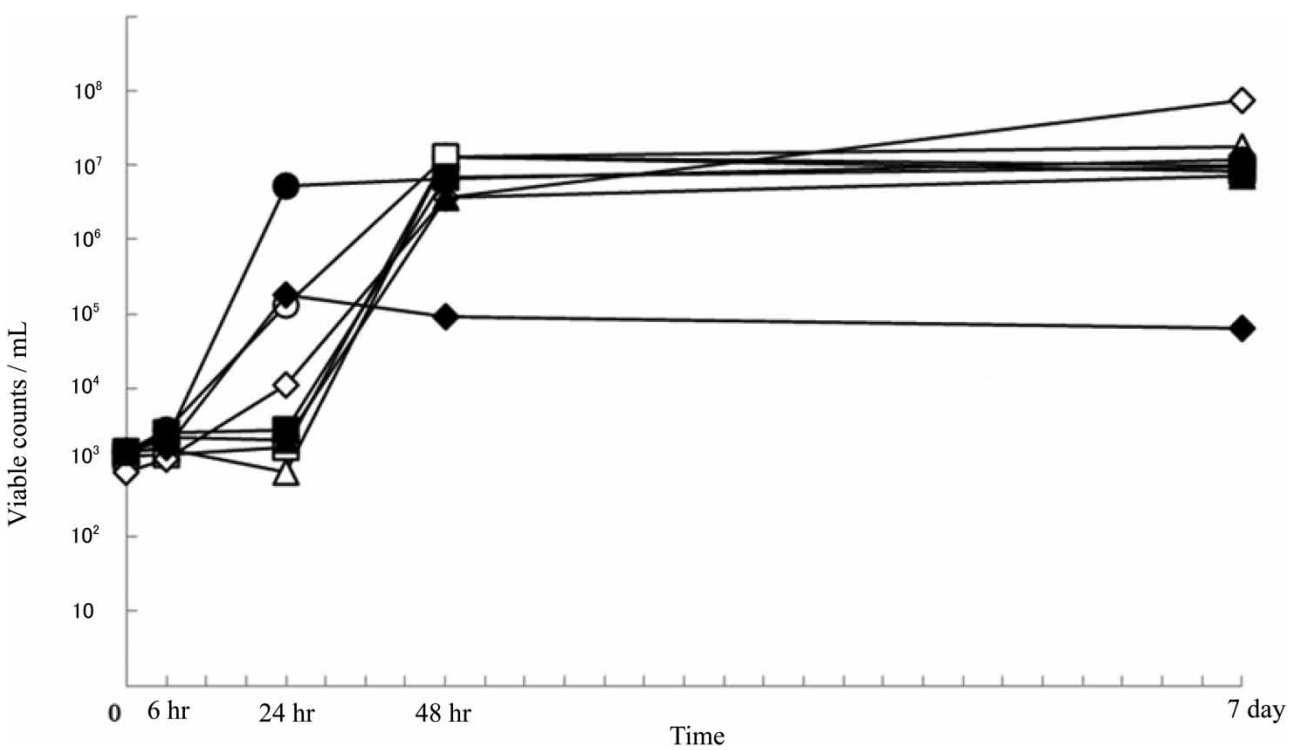

Figure 4 | Viability of *Pseudomonas aeruginosa* IFO3919 at 30 °C in seven commercially available brands of green tea A (○), B (△), C (□), D (◇), E (●), F (▲), G (■) and one brand of mineral water (◆).

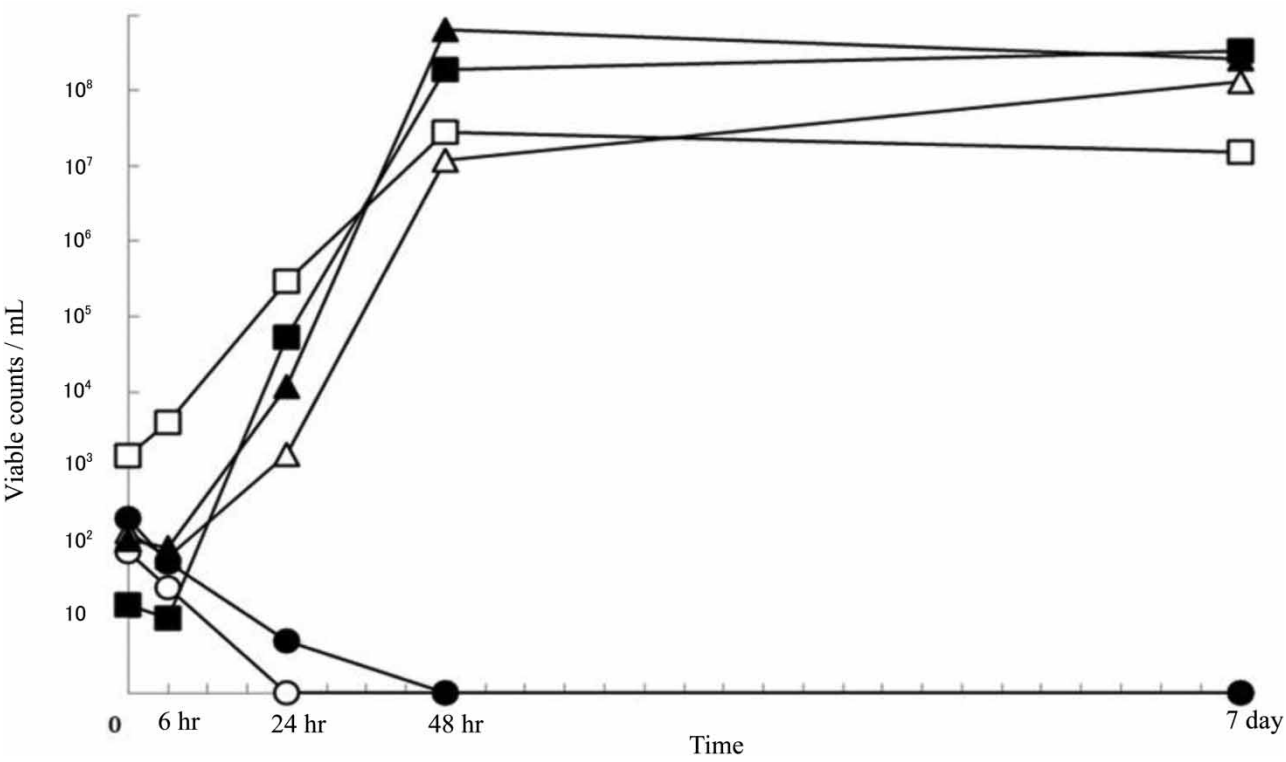

Figure 5 | Viability of six species of microorganism at 30 °C in one brand of green tea A. *Staphylococcus aureus* (○), *Escherichia coli* (△), *Pseudomonas aeruginosa* (□), MRSA (●), *Serratia marcescens* (▲), *Candida albicans* (■).

IFO3936, and *Candida albicans* IFO1594 proliferated in green tea, whereas *Staphylococcus aureus* 209P and the clinical isolate MRSA did not.

DISCUSSION

P. aeruginosa can proliferate using a small amount of nutrients in humid conditions, and therefore proliferates robustly in kitchen sponges, bath chair padding, shaving brushes, antiseptic-soaking cotton, enteral feeding solution, and fruits and vegetables (Oie & Kamiya 1992, 1996, 2001a, 2001b; Oie *et al.* 2005, 2008). It is also known that intravenous infusion and medical equipment and devices that are contaminated by *P. aeruginosa* become sources of nosocomial infection (Archibald *et al.* 1998; Prospero *et al.* 2006; Kayabas *et al.* 2008). Therefore, it is imperative that contamination of *P. aeruginosa* in medicine, food, and equipment be prevented, especially in the environment of compromised hosts.

We reported a patient who kept green tea in a spray bottle, sprayed it in the oral cavity, developed pneumonia caused by *P. aeruginosa*, and clinical course is not favorable as usual treatment (third-generation cephems) for pneumonia caused by *P. aeruginosa*. However, the patient recovered from pneumonia by discontinuing spraying the green tea in the oral cavity and by continuing administering the antibiotics. As *P. aeruginosa* isolated from the sputum of the patient was identical to that isolated from the green tea on pulsed-field gel electrophoresis fingerprinting, contamination of the green tea with *P. aeruginosa* was considered the possible cause of infection. Infection does not usually occur just by drinking green tea contaminated with *P. aeruginosa*, but the patient frequently sprayed this green tea in the oral cavity, which may have caused infection.

As this patient wanted to continue spraying green tea in the oral cavity to hydrate, this request was investigated. First, the spray bottle contaminated with *P. aeruginosa* was disinfected with alcohol but was ineffective to suppress microbial growth. We hypothesized that disinfection was not possible due to a possibility of *P. aeruginosa* forming biofilm in the nozzle of the spray bottle. As it is not structurally feasible to wash or dry the nozzle region of a spray bottle easily, it is a region that is readily contaminated by microorganisms. Next, the growth of the bacteria was investigated by adding the contents of the spray bottle containing *P. aeruginosa* into four green tea products, one sugar-free black tea product, and one sugar-free

coffee product. No growth of *P. aeruginosa* was noted in sugar-free black tea or sugar-free coffee, but growth was detected in the four green tea products (Figure 3). The green tea contains catechins, coffee contains caffeine, and the black tea contains theaflavin, all of which have antibacterial activity (Toda *et al.* 1989; Daglia *et al.* 1994, 2007; Hamiton-miller & Shah 2000; Hu *et al.* 2002; Cantatore *et al.* 2013; Kaur *et al.* 2019). However, green tea contains abundant nutrients, such as electrolytes, and these nutrients may counteract the antibacterial effects of catechin and enable *P. aeruginosa* to grow in the green tea. On the other hand, black tea and coffee contain less nutrients, such as electrolytes compared to green tea, and the antibacterial effects of theaflavin and caffeine may have fuller effect.

As the catechin content varies among commercial green tea products, the viability of *P. aeruginosa* IFO3919 in seven green tea products was investigated (Figure 4). *P. aeruginosa* grew in all products. In contrast, *P. aeruginosa* was unable to grow in any of three sugar-free black tea products or the three sugar-free coffee products (data not shown). In addition, the viability of six species of microorganism in a green tea product were investigated. *S. aureus* and MRSA did not grow, but *E. coli*, *S. marcescens*, and *C. albicans* did (Figure 5), confirming that not only *P. aeruginosa*, but also other bacteria and fungi can grow in green tea. A spray bottle is easy to be contaminated and may be a possible source of pneumonia in an immunodeficient host. Considering that some patient care settings and personal preferences may opt for the use of green tea for hydration or any medical uses that presumes antibacterial effect of catechins contained in green tea, it is necessary to be aware that green tea can also be a medium that is readily contaminated by opportunistic microorganisms.

CONCLUSION

Green tea in a spray bottle should not be used because pathogenic microorganisms such as *P. aeruginosa* rapidly grow green tea, and a spray bottle is not structurally possible to wash or dry the nozzle region.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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