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Iotex Bubbler Antimicrobial Efficacy
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Purpose The primary purpose of this investigation was to study the antibacterial efficacy of the **Iotex Bubbler** in delivering low-dose, free iodine via infusion into contaminated self-contained water reservoirs. Experiments were performed evaluating the bactericidal ability of deliverable free iodine when tested against representative gram-positive and gram-negative pathogens.

Materials and Methods Test bacteria for experiments were *Staphylococcus aureus* ATCC #29213, *Escherichia coli* ATCC #25922, and *Pseudomonas aeruginosa* ATCC #27853. Suspensions of each were initially prepared from maintained stock cultures by inoculating 100 mL of tryptic soy broth with incubation at 37C for 24 hours. Resultant growth was assessed by plating aliquots of broth cultures onto tryptic soy agar plates containing 5% sheep blood. 1 mL samples of each test organism were then added to 750 mL of sterile distilled water in plastic reservoirs used to deliver dental water. An **Iotex Bubbler** straw was attached to the diaphragm air pump, and a flow rate of 30 psi was set as the standardized pressurization used to assess free iodine release from the straw. The straw was inserted into the contaminated reservoir and the diaphragm pump turned on to release gaseous iodine through the bubbler. A new straw was used for each bacterial species tested. Preliminary studies revealed that when this system was activated, resultant agitated bubbling of gas caused the water in the reservoir to spill outside the bottle. As a result, studies reported here used 450 mL of the original 750 mL seeded volume as the contaminated water source. Aliquots of water exposed to gaseous iodine were collected following 1- and 5-minute treatment intervals. Water samples were assayed in quintuplicate by pipetting either 0.1 mL or 0.5 mL of fluid onto tryptic soy agar plates containing 5% sheep blood, and incubating them aerobically for 24-48 hours. Positive controls for bacterial growth were included by sampling the original contaminated water reservoirs; these served as 0 minute controls.

A separate experiment was performed by seeding a water reservoir with a 1.0 mL culture containing equal volumes of the 3 test gram-positive and gram-negative bacteria (0.33 mL each). Antimicrobial efficiency of gaseous iodine at 0-, 1-, and 5-minute exposure intervals. All culture procedures were performed within 1 hour of sample collection. Culture findings were reported as colony forming units (CFU)/mL.

Results

As expected, samples of bacterial contaminated water that were collected and cultured prior to exposure of gaseous iodine yielded substantial levels of test bacteria. These are shown in Tables 1-4 as 0 minute exposure control data. When contaminated reservoirs were exposed to bubbled iodine for two time intervals, it was found that even brief contact times resulted in significantly lower microbial levels. The gram-negative bacterium *Escherichia coli* appeared to be the most susceptible, as a 1-minute treatment destroyed all bacterium present in the reservoir (Table 2). Of the three bacterial species used in the study, *Pseudomonas aeruginosa* was the most resistant to iodine following 1-minute exposure when compared to the controls (Table 3). CFU data for *Pseudomonas* colonies following 1-minute exposure were reported as Too Numerous To Count (TNTC). This finding was due in part because of the microbe's motility resulting in the appearance of diffuse, swarming colonies on solid media. In contrast, the results for the individual test bacteria cultures and the mixtures were consistent after 5-minute exposure to the bubbled free iodine. No viable bacteria were isolated from any of the 20 incubated agar plates.

Table 1: Inhibition *Staphylococcus aureus* after exposure to gaseous iodine.

<u>Replications</u>	<u>Mean CFU/mL (range)</u>
0 minute exposure (control) – 5	> 7,000 (3,500 - 14,000)
1 minute exposure – 5	1,135 (400 - 3,700)
5 minute exposure – 5	0

Table 2: Inhibition of *Escherichia coli* after exposure to gaseous iodine.

<u>Replications</u>	<u>Mean CFU/mL (range)</u>
0 minute exposure (control) – 5	> 8,000 (5,400 - 14640)
1 minute exposure – 5	0
5 minute exposure – 5	0

Table 3: Inhibition of *Pseudomonas aeruginosa* after exposure to gaseous iodine.

<u>Replications</u>	<u>Mean CFU/mL (range)</u>
0 minute exposure (control) – 5	confluent
1 minute exposure – 5	TNTC*
5 minute exposure – 5	0

*TNTC = Too Numerous To Count

Table 4: Inhibition of growth of bacterial mixture after exposure to iodine.

<u>Replications</u>	<u>Mean CFU/mL (range)</u>
0 minute exposure (control) – 5	> 8,000
1 minute exposure – 5	6 (0 - 20)
5 minute exposure – 5	0

Conclusions

Iodine has been used for many decades as a broad spectrum antiseptic and disinfectant. In the present investigation, the antimicrobial efficacy of this halogen was evaluated using iodine released from a pressurized bubbler, which generated iodinated air bubbles released into volumes of bacterial-contaminated water. A brief 1-minute exposure to gaseous iodine was found to destroy the majority of the bacteria present in contaminated water bottles. Aliquots of treated water cultured after a 5-minute exposure period failed to yield any cultivable bacteria. In summary, **lotex Bubbler** cartridges were found to be highly effective in destroying bacterial contaminants in self-contained dental water bottles.