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1 **Droplet rather than Aerosol Mediated Dispersion is the Primary Mechanism of Bacterial**
2 **transmission from Contaminated Hand Washing Sink Traps**

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20 **ABSTRACT**

21 An alarming rise in hospital outbreaks implicating hand-washing sinks has led to widespread
22 acknowledgement that sinks are a major reservoir of antibiotic resistant pathogens in patient-care
23 areas. An earlier study using a GFP-expressing *Escherichia coli* (GFP-*E. coli*) as a model
24 organism demonstrated dispersal from drain biofilm in contaminated sinks. The present study
25 further characterizes the dispersal of microorganisms from contaminated sinks. Replicate hand-
26 washing sinks were inoculated with GFP-*E. coli*, and dispersion was measured using qualitative
27 (settle plates) and quantitative (air sampling) methods. Dispersal caused by faucet water was
28 captured with settle plates and air sampling methods when bacteria were present on the drain. In
29 contrast, no dispersal was captured without or in between faucet events amending earlier theory
30 that bacteria aerosolize from P-trap and disperse. Numbers of dispersed GFP-*E. coli* diminished
31 substantially within 30 minutes after faucet usage, suggesting that the organisms were associated
32 with larger droplet-sized particles that are not suspended in the air for long periods.

33

34 **IMPORTANCE**

35 Among the possible environmental reservoirs in a patient care environment, sink drains are
36 increasingly recognized as potential reservoir of multidrug resistant healthcare-associated
37 pathogens to hospitalized patients. With increasing antimicrobial resistance limiting therapeutic
38 options for patients, better understanding of how pathogens disseminate from sink drains is
39 urgently needed. Once this knowledge gap has decreased, interventions can be engineered to
40 decrease or eliminate transmission from hospital sink drains to patients. The current study further
41 defines the mechanisms of transmission for bacteria colonizing sink drains.

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42

43 **INTRODUCTION**

44 Recent reports have implicated hand-washing sinks as a primary reservoir of antibiotic
45 resistant pathogens within patient-care environments(1-27). Many of these reports have been
46 published since 2016 highlighting the global recognition that biofilms located in and on sinks can
47 have in disseminating clinically important drug resistant gram-negative bacteria(14-27).
48 Retrospective and prospective surveillance investigations affirm that hospital sinks provide
49 habitats for several opportunistic pathogens, raising serious concerns(8, 24, 26, 28)**.** It is not the
50 mere presence of these drug-resistant pathogens in the hospital wastewater that is of concern, but
51 the ability of these organisms to colonize biofilms on the luminal surfaces of wastewater
52 plumbing and thereby withstand routine cleaning practices. While several gammaproteobacteria
53 detected from the sinks in hospitals have been linked to healthcare-associated infections**,**
54 opportunistic pathogens like *Pseudomonas aeruginosa, Acinetobacter baumannii* and
55 *Stenotrophomonas maltophilia* are typically known to be found in water environments(29-31). In
56 contrast, emerging pathogens such as the carbapenemase-producing *Enterobacteriaceae,* many
57 having fecal origin, may survive within the biofilm formed on sink surfaces and wastewater
58 premise plumbing(32, 33). Often by acquiring mobile resistance elements through horizontal
59 gene transfer, carbapenemase-producing *Enterobacteriaceae* (CPE) infections are especially
60 threatening because they are more frequent causes of highly antibiotic resistant infections with
61 reduced treatment options.

62 In outbreak investigations species and strain matching between patient and sink isolates is
63 often attributed to sink source contamination, however direction (sink to patient versus patient to
64 sink) and precise mode of transmission remains inconsistent and elusive(29, 30). Even with

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65 increased recognition of transmission, a knowledge gap exists with regards to the precise
66 mechanism of transmission from sink reservoirs to the patient. Using a model of sink
67 colonization with green fluorescent protein (GFP)-expressing *Escherichia coli* (GFP-*E.coli)*, we
68 recently demonstrated the source and the degree of dispersion from sink wastewater to the
69 surrounding environment(34)**.** Factors effecting the rate and extent of droplet-mediated
70 dispersion were investigated, but particle size involved in dispersion was not measured in this
71 study. Studies that claim aerosols as the primary dispersion mechanism from sinks are based on
72 rudimentary findings (2, 23, 35, 36) or assumptions drawn based on these unsubstantiated
73 findings(3, 6, 10, 13, 21, 37). Airborne particles originating from sinks can have varied sizes and
74 compositions. The World Health Organization and Healthcare Infection Control Practices
75 Advisory Committee (HICPAC) guidelines use a particle diameter of 5 μm to delineate between
76 bioaerosol (≤5 μm) and droplet (>5 μm) transmission (38, 39).

77 Aerosol-mediated transmission and droplet-mediated transmission in the healthcare
78 environment will require conceptually different infection control strategies. Clarity regarding
79 aerosol versus droplet mediated dispersion in the context of sinks is critical. In the present study,
80 we aim to further define several outstanding knowledge gaps: i) dispersion mechanism of
81 bacteria, aerosol sized particles or droplets, from biofilms in handwashing sinks, ii) factors
82 triggering dispersion from a colonized sink drain and iii) role of biologically active aerosols
83 spontaneously dispersing from drain or P-trap without a triggering event. GFP-*E. coli* as the
84 surrogate organism for Enterobacteriaceae was used in this model study to investigate these
85 questions.

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88 **MATERIALS AND METHODS**

89 **Sink Gallery Operation and Automation.** A dedicated sink gallery at the University of
90 Virginia and described in an earlier study(34) was used in the present study. Sinks were
91 operated in automated mode using a microprocessor. The microprocessor activated the faucets
92 for 30 s each hour via an inline solenoid valve in the hot water supply line (faucet event) and also
93 turned on a peristaltic pump (Masterflex Pump #HV-77120-42, Cole-Parmer, Vernon Hills, IL)
94 to dispense 1ml soap (Kleenex Foam Skin Cleaner, Kimberly-Clark Worldwide Inc., Roswell,
95 GA). A steel tube discharging the soap was held in a clamp attached to the faucet and positioned
96 directly below the discharge of the faucet water. Water flow rate from the faucet was 8 L/min. In
97 one experiment, mannequin hands (Dianne Practice Hand, D902, Fromm International, Mt.
98 Prospect, IL) attached to a metal rack and positioned between the faucet head and sink drain
99 were used (Figure 1). To facilitate access to the luminal surface of the drain line, sampling ports
100 were drilled along the length of the tailpiece (between the P-trap and the drain), and the trap arm
101 (between the P-trap and the common line). These holes were fitted with size 00 silicone stoppers
102 (Cole-Parmer, Vernon Hills, IL). Temperature and total and free chlorine residual concentrations
103 of the faucet water i) first catch and ii) after 2 min flushing of faucets were measured at regular
104 intervals using the DPD method (Hach Model, Hach, Loveland, CO).

105 **Inoculation, Growth and Establishment of GFP-*E.coli* in Sink P-traps.** A single isolated
106 colony of GFP-*E.coli* (ATCC® 25922GFP™) grown from -80ºC stock was inoculated in 5 ml
107 Tryptic Soy Broth (TSB) containing 100µg/ml ampicillin (ATCC® Medium 2855). The method
108 of inoculation varied for each experiment. For P-traps a 10ml mid-log phase culture of GFP-
109 *E.coli* (10^9 CFU/ml) was added into the P-trap water (~150ml) through the lower- most sampling
110 port on the tailpiece using a 60ml syringe attached to silicone tubing (Cole-Parmer, Vernon Hills,

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111 IL). The inoculum was mixed with the P-trap water by repeated withdrawal and injection of the
112 inoculum, with precautions taken to avoid unintentional inoculation of drain (strainer) or sink
113 bowl (bowl). For the drain inoculation, a 10ml mid-log phase culture of GFP-*E.coli* (10^9
114 CFU/ml) was evenly applied on the surface of the drain using a sterile pipet. For establishment
115 of GFP-*E.coli* P-trap biofilm, a 10 ml mid-log phase culture of GFP-*E.coli* (10^9 CFU/ml) was
116 added into an unused P-trap and following inoculation, 25ml TSB and 25ml (x2) 0.85% saline
117 was added on a daily basis through the drain for 7 days to facilitate biofilm growth on the
118 luminal surface of the drain line without additional water every hour. Seven days later, P-trap
119 water and swab samples from the inner surface of the drain, tailpiece, and the P-trap were plated
120 on Tryptic soy agar containing 100μg/ml ampicillin (TSA). TSA plates were incubated overnight
121 at 37ºC and colony-forming units (CFUs) fluorescing under UV light were enumerated. All
122 preparatory culturing of GFP-*E.coli* took place in a separate room from the sink gallery to avoid
123 unintentional contamination.

124 **Sampling and Enumeration of *GFP-E.coli*.** To monitor the growth of GFP-*E.coli* within the
125 plumbing, sterile cotton swabs (Covidien™, Mansfield, MA) presoaked in 0.85% sterile saline
126 were inserted through sampling ports and biofilm samples were collected by turning the swab in
127 a circular motion on the inner surface (~20 cm^2). Sample swabs were pulse-vortexed in 3ml
128 saline and serial dilutions were plated on TSA. The entire surface area of the drain, faucet
129 aerator, and sink bowl surfaces were sampled using environmental sponge wipes (3M Sponge-
130 stick with neutralizing buffer, 3M, St. Paul, MN) using overlapping and multidirectional
131 motions. The sponge-wipes were expressed in 90 ml of Phosphate Buffered Saline containing
132 Tween 80 (0.02%) (PBST) using a Stomacher (400 Circulator (Seward Ltd., UK). The eluate
133 was concentrated by centrifugation and plate counts were performed on TSA and R2A agar

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134 (Becton Dickinson and Company, Franklin Lakes, NJ). TSA plates were incubated at 35°C for
135 48hr and fluorescent CFUs were enumerated; R2A plates were incubated at 25°C for 7d and
136 CFUs were counted.

137 **Experimental Approach for Dispersion Studies.** Each dispersion experiment comprised a 30 s
138 faucet event repeated three times at 60 min intervals (Fig. 2). Each experiment also comprised a
139 control (without faucet events) followed by a test (with faucet events). Three sinks were tested
140 concurrently but staggered by a few minutes, to account for the variability in dispersion driven
141 by faucet water flowrate, air flow dynamics in the room, contact angle and wastewater drainage /
142 water backup rate.

143 **Sampling Droplet Dispersion.** TSA settle plates were used to capture the droplet dispersion.
144 Numbered TSA plates were laid out radially around the sink bowl. A fixed layout and number of
145 settle plates around the sinks was used for each dispersal experiment (Fig. 3). The counter space
146 of each sink was thoroughly disinfected with Caviwipes-1 (Metrex Research, LLC, Orange, CA)
147 prior to each experiment. TSA plates were then positioned on the sink counter surrounding the
148 sink bowl. Additional plates were attached to the faucets, plexiglass partitions, and faucet
149 handles using adhesive tape. Plates were not placed in the sink bowl. TSA plates were also
150 placed >3 m away from the sink as negative controls. Lids of the TSA plates were removed only
151 for the duration of the dispersal experiment. Dispersion per defined area (CFU/cm^2) for settle
152 plates was determined by dividing the CFU counts in the TSA plate by the surface area of the
153 plate.

154 **Air Sampling and Particle Counts.** Each dispersion experiment comprised the collection of air
155 samples at five separate time points: time t=0 (first faucet event), t=30 minutes after the first

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156 faucet event, t=60 minutes (second faucet event), t=90 minutes, and t=120 minutes (third faucet
157 event) (Fig. 2). Individual sink sampling was staggered by a few minutes to provide time for air
158 sampler installation and sampling of each sink. A control experiment period, in which faucets
159 were not activated for the entire 120 minutes was also performed for each sink. Three air
160 sampling methods were tested: impaction, impingement, and filtration. For the impaction
161 method, two SAS90 air samplers (Bioscience International, Rockville, MD) containing one TSA
162 plate and one R2A plate each were positioned 12 inches from the sink bowl and set for a 300L
163 sample (at 90L/min for 200 seconds) (Fig. 2a). TSA and R2A plates from each air sampling
164 event were incubated as described earlier. A gel filtration device (MD8 Portable Air Sampler-
165 Sartorius AG Goettingen, Germany) fitted with disposable gelatin filters (Sartorius AG
166 Goettingen, Germany) was positioned 12 inches from the sink bowl and set for a 300L sample
167 (at 100L/min for 180 seconds) (Fig. 2c). Gelatin filters were carefully overlaid on TSA plates,
168 which were as already described. Liquid impingers (Ace Glass Inc. Vineland, NJ) were
169 autoclaved and filled with 20ml sterile Phosphate Buffered Saline (PBS) prior to each
170 experiment. Each was connected via a flowmeter (Cole-Parmer, Vernon Hills, IL) and vacuum
171 pump (Cole-Parmer, Vernon Hills, IL). The impinger was positioned 12 inches from the sink
172 bowl (set at 6L/min for 50min) to collect a 300L air sample (Fig 2b). In a biological safety
173 cabinet, the liquid from the impinger was transferred to a sterile tube, vortexed, filtered through
174 0.22μm membrane filters (Pall Laboratories, Port Washington, NY), and 5 ml duplicate samples
175 were plated on TSA and R2A plates. Fluorescent CFUs were enumerated after TSA plates were
176 incubated at 35°C for 48 hours and counted. R2A plates were incubated for 7 days at 25°C and
177 counted. Plates from air impaction samples and samples collected from liquid impingement were
178 shipped via overnight courier to CDC laboratories for processing and counting. Gel filtration

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179 plates were processed and counted at University of Virginia. Paired with air sampling particles in
180 size range 0.3, 0.5, 0.7, 1.0, 2.0 and 5.0 μm were measured using particle counter (GT-526, Met
181 One Instruments, Inc. Grants Pass, OR) placed 12 inches from the sink bowl (Fig 2.). With a
182 runtime of 660 seconds each, 3 successive runs of particle counter were performed, first run
183 coinciding with t=0. Particle counter also recorded relative humidity and air temperature.

184 **Verification of GFP-*E. coli*.** Fluorescent colonies on TSA-amp plates were counted under a
185 long-wavelength UV light source. To verify that fluorescent colonies were GFP-*E.coli*, two
186 fluorescent colonies from each sample were randomly selected and first screened on MacConkey
187 II agar (BD, Franklin Lakes, NJ). The MacConkey II plates were incubated at 35°C for 24 hours,
188 and lactose fermenters were isolated on Tripticase Soy Agar with 5% Sheep Blood (TSA II)
189 (BD, Franklin Lakes, NJ) and incubated under the same conditions. Once colonies were isolated,
190 they were identified using matrix assisted laser desorption ionization-time of flight mass
191 spectrometry (MALDI-TOF MS) (Bruker, Billerica, MA), or using the Vitek 2 system
192 (bioMérieux Durham, NC).

193 **GFP-*E.coli* Detection in Sink Plumbing and faucet water.** Prior to each experiment, 500 mL
194 of first-catch faucet water and a 500 mL sample collected after two minutes of flushing were
195 collected in sterile bottles containing sodium thiosulfate (0.18 g/l) for dechlorination. Samples
196 were plated on TSA and incubated at 35°C for 48 hours to test for GFP-*E.coli* in the faucet water
197 supplied to each sink and to ensure cross contamination of GFP-*E.coli* had not occurred. First-
198 catch and two-minute flush samples were diluted and plated on R2A, which were incubated at
199 25°C for 7 days and counted. After the experiment was completed, samples were collected and
200 processed to detect GFP-*E.coli* in the sink plumbing. The P-trap water was collected, and
201 processed by vortexing and filtration, and plated in duplicate on TSA and R2A media as already

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202 described. The sink P-trap and tailpiece were removed from the sink lab units, filled with faucet
203 water, plugged, and shipped by overnight courier to CDC for analysis. Swab sampling from the
204 tailpipe and P-trap, and sponge wipes were processed as already described to recover and
205 quantify biofilm organisms. Samples were plated on TSA and R2A media, and counted, as
206 described.

207

208 **RESULTS**

209 Free and total chlorine concentrations in the faucet water were consistent across the experiments
210 (Table 1). So were the water and air temperatures (Table 2). In comparison, the relative humidity
211 recorded across the experiments varied, with highest recorded in case of P-trap inoculation
212 experiments (Table 2).

213 **Dispersion immediately following P-trap inoculation.** No GFP-*E. coli* dispersion was detected
214 on settle plates immediately following inoculation of P-traps with 10^{10} CFU GFP-*E. coli* (Fig.
215 4a). No dispersion was detected using impaction, impingement, or filtration air sampling
216 methods (Fig. 5). Further, no GFP-*E.coli* were recorded in the P-trap water, P-trap, tailpiece or
217 sink bowl surface samples collected at the end of dispersion experiment.

218 **Dispersion immediately following Drain inoculation.** When sink drains were inoculated with
219 10^{10} CFU GFP-*E. coli*, dispersion was detected on settle plates and by impaction and filtration.
220 Dispersion detected by settle plates across the three sinks ranged from 35-107 CFU/plate. GFP-
221 *E.coli* levels were higher on the counter space surrounding the sink bowl compared to lower
222 counts near the faucets (Fig 4b). Dispersion was not detected on side-splatter shields. With the
223 exception of the detection of 1 CFU/m^3 at t=60 min using the impaction, dispersion of GFP-*E.*

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224 *coli* was detected only at the first faucet event (t=0 min) using both impaction and filtration
225 methods (Fig 5). Average dispersion captured at the first faucet event (t=0min) was 77 and 83
226 CFU/m^3 using impaction and filtration methods respectively. GFP-*E. coli* was not detected at
227 any time point using liquid impingement.

228 **Dispersion following growth for 7 days in an amended P-trap biofilm.** Allowing colonization
229 of GFP-*E. coli* in drainlines between strainer and P-trap with nutrient exposure over time,
230 dispersion was detected on settle plates (Fig. 4c), with counts ranging from 49-107 CFU/plate.
231 The counter space surrounding the sink bowl received the largest amount of droplet dispersion,
232 followed by faucet, faucet handle surfaces and splatter shields. GFP-*E. coli* levels were highest at
233 the first faucet event (t=0 min) and not detectable afterwards, with the exception of a 2 CFU/m^3
234 count at 60 min using air impaction and 1 CFU/m^3 count at 90 min filtration similar to what was
235 observed for drain inoculation (Fig. 5). Dispersion captured at the first faucet event (t=0 min)
236 was 138 and 29 CFU/m^3 using impaction and filtration methods, respectively. GFP-*E.coli* was
237 not detected using liquid impingement. GFP-*E.coli* was detected on the sink bowl, drain grate,
238 tailpiece, P-trap, and P-trap water at the completion of this experiment (data not shown).

239 **Dispersion without faucet events (control experiments).** Without a faucet event, GFP-*E. coli*
240 was not detected on settle plates, or by impaction, impingement, or filtration air sampling
241 methods in any experiment. Without a faucet event fungal and non-fluorescent CFUs were
242 occasionally recorded on settle plates, subsequently identified as *Staphylococcus sp.,* non-
243 hemolytic Streptococci, *Paenibacillus sp.,* yeast and small gram positive rods.

244 **Total viable heterotrophic organisms in laboratory air.** No GFP-*E. coli* was detected in the
245 faucet water at any time during experiments. Air samples were collected by impaction on R2A
246 medium with and without faucet events, in order to quantify total heterotrophic organisms in the

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247 air space in proximity to the sink during each experiment. Across the experiments the
248 heterotrophic organisms in the air ranged from 4-578 and 2-69 CFU/m^3 with (test) and without
249 (control) faucet events, respectively (Fig. 6). Dispersion captured at the first faucet event
250 (t=0min) when the faucets were turned on (test) was 1 log_{10} higher than the same recorded in
251 case of control experiment (without faucet event). Heterotrophic organisms captured from the air
252 steadily declined along the time points 0, 30, 60, 90 and 120 min with and without faucet events.
253 Particle concentrations in the air with and without faucet events were found to be consistent
254 during the day of the experiment. However, when compared across the experiments, no
255 correlation could be established (Supplemental Figure S1).

256 **Dispersion in the Presence of Mannequin Hands**

257 When mannequin hands, which created a barrier for water flow onto the drain, were positioned
258 under the faucet and the sink drain was inoculated with ~1E+10 CFUs GFP-*E.coli*, 10-fold lower
259 dispersion was captured on settle plates. No dispersion in air sampling using impaction or
260 filtration methods was observed. Dispersion pattern and load on settle plates varied considerably
261 across the three sinks and experiments with the mannequin hands in place.

262

263 **DISCUSSION**

264 The objective of the present study was to characterize the mechanism of bacterial dispersion
265 from handwashing sinks, using a GFP plasmid-containing *E. coli* strain as a surrogate for
266 multidrug resistant Enterobacteriaceae. Very few studies have investigated the dispersion from
267 sinks using methods to sample aerosol-associated microorganisms(23, 36, 40, 41); however,
268 several studies have drawn subjective interpretations about aerosol-mediated transmission from

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269 contaminated sinks(3, 6, 10, 13, 21, 37). Doring et al. used an impaction method to examine *P.*
270 *aeruginosa* dispersion from the sink bowl surface during faucet use. *P. aeruginosa* was detected
271 15 cm from the sink drain when counts in the sink drains exceeded 10^5 CFU/ml (41). Kramer et
272 al. detected 439 CFU/m^3 *P. aeruginosa* in the air sampled 10cm above the sink drain with 10^5
273 CFU/ml bacteria in the 'sink fluid' (P-trap water)(40). In these studies, dispersion was not
274 measured without faucet usage (control samples). De Geyter et al. used an MAS-100 air sampler
275 to measure CRE dispersal from contaminated sinks with and without faucet usage. Several
276 species in the family Enterobacteriaceae were detected during faucet usage but results for control
277 samples (without faucet usage) were not provided(23). Fusch et al. in contrast sampled air
278 around sinks with and without faucet event (control) and did not detect *P. aeruginosa* in air
279 samples collected without running water(36). We had previously provided a quantitative
280 assessment of dispersal as a function of faucet usage and reported GFP-*E. coli* could be dispersed
281 up to 30 inches beyond the sink drain during faucet usage(34). However, dispersion was
282 assessed using a gravity method, and bioaerosol production was not evaluated.

283 The air sampling methods chosen and tested in the present study were three of the most widely
284 used methods previously reported(23, 35, 36, 40, 42, 43) and were selected to assess bioaerosol
285 production during sink usage. In the present study, GFP-*E. coli* dispersion was detected during a
286 faucet event but was not detected in the absence of faucet events using either settle plates or
287 impaction and filtration air sampling methods. This finding corroborates previous studies (2, 21,
288 34, 36). It also implies that shear forces of the faucet water flowing onto the sink drain and/or
289 bowl surfaces results in dispersion of bacteria. Detection of dispersed GFP-*E. coli* during a
290 faucet event and non-detection at subsequent time points (after 30 minutes) suggested that
291 dispersed cells were associated with larger heavier droplets that would quickly settle onto

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292 surfaces due to gravity rather than aerosol sized particles which remain in the air(44)**.** Dispersion
293 of GFP-*E. coli* from sinks does not appear to be associated with the production of bioaerosols,
294 that is, particles smaller than 5 μm (35, 40, 45, 46). Studies that measured air sampling lacked
295 the resolution between aerosols and droplets(35, 40, 41). Air was sampled significantly closer
296 (~4 inch) to the sink drain or impact point of faucet water on the sink and therefore, might have
297 picked droplets rather than aerosols.

298 A consistent result from this work which is worth reemphasizing is the finding that for dispersion
299 to occur the presence of bacteria on drain and/or bowl surface is necessary(34). When GFP-*E.*
300 *coli* was inoculated into a new P-trap, dispersion was not detected using settle plates or air
301 sampling methods. This underscores the fact that as long as the sink drain and bowl remain free
302 of the target organisms (e.g., CPE or other antibiotic resistant Gammaproteobacteria), dispersion
303 can be controlled. However, under favorable conditions bacteria can grow or mobilize from the
304 P-trap into the drain piping (tailpiece) and colonize the sink drain surfaces, with the potential for
305 a dispersion event to occur. This further underlines the importance of sanitary hygiene practices,
306 strategic surveillance paired with hand washing only use of hand-washing sinks in the patient
307 care environment to reduce the risk of hand-washing sink contamination by the multi-drug-
308 resistant microorganisms that can colonize ICU patients(8). This also emphasizes the necessity to
309 implement stricter measures to prohibit disposal of nutrients, body fluids and anything into the
310 sinks that could be a nutrient source for maintenance of microorganism biofilms in drains(23).
311 Dispersion from a contaminated sink reservoir can result in transmission to patients either
312 directly or indirectly mediated through numerous contact surfaces. Herruzo and colleagues
313 demonstrated the potential for microbial transfer from contaminated hands, which continued to
314 disperse microorganisms after more than 10 successive contacts with surfaces (25).

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315 The droplet dispersion load observed on settle plates was similar and consistent with our
316 previous work(34). Total dispersion measured in corresponding experiments in the previous
317 study was higher, which may be attributed to one or more of the following factors: i) fewer settle
318 plates were used in the present study (22 vs 90), ii) a higher water flow rate was used in the
319 present study (8 vs 1.8-3.0 L/min) and iii) air sampling methods performed in conjunction with
320 the settle plate method may have captured a portion of the dispersed droplets. Settle plates were
321 found to be a reliable method to assess the large-droplet dispersion from sinks. In this study 22
322 settle plates (=11.24 m^2) were used which accounted for a defined surface area and locations on
323 the sink counter. Dispersion could have been higher in locations of the sink counter other than
324 those chosen in the present study, and the dispersion load recorded in this study may not be the
325 absolute value. Of the three methods investigated for air sampling, impaction and filtration were
326 found to be reliable and consistent. In the same amount of air sampled using impaction and
327 filtration, comparable counts were recorded; however, air sampled using the impinger method
328 was unable to capture the dispersion of GFP-*E.coli* under similar testing conditions.

329 Mannequin hands functioned as obstruction to direct impact of faucet water on the sink drain,
330 and therefore no dispersion was detected. This rationale behind testing mannequin hands was to
331 simulate hand washing, but in reality the water would be flowing before, after and during a hand
332 washing event. In other words, an actual handwashing event is more dynamic than static
333 mannequin hands and there is likely direct impact of water on the sink drain at least for brief
334 periods when the water is running. There is also the scenario where the sinks and faucets may be
335 used outside of hand washing (e.g. dumping liquid wastes)(5, 8, 23). This finding we think
336 further defines and supports another important dynamic that may minimize dispersion in
337 healthcare settings (i.e., avoid faucet water flow directly onto drains to minimize dispersion). All

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338 of these findings must be taken in the context of an experimental water stream which directly hits
339 the drain which is outside FGI guidance but thought to be frequently found in health care sink
340 design.

341 This study has several limitations. First, the dispersion experiments were not performed in a
342 controlled environment. Each dispersion experiment lasted at least 12h, therefore it was not
343 possible to maintain precisely the same conditions with regards to air flow velocity, air
344 temperature, relative humidity, and bacterial and/or fungal burden in the laboratory space
345 harboring the sinks. These parameters may have direct or indirect influence on the dispersion
346 pattern and load recorded across experiments(47). To address this issue, we monitored the
347 heterotrophic plate counts, relative humidity and particle concentration in the air. Particle counts
348 recorded in the absence of faucet event (control) were higher or equal to that in the presence of
349 faucet event (test). This observation implies that particle concentrations in the air were driven by
350 relative humidity and/or temperature of the air. This trend was observed in all the experimental
351 methods (Drain, P-trap inoculation and Drain colonization) (Supplemental Figure S1). In other
352 words, particle counts were largely consistent across the day for a given experiment (control
353 preceding test). Further particle counter used in the study could not resolve or measure particles
354 >5µm, which defined droplet particles. Another limitation was that air samples were collected at
355 only one location relative to the sink bowl, so it is not possible for this data set to define a
356 "splash zone" pattern without additional measurements collected from various positions and
357 distance from the source of dispersed organisms.

358 We have provided data to support the position that microorganisms will disperse from
359 contaminated sink bowl and drain surfaces primarily as large droplets that are generated during
360 faucet usage. These droplet-associated organisms remain viable with the potential to contaminate

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361 surfaces surrounding the sink bowl. However, it does not appear that dispersion results in the
362 production of bioaerosols with sustained dispersion characteristics into the patient's room space
363 with this simulated sink design and study.

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382

383 **Table 1.** Total and Free Concentrations and temperature in the Faucet water

	Initial (First Catch)	Final (After 2min)
Total Chlorine	0.77 ±0.26 mg/L	0.63 ±0.07 mg/L
Free Chlorine	0.62 ±0.25 mg/L	0.54 ±0.13 mg/L
Water Temperature	22.53 ±1.87°C	36.69 ±2.17°C

384

385 Mean and standard deviation of total and free chlorine residual and water temperature, measured at
386 different time points and from water collected from faucets supplying different sinks over the course of
387 this study.

388

389 **Table 2.** Air temperature and relative humidity recorded across experiments.

390

	Air Temperature (°C)	Relative Humidity
Drain Inoculation	19.8	31.4
P-trap Inoculation	19.5	54.4
Drain + P-trap Colonization	20.0	44.7
Control experiments	19.7	46.5

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398 **FIGURE LEGENDS**

399 1. Test sink, showing mannequin hands positioned directly below faucet (A), and a
400 peristaltic pump on the left to deliver hand-soap (B), via steel tube attached to the faucet
401 (C).
402 2. Experimental set-up used for different air sampling methods: a) Impaction, b) Impinger,
403 and c) Gel Filtration. Air samples were collected at the initial faucet event (0 min) and
404 every 30 minutes thereafter. Faucet events (faucet activation) occurred at 0, 60, and 120
405 minutes under test conditions. Faucets were not activated in control experiments.
406 3. Graphical representation depicting the layout of the settle plates positioned around the
407 sink used to capture droplet dispersion.
408 4. Heat map representation of GFP-*E.coli* dispersion captured on TSA settle plates
409 following (a) P-trap, (b) sink drain inoculation and (c) drainline colonization.
410 5. GFP-*E. coli* as measured by impaction [SAS90], and gel filtration [MD8] across D-Drain
411 inoculation, D7-Drainline colonization and P-P-trap inoculation methods.
412 6. Heterotrophic Plate Counts as measured by impaction air sampling a) without faucet
413 event (Control) and b) with faucet events (Test).

414

415 Supplemental Figure S1: Particle Counts (measured with MetOne GT-526) across Control
416 (without faucet) and Test (with faucet) experiments of D-Drain inoculation, D7-Drainline
417 colonization and P-P-trap inoculation methods.

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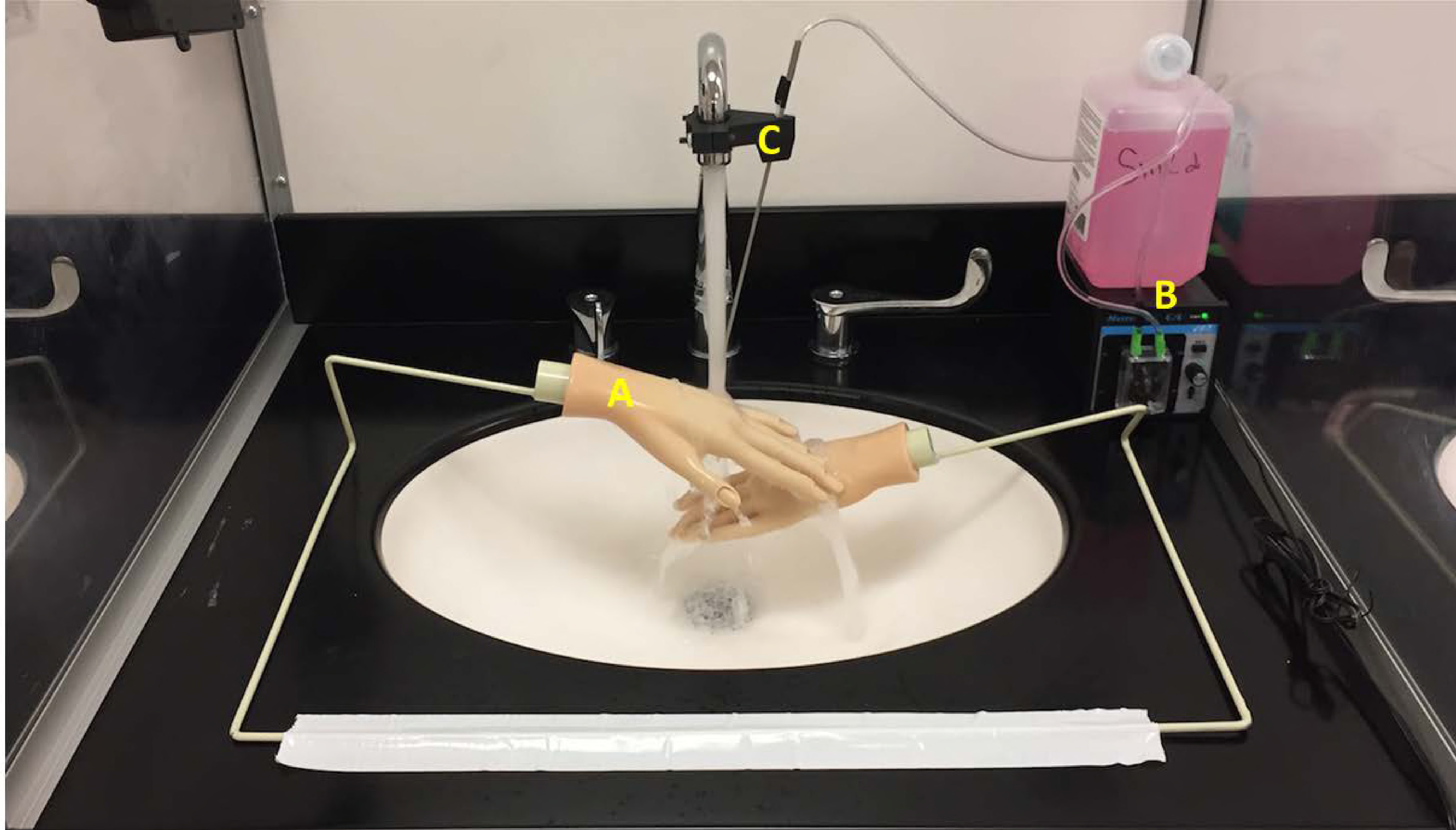
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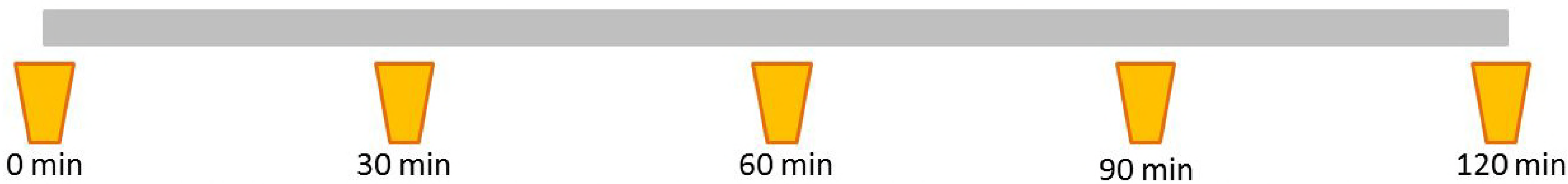
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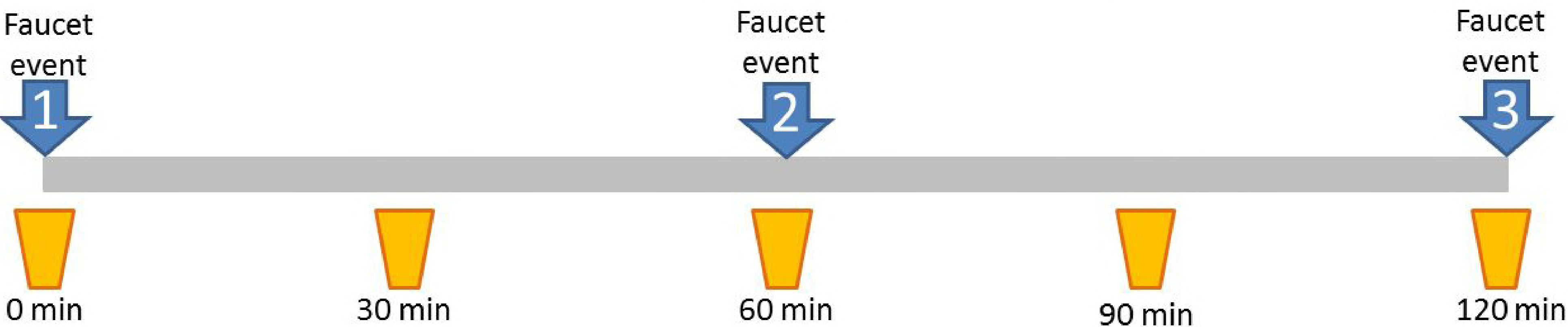
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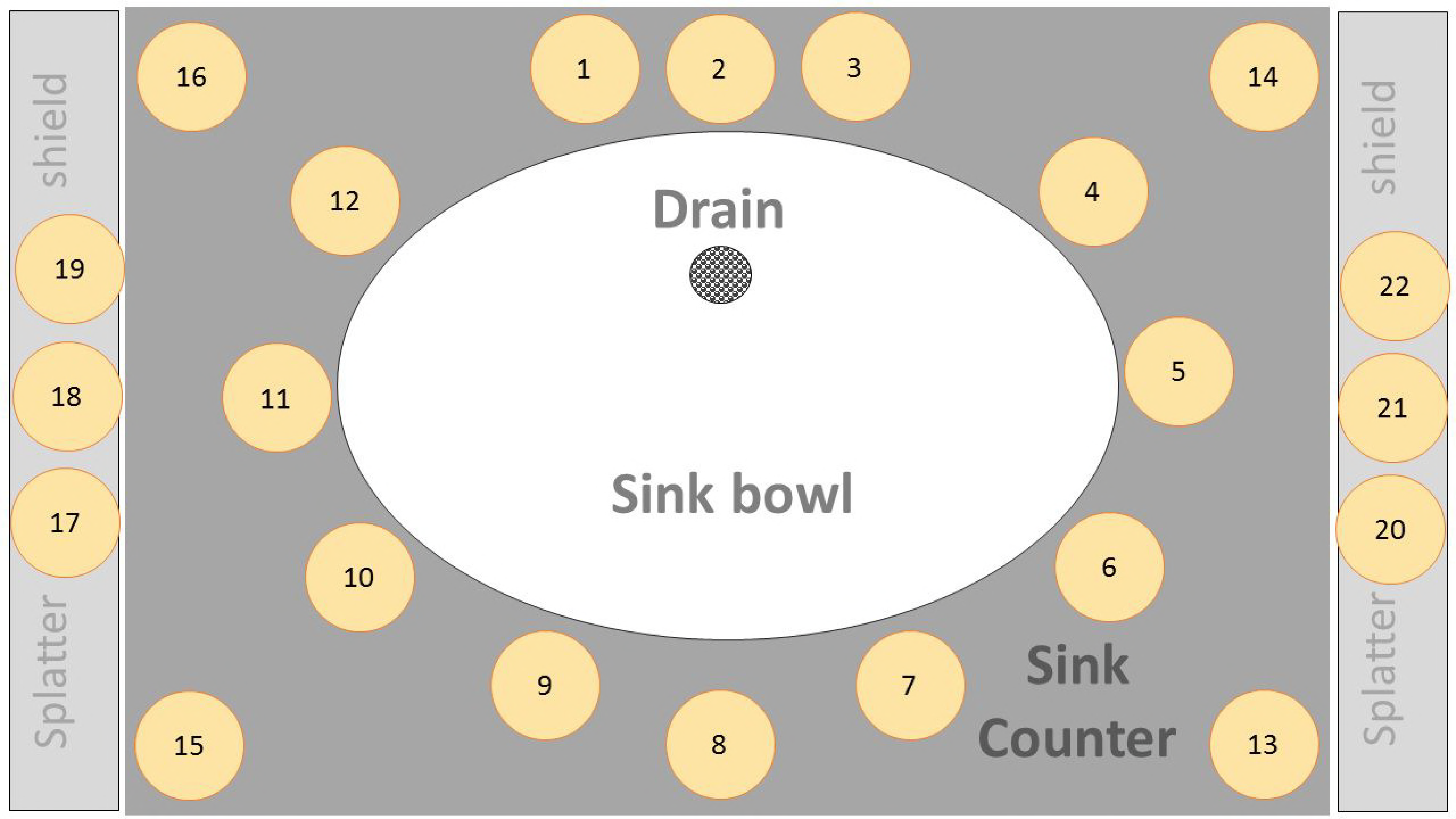

Splatter shield
Drain
Sink bowl
Sink Counter
Splatter shield
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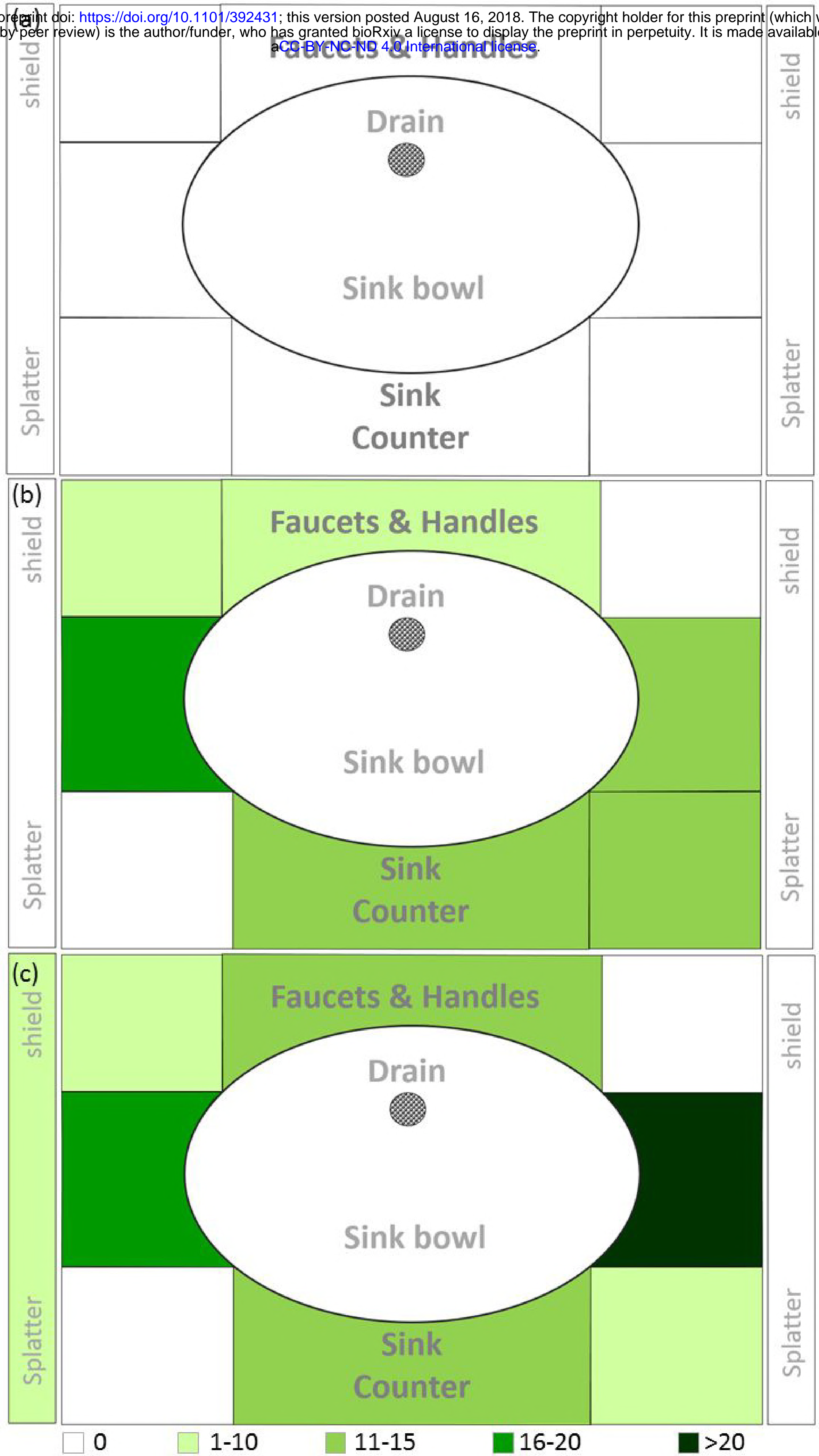

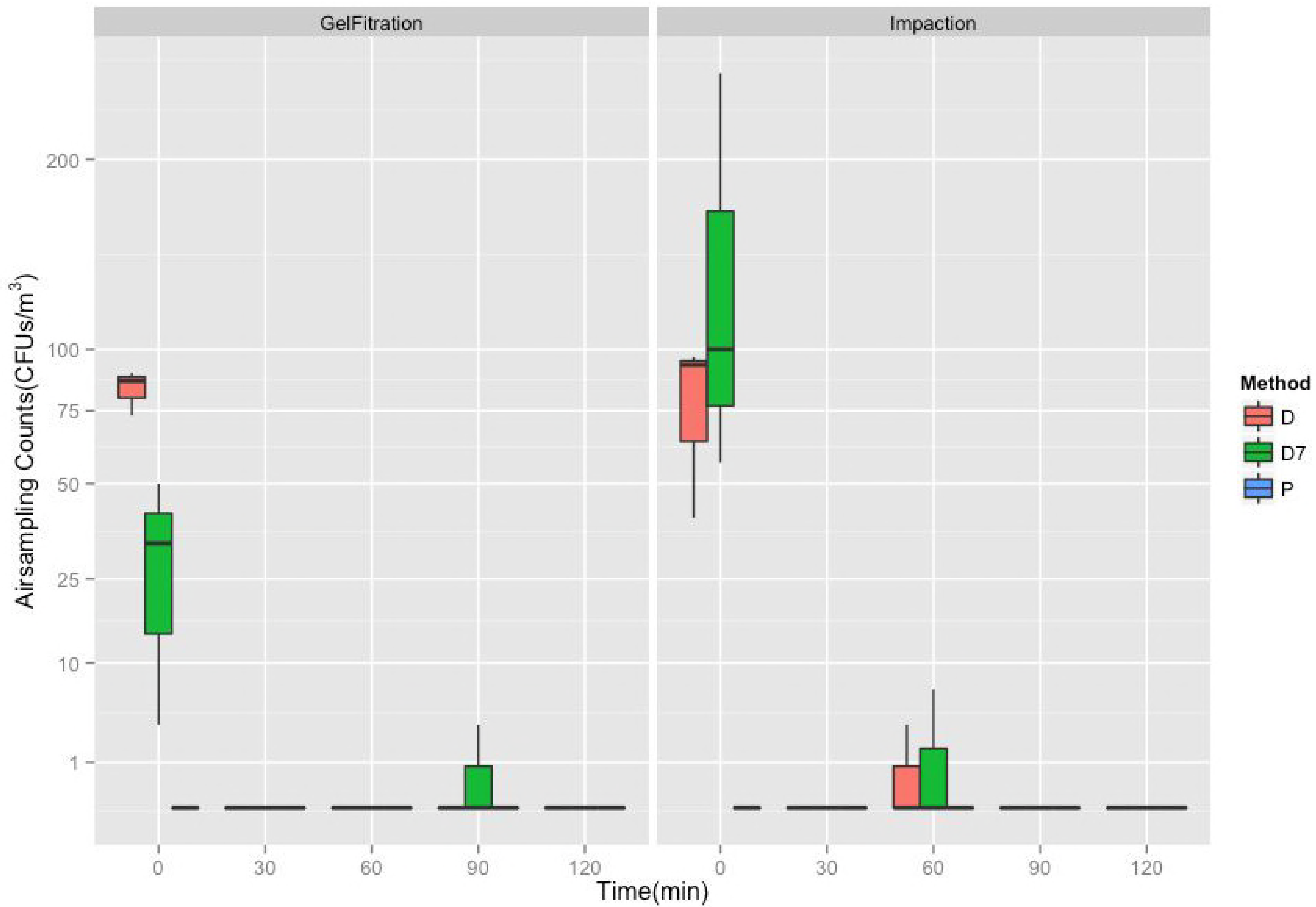
GelFitration
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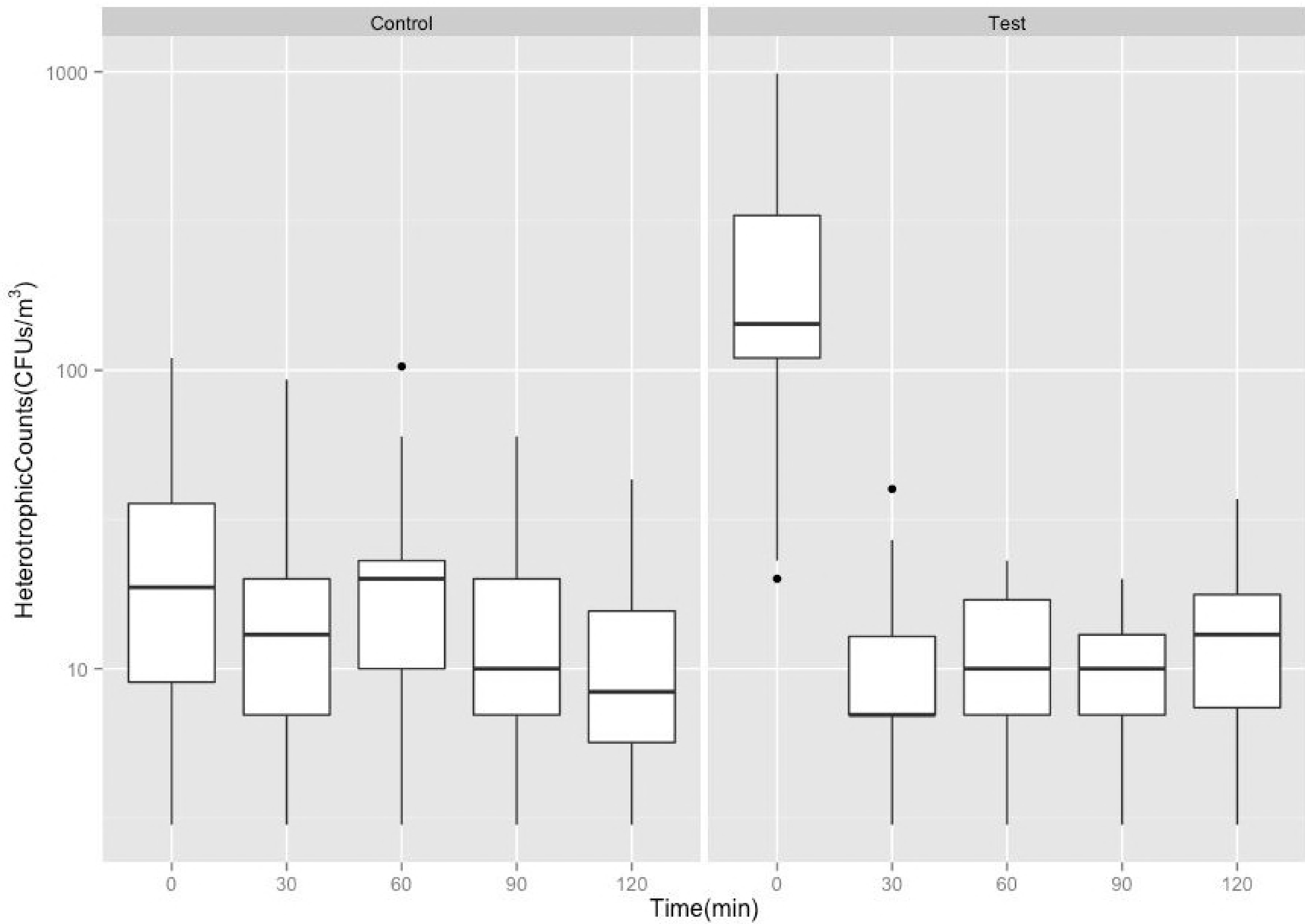
Control
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