Spread from the Sink to the Patient: *in situ* Study Using Green Fluorescent Protein (GFP) Expressing- *Escherichia coli* to Model Bacterial Dispersion from Hand Washing Sink Trap Reservoirs

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19 **Abstract**

20 There have been an increasing number of reports implicating Gammaproteobacteria often
21 carrying genes of drug resistance from colonized sink traps to vulnerable hospitalized
22 patients. However, the mechanism of transmission from the wastewater of the sink P-
23 trap to patients remains poorly understood. Herein we report the use of a designated hand
24 washing sink lab gallery to model dispersion of green fluorescent protein (GFP)-
25 expressing *Escherichia coli* from sink wastewater to the surrounding environment. We
26 found no dispersion of GFP-*E.coli* directly from the P-trap to the sink basin or
27 surrounding countertop with coincident water flow from a faucet. However, when the
28 GFP-*E.coli* were allowed to mature in the P-trap under conditions similar to a hospital
29 environment a GFP-*E.coli* containing putative biofilm extended upward over seven days
30 to reach the strainer. This subsequently resulted in droplet dispersion to the surrounding
31 areas (<30 inches) during faucet operation. We also demonstrated that P-trap colonization
32 could occur by retrograde transmission along a common pipe. We postulate that the
33 organisms mobilize up to the strainer from the P-trap resulting in droplet dispersion
34 rather than directly from the P-trap. This work helps to further define the mode of
35 transmission of bacteria from a P-trap reservoir to a vulnerable hospitalized patient.

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Importance

Many recent reports demonstrate that sink drain pipes become colonized with highly consequential multidrug resistant bacteria, which then result in hospital acquired infections. However, the mechanism of dispersal of bacteria from the sink to patients has not been fully elucidated. Through establishment of a unique sink gallery this work found that a staged mode of transmission involving biofilm growth from the lower pipe to the sink strainer and subsequent splatter to the bowl and surrounding area occurs rather than splatter directly from the water in the lower pipe. We have also demonstrated that bacterial transmission can occur via connections in wastewater plumbing to neighboring sinks. This work helps to more clearly define the mechanism and risk of transmission from a wastewater source to hospitalized patients in a world with increasingly antibiotic resistant bacteria which can thrive in wastewater environments and cause infections in vulnerable patients.

Introduction

Despite early reports (1-5), the premise that hand wash sink strainers can act as reservoirs of bacteria that cause nosocomial infections has been frequently overlooked. There has recently been an alarming increase in sink related outbreaks worldwide with many reports establishing an observational link (6-13). A sink often operates as an open conduit to wastewater in a patient care area which is often in the same room as the patient.

Healthcare establishments often invest in desperate interventions to deal with nosocomial outbreaks. The preferred method for addressing most of the environmental related

59 transmission is to employ enhanced cleaning using chemical and physical agents (14, 15).
60 Unfortunately, routine approaches are inefficient in completely eliminating drug resistant
61 Gammaproteobacteria in an inaccessible microbiologically active area such as a sink trap
62 (6, 16-20). The wet, humid and relatively protected environment in a sink trap favors the
63 formation of rich stable microbial communities (16, 21, 22). These communities will be
64 exposed to liquids and waste that are discarded in a sink, and may include antimicrobials,
65 discarded beverages, soap, presumably pathogenic bacteria from health care workers
66 hands, and other items. In short, sink traps could serve as a breeding ground for
67 opportunistic and highly antimicrobial resistant bacteria which cannot be easily cleaned
68 or removed (23-28).

69 There are many reports of a genetic association between pathogens found in sink traps
70 and those found in patients (29, 30). However, surprisingly little work has been done to
71 understand the microscale transmission dynamics. It was previously demonstrated using
72 a suspension of fluorescent particles (GloGerm™ GloGerm Co., Moab, UT) that material
73 injected into the P-trap gets dispersed around a hand washing sink (6). This result
74 however has not been replicated hitherto in the follow-up studies. Dispersion has never
75 been investigated with living organisms. Ultimately, many details remain unaddressed
76 surrounding the spread of *Enterobacteriaceae* in sink trap wastewater systems; 1) can
77 organisms grow retrograde from the P-trap water to the sink strainer, 2) can organisms
78 spread from one sink to another along the internal surfaces of pipes with shared drainage
79 systems, and 3) which portion of a colonized drain pipes results in dispersion into the
80 sink bowl during a hand washing event. We aim to better understand the dispersion
81 dynamics of Gammaproteobacteria living in the wastewater of a sink strainer and P-trap

into an area where patients and healthcare workers could be exposed. To study this dynamic we used a surrogate organism that could be easily tracked while remaining in the Enterobacteriaceae family, where some of the most concerning threats in antimicrobial resistance are developing (30).

Materials & methods

Sink Gallery design

A dedicated sink gallery was set up to simulate hospital hand washing sinks. The gallery was comprised of five sink modules assembled next to each other (Fig. 1). The five hand wash sink stations were identical in bowl design and dimensions and were modeled from the most common intensive care unit hand washing sink type in the acute care hospital at University of Virginia Medical Center. Partitions made of 24 inch high Plexiglas sheet were installed between the sinks to prevent splatter and cross contamination. Each sink module was built with Corian integrated sink/countertops without an overflow and fitted with 8 inch Centerset 2-handle Gooseneck Faucet (ELKAY®, Oak Brook, IL). Drain line under each sink comprised of flat-top fixed strainer (drain size -2 inch x 3 inch), 17 gauge (1.47 mm thickness) 8-10 inch long tailpipe, P-trap and trap-arms of 1¼ inch OD (Dearborn Brass®-Oatey, Cleveland, Ohio). All the fixtures were made of brass with chrome plating. Each of the sink P-traps was connected to a 3 inch common cast iron pipe sloping into a T-joint leading into the building sanitary line located behind Sink 3 (Fig. 1).

Inoculation, growth and establishment of GFP-*E.coli* in Sink P-traps

For the GFP-*E.coli* strain (ATCC® 25922GFP™) the Green Fluorescent Protein (GFP) gene is contained on a plasmid which also contains an ampicillin resistance gene. A single isolated colony of GFP-*E.coli* grown from -80ºC stock was inoculated in 5 ml Tryptic soy broth (TSB) (Becton, Dickinson and Company Sparks, MD) containing 100 µg/ml ampicillin (ATCC® Medium 2855). Inoculum concentration and method varied for each experiment. For establishment of GFP-*E.coli* in Sink P-traps, new autoclaved P-traps were filled with 100 ml 0.1X strength TSB and inoculated with ~10^3 CFUs/ml GFP-*E.coli*. Following inoculation, both the ends of the P-traps were covered with perforated Parafilm (Bemis Inc. Oshkosh, WI) and allowed to incubate at room temperature (22±2 °C) for 14 days to facilitate adherent bacterial growth. The media in the P-trap was decanted and replaced with fresh 0.1X TSB every 48 h. An aliquot of decanted media and a swab sample from the inner surface of the P-trap were plated on Tryptic soy agar (Becton, Dickinson and Company Sparks, MD) plates containing 100 µg/ml ampicillin (TSA) to monitor the growth GFP-*E.coli* in the P-traps. TSA plates were incubated overnight at 37ºC and colony-forming units (CFUs) fluorescing under UV light were enumerated. All preparatory culturing of GFP-*E.coli* took place in a separate room from the sink gallery to avoid unintentional contamination.

Installation of P-traps colonized with GFP-*E.coli*

After the 14-day incubation, P-traps were fastened into the plumbing of the sinks (Fig. 2a). The remainder of the drain-line was either autoclaved (strainer, tailpipe, and trap-arms) prior to installation or surface disinfected (sink bowl, countertop and faucets) with Caviwipes-1 (Metrex Research Romulus, MI) maintaining at least 1 minute contact time. After the P-trap was installed, a daily regimen comprised of 25 ml of TSB followed by 25

ml of 0.9% NaCl solution (saline) were added in the ratio 1:3 via the strainer (Fig. 2b) to mimic the potential nutrient exposure in the hospital.

Sampling and enumeration of *GFP-E.coli*

To monitor the growth of GFP-*E.coli* in the plumbing, sampling ports were drilled along the length of the tailpiece (between the P-trap and the strainer), and the trap arm (between the P-trap and the common line). These holes were fitted with size 00 silicone stoppers (Cole-Parmer Vernon Hills, IL) (Fig. 2a). Sterile cotton swabs (Covidien™, Mansfield, MA) presoaked in saline were inserted through these sampling ports and samples were collected by turning the swab in a circular motion on the inner surface (~20 cm^2) of tailpipes. Sample swabs were pulse-vortexed in 3 ml saline and serial dilutions were plated on TSA. Strainer, faucet aerator and bowl surface were sampled with presoaked swabs and processed as described earlier.

Sink-to-sink transmission of bacteria

To investigate sink-to-sink transmission of bacteria, a distal sink (Sink 5) (Fig 1) was fitted with a P-trap inoculated with GFP-*E.coli*. Effect of different inoculum concentrations of GFP-*E.coli* -10^3, 10^6 and >10^{10} CFUs/ml (colonized for 14days) were investigated. Speciation of fluorescent and non-fluorescent colonies identified from mixed pipe cultures was performed using a Matrix-Assisted Laser Desorption/Ionization (MALDI)–Time of Flight (TOF) (VITEK-MS, Biomérieux Durham, NC). The wastewater paths of Sinks 1 to 4 were either autoclaved (strainer, tailpipe, P-traps and trap-arms) prior to installation, or surface disinfected (sink bowl, countertop and faucets) with Caviwipes-1 (Metrex Research Romulus, MI). Faucets on each of the five sinks

were turned on simultaneously for 1 min supplying water at a flow rate of 8 L/min once every 24 h for 7 days. No additional feed to any of the sinks was added during this 7 days. On day-0 and day-7 P-traps on each of the five sinks were unfastened, and swab samples of the P-trap were collected and processed as described earlier.

Dispersion measured using fluorescent microspheres

Fluoresbrite® YO carboxylate microspheres (Polysciences, Inc.) which had 1 µm diameter, maximum excitation and emission of 529 nm and 546 nm respectively were used as tracer in the preliminary experiments to understand droplet dispersion from the hand wash sinks.

To test whether microspheres could be dispersed from below the sink strainer, 1 ml suspension of microspheres (~10^{10} particles) was injected through a strainer attached to a Hert 4½" Offset Drain-tailpiece typically used for wheelchair accessible sinks (American Standard-Model #7723018.002) (Fig. 2c). The vertical distance between the strainer and microsphere suspension injected into the tailpipe was ~4 inches. Counter space around the sink bowl was thoroughly wiped with alcohol wipes (Covidien Webcol™ 6818, Kendall) and polyester sheets precut to appropriate shapes were placed on the counter to cover the entire sink counter and labeled according to position (Fig. 3a). The faucet was turned on for 5 min at a water flow rate of 1.8-3.0 L/min. Polyester sheets were harvested and immediately analyzed using a ChemiDoc MP system (Bio-Rad Laboratories, Inc.) with an exposure time of 5 s. Fluorescent microspheres were enumerated from the digital micrographs using the Image Lab™ Software (Bio-Rad Laboratories, Inc.).

To test whether microspheres could be dispersed from the surface of the sink bowl, 20 ml microsphere suspension (~10^{10} particles/ml) was evenly coated onto the sink bowl using disposable swab (SAGE Products Inc. Cary, IL) and dispersion experiment was repeated following the protocol described above. To ascertain there was no non-specific background fluorescence in the sink and/or the water from faucet a control using the same protocol but without the fluorescent microspheres was performed before each experiment.

Dispersion measured using *GFP-E.coli*

Dispersion using GFP-*E.coli* was investigated in three experiments. To test whether live organisms in the P-trap could be dispersed by running water, ~10^{10} CFUs/ml GFP-*E.coli* in saline was added to an autoclaved P-trap and fitted into the drain line that was pre-autoclaved (strainer, tailpipe, and trap-arms). Similarly, to test whether live organisms could be dispersed from the tailpieces of wheelchair accessible sinks, ~10^{10} CFUs/ml GFP-*E.coli* suspension was added into the Hert 4½" Offset Drain-tailpiece (Fig 2c) through the strainer using a syringe. Just as in the microsphere dispersion experiment, the vertical distance between the strainer and GFP-*E.coli* suspension injected into the tailpipe was ~4 inch.

We next tested whether live organisms from the surface of the sink bowl could be dispersed by running water. Approximately 20 ml suspension of 10^{10} CFUs/ml GFP-*E.coli* was evenly coated onto the sink bowl surface.

Finally, to mimic all these conditions, P-trap colonized with GFP-*E.coli* (for 14 days) was installed and a nutrient regimen (Fig. 2b) was followed for 14 days to intentionally

promote the GFP-*E.coli* colonization in the attached tailpipe and strainer. On day-15 the dispersion experiment was performed.

Before each of the GFP-*E.coli* dispersion experiment the counter space was thoroughly disinfected with Caviwipes-1. TSA plates were then positioned on the sink counter surrounding the bowl and an extension platform (Fig 3b). Additional plates were attached to the sink bowl, faucets, Plexiglas partitions, and faucet handles using adhesive tape. TSA plates were also placed 3 m away from the sink as negative controls. The faucet was turned on for 5 min with water flow rate of 1.8-3.0 L/min. Lids of the TSA plates were removed only during faucet operation. Swab samples of the faucet aerators before and after operation were collected and plated on TSA. Prior to the each dispersion experiment, 50 mL water from the faucet was also collected and aliquots were plated to assess for the presence of GFP-*E.coli* in source water and ensure cross contamination of GFP-*E.coli* had not occurred. A control dispersion experiment was also performed using the same protocol prior to GFP-*E.coli* inoculation in each case. Dispersion per defined area (CFU/cm^2) was deduced by dividing the CFU counts in the TSA plate with the surface area of the TSA plate.

Results

Growth and Colonization of GFP-*E.coli* in P-trap

In the first 14 days following the installation of the P-trap with established GFP-*E.coli* and just water running from the faucet, GFP-*E.coli* was not detected in the tailpipe

beyond 1.5 inch above the liquid level in the P-trap. GFP-*E.coli* however was found to be viable in the P-trap without any nutrients added. A nutrient regimen was then instituted to understand the influence of nutrients on mobility and upward growth. The addition of TSB promoted GFP-*E.coli* growth as early as day-1, with growth observed in the tailpipe 2 inches above the liquid surface in the P-trap (Table 1). On day-7, the strainer (~8" above the liquid in the p-trap) was found to be colonized with GFP-*E.coli*. This translates to an average growth rate of 1 inch/day along the length of the tailpipe with the addition of nutrients and without faucet operation. GFP-*E.coli* was not detected in the faucet water.

Sink to sink transmission of bacteria

. In these experiments a flanking sink (Sink 5) was the only P-trap inoculated with GFP-*E.coli* and therefore was the sole source for transmission to the connected sinks. Starting with lower inoculum concentration (10^3 CFUs/ml) in Sink 5, on day-7 GFP-*E.coli* was detected in the Sink 2 and Sink 3 P-traps (Fig. 4a). With 10^6 CFUs/ml and $>10^{10}$ CFUs/ml inoculum concentrations in Sink 5, all the sink P-traps in the sink gallery with the exception of Sink 1 were found to be colonized with GFP-*E.coli* after 7 days (Fig. 4b and c). Faucet water and aerators tested negative for GFP-*E.coli.* Irrespective of starting inoculum concentration, on day-7 the highest level of colonization was recorded in the Sink 3 P-trap. After day-7 when the nutrient regimen (described previously) was followed for additional 7 days in each of the sinks in the sink gallery with inoculum concentration $>10^{10}$ CFUs/ml, GFP-*E.coli* was detected in the strainers of Sink 2 and Sink 3 on day-14. This finding validated the upward growth and growth rate in the tailpipe when nutrients were added. Non-fluorescent colonies were occasionally observed in the P-trap water samples collected from the sinks, which were subsequently identified

to be *Pseudomonas sp.* or *Stenotrophomonas maltophilia* and fluorescent colonies were confirmed to be *E. coli.*

Dispersion of microspheres from sinks

In the first dispersion experiment, when the fluorescent microspheres were inoculated into the offset drain-tailpiece only 4 inches below the strainer, no microspheres were detected on the polyester sheets placed on the counter space.

However, when the sink bowl was coated with the microspheres, polyester sheets overlaid on the counter space captured the dispersed microspheres caused by the faucet operation. Dispersion was observed on almost all zones of the sink counter space (Fig 5). Relatively higher dispersion were observed along the major and minor axes of the elliptical sink bowl (zone # 2, 5, 6, 9, 11 and 12). Anterior corners of the sink counter space (zone # 4 and 7), which were most distant from the impact of water in the sink bowl received lowest dispersion.

Dispersion of GFP-*E.coli* from sinks

Initially the P-trap alone was inoculated with GFP-*E.coli* and carefully installed keeping the tailpipe and strainer free of GFP-*E.coli* before operating the faucets. No fluorescent CFUs were observed on the plates placed on the counter or attached to the bowl surface after faucet operation. Similarly, no fluorescent CFUs were detected when GFP-*E.coli* was inoculated into the offset drain-tailpiece only 4 inches below the strainer. Interestingly, when there was conspicuous water backup over the strainer as a result of

higher water flow rate from the faucet than the drainage rate from the P-trap, dispersal was detected on the plates attached to the bowl surface.

The dispersion pattern recorded when the sink bowl was coated with GFP-*E.coli* was comparable to the pattern recorded when fluorescent microspheres were coated on the sink bowl (Fig. 5). Dispersion was significantly higher along the axes (zones 6, 9, 11, 12) and lower at the corners of the sink counter space (zones 4, 7 and 10).

In contrast, dispersion of GFP-*E.coli* caused by the faucet operation was much more extensive when the strainer was allowed to colonize with GFP-*E.coli* prior to the dispersion experiment. In addition to the sink counter space, we also measured dispersion to the sink bowl, faucet, faucet-handles, splatter shields, and the extended counter surface. Dispersion of GFP-*E.coli* was highest on the plates attached to the sink bowl (Fig. 6b). Further, dispersion was greater along the minor axis of the sink bowl (Figure 6b, zones B3, B4 and B10) than along the major axis of the sink bowl; associated with a shorter distance from the strike point of the faucet water to the bowl along this axis. The next highest CFU count from the dispersal was recorded on the counter area near the faucets (Fig. 6a, zones 12 and 11). Similar pattern of higher dispersion near the faucets and lower dispersion at the corners of the counter space (Fig. 6a, zones 4, 7 and 10) was also observed using microspheres. Dispersion was also recorded in other zones of the counter space, on the Plexiglas splatter shields, faucets, faucets handles and extended surface (Fig. 6c). There were no GFP-*E.coli* CFUs recorded on plates placed beyond 30 inches from the strainer, demarcating the range of dispersion under these experimental conditions.

Table 2 gives a summary of the total distribution load recorded using fluorescent microspheres and GFP-*E.coli* across each experiment. The load of dispersion on the sink counter was comparable when the microspheres or GFP-*E.coli* was coated on the sink bowl before the faucet operation. Although, dispersion load on the sink counter was lower when sink strainer was colonized, it is interesting to note that the sink bowl received highest dispersion.

Discussion

To mimic hospital dispersion, we first investigated whether GFP-*E.coli* would establish consistent colonization in a sink trap as many other Gammaproteobacteria implicated in nosocomial outbreaks have done (6, 28). Many recent reports demonstrate that P-traps become colonized with highly consequential Gammaproteobacteria, which then result in nosocomial transmission (29, 31, 32) The retained water in a sink P-trap is present to provide a water barrier to prevent off-gassing of sewer smell but it may inadvertently provide favorable conditions for pathogenic and opportunistic antibiotic-resistant microorganisms to survive and develop resilient biofilms (3, 33). However, the mechanism of dispersal of the bacteria in the P-trap to patients or the surrounding healthcare area had not been fully elucidated. We began with the hypothesis that the bacteria originate from the P-trap via droplet creation when the water from the faucet hits the P-trap water thus contaminating sink bowl and the surrounding area. The finding supporting this theory had been previously reported using GlowGerm particles (6). However, in the present study with careful attention to avoid strainer and tail piece

297 contamination the dispersal directly from the sink P-trap with either microspheres or
298 GFP-*E.coli* could not be reproduced as previously reported (6).

299 Rather this work demonstrates a different more staged mode of transmission from a P-
300 trap reservoir to the sink and surrounding environment. GFP-*E.coli* in the P-trap alone
301 sustained for 14 days but did not grow or mobilize up the tailpipe to the strainer with just
302 intermittent water exposure. However, when nutrients were subsequently added to the
303 system the organisms rapidly grew up the tailpipe to the strainer at approximately an inch
304 per day. In a real-world setting motility of bacteria inside the tailpipe is restricted to
305 relatively sporadic and short-lasting wetting events in which swimming is an opportunity
306 to colonize new surfaces. It is assumed that once established, the biofilm promotes the
307 upward growth of GFP- *E.coli* in the tailpipe at an accelerated rate. The nutrient regimen
308 which promoted colonization in our model reflects our observations and others of items
309 commonly disposed of in hospital sinks (intravenous fluids, feeding supplements, and left
310 over beverages) (5, 32).

311 Transmission of bacteria between sinks via a common pipe was a key finding in this
312 study as this highlights the concept that premise plumbing may be a more continuous
313 system with shared microbiology rather than a single isolated sink. The sink gallery used
314 in this study provided a unique *in situ* advantage to investigate sink-to-sink transmission
315 of bacteria through common drains. The two possible mechanisms for P-trap strainers
316 becoming colonized are seeding of organisms from above, and retrograde spread of
317 organism along common pipes in a hospital wastewater infrastructure. Here we
318 demonstrate that it is possible for GFP-*E.coli* to contaminate adjacent P-traps with just
319 time and water given a standard US code piping rise of ¼′ per foot. Sink-to-sink or

retrograde transmission may explain the recurrence of pathogen colonization following intervention strategies like disinfection or replacement of plumbing (23). Sink 3 was lowest on the slope in the drain-line (Fig. 1) with arguably the most opportunity for reflux and retrograde wetting. Sink 1, on the other hand, was farthest away from the source (Sink 5) and its P-trap had the greatest incline in the drain-line connecting the sinks, which could perhaps contribute to the reasons there was no GFP-*E.coli* colonization detected in it after 7 days. There has been more investigation about microbiologic dynamics of infectious viral particles such as SARS and Ebola through premise plumbing systems (34-36). However, the microbiology, sustainability and dynamics might be very different but the backflow and inoculation issues could have some parallels when comparing viruses to bacteria. As *Enterobacteriaceae* can either multiply or remain viable for long periods of time in biofilms coating the interior of P-traps and the connected plumbing it may not be sustainable to target any intervention limited to a single isolated sink as a source of a particular pathogen.

Data from different dispersal experiments suggest that although P-traps can act as the source or the reservoir of pathogens, physical presence of the organism in the sink bowl or colonization of strainer is necessary for the dispersal to occur. Colonization of strainers or drains reported in earlier studies (7, 10, 13, 24, 37) was perhaps a result of ascending biofilm growth from the P-trap to the strainer or introduction through contaminated fluids. Many of the studies used swab samples, which likely sampled the strainer rather than P-trap water (17, 20). Once the strainer was colonized, the water from the faucet resulted in GFP-*E.coli* dispersion in the bowl and to the surrounding surfaces of up to 30 inches. The range of dispersal recorded in this study was comparable that reported earlier (6). Greater

343 dispersal near the faucet may be attributed to the specific designs of the sink bowl and
344 faucet in this study which determine the contact angle of water impact. It is an important
345 finding since many sinks in hospitals have a similar in design with faucet handles
346 representing a high-touch surface for the sink users (38). It can also be concluded from
347 the dispersion experiments that secondary and successive dispersals would likely increase
348 the degree and the scope of dispersion.

349 There are several limitations to this work. First the similar sink bowl across these sinks
350 only examines a dispersion pattern of this particular sink design. Similarly the sink-to-
351 sink transmission may not be applicable to all wastewater plumbing systems as the
352 fixtures on the pipe are very close together unlike most layouts in healthcare settings.
353 However we speculate that transmission could occur on larger systems over greater time
354 scales especially if heavy nutrient and contamination loads were also included. GFP-
355 *E.coli* is a laboratory surrogate, and the putative biofilms established in the short time
356 frame of our experiments are unlikely to be as complex or stable as biofilms developed in
357 a hospital wastewater system over many years. However, to address the mono-microbial
358 dominance of the GFP-*E.coli* added to the system we kept the system open and other
359 environmental organisms were able to co-colonize in an attempt to mimic the hospital
360 system. Another limitation was the need to add nutrients to the drain to ensure rapid and
361 robust colonization. We are not clear how widespread the practice of disposing dextrose
362 containing intravenous fluids or left over beverages in the hand wash sinks is however we
363 have observed this practice and anecdotally it appears to be a relatively common in the
364 United States. We also did not completely characterize the droplet sizes nor do we
365 demonstrate air sampling to understand if the dispersion is only droplet or if there are

366 also aerosols, which contain GFP-*E.coli*. This would require additional testing and is
367 planned as future work.

368 In summary, this work for the first time better models the mechanisms of spread of multi-
369 drug resistant pathogens arising from the sink drain and infecting patients. Droplet
370 dispersion from the P-trap does not happen directly. Rather it is a multi-stage process;
371 dispersal originates from the strainer and/or the bowl after growth of the biofilm up from
372 the microbial reservoir of the P-trap. We also demonstrate sink-to-sink transmission via
373 common sanitary pipe. This work could have implications for patient safety, infection
374 control and interventions as well as the design of future hospital plumbing systems to
375 eliminate this mode of transmission to vulnerable hospitalized patients.

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520 Figure 3. a) Layout of the zones of sink counter, bowl and extension surface designated
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523 Figure 4. GFP-*E.coli* detected in the P-traps attached to each of the sinks on day-0 (black
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526 Figure 5: Dispersion of microspheres (grey bars) and GFP-*E.coli* (black bars) on the area
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528 of the sink counter.

529 Figure 6. Dispersion of GFP-*E.coli* on the area surrounding the sink when strainer,
530 tailpipe and P-trap were colonized. (a) Sink Counter (b) Sink bowl and (c) Other
531 surrounding area. X-axis represents the designated zones of the sink counter.

532

533 Table 1. Growth in the tailpipe connected to the p-trap colonized with GFP-*E.coli* biofilm.
534 '-' and '+' denote absence and presence of GFP-*E.coli* respectively.

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Strainer (8" above P-trap water)	-	-	-	-	-	-	-	+
Tailpipe (6" above P-trap water)	-	-	-	-	+	+	+	+
Tailpipe (4" above P-trap water)	-	-	-	+	+	+	+	+
Tailpipe (2" above P-trap water)	-	+	+	+	+	+	+	+
P-trap	+	+	+	+	+	+	+	+

535

536 Table 2. Comparison of dispersion load across different experiment

Dispersion Experiment	Dispersion load (microspheres/cm^2 or CFUs/cm^2)			
	Sink counter (>30inch)	Sink bowl	Faucets & Faucet handles	Splatter shields
Microsphere inoculated in Offset Drain	0	NA	NA	NA
Microsphere coated on sink bowl	206±10	NA	NA	NA
GFP-*E.coli* inoculated in P-trap	0	0	0	0
GFP-*E.coli* inoculated in Offset Drain	0	NA	NA	NA
GFP-*E.coli* coated on sink bowl	232±17	NA	NA	NA
Strainer colonized with GFP-*E.coli*	171±15	342±17	17±3	3±1

537