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Nutrients influence the dynamics of *Klebsiella pneumoniae* carbapenemase producing enterobacterales in transplanted hospital sinks

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ABSTRACT

Antimicrobial resistance has been recognized as a threat to human health. The role of hospital sinks acting as a reservoir for some of the most concerning antibiotic resistant organisms, carbapenemase producing Enterobacterales (CPE) is evident but not well understood. Strategies to prevent establishment, interventions to eliminate these reservoirs and factors which drive persistence of CPE are not well established. We use a uniquely designed sink lab to transplant CPE colonized hospital sink plumbing with an aim to understand CPE dynamics in a controlled setting, notably exploiting both molecular and culture techniques. After *ex situ* installation the CPE population in the sink plumbing drop from previously detectable to undetectable levels. The addition of nutrients is followed by a quick rebound in CPE detection in the sinks after as many as 37 days. We did not however detect a significant shift in microbial community structure or the overall resistance gene carriage in longitudinal samples from a subset of these transplanted sinks using whole shotgun metagenomic sequencing. Comparing nutrient types in a benchtop culture study model, protein rich nutrients appear to be the most supportive for CPE growth and biofilm formation ability. The role of nutrients exposure is determining factor for maintaining a high bioburden of CPE in the sink drains and P-traps. Therefore, limiting nutrient disposal into sinks has reasonable potential with regard to decreasing the CPE wastewater burden, especially in hospitals seeking to control an environmental reservoir.

1. Introduction

Escalating number of reports on contamination of hospital sinks with multidrug-resistant gram-negative organisms, and recent scientific investigations establishing their link to outbreaks and transmissions to patients, underline the role of these environmental reservoirs in managing healthcare associated infections (Amoureux et al., 2017; Bousquet et al., 2017; De Geyter et al., 2017; Herruzo et al., 2017; Hopman et al., 2017; Leitner et al., 2015; Lowe et al., 2012; Regev-Yochay et al., 2018; Roux et al., 2013; Starlander and Melhus, 2012; Tofteland et al., 2013; Varin et al., 2017; Vergara-Lopez et al., 2013; Yablon et al., 2017). The primary mechanism of transmission from sinks to patients has recently been demonstrated in a model lab using a model organism (Kotay et al., 2017). We presented evidence that bacteria can colonize the standing water in the sink P-trap, under favorable conditions grow up to the sink drains, and driven by the faucet water impact disperse onto the surrounding touchable surfaces (Kotay et al., 2017). A recent study in this direction has also suggested that dispersal from sinks is influenced by drain position and drainage rates (Aranega-Bou et al., 2018). Our further work has also demonstrated that transmission from a contaminated sink drain was primarily droplet-mediated rather than aerosols (Kotay et al., 2019).

It is reasonable to speculate that non-native highly resistant bacteria like CPE have evolved to colonize and persist within the biofilms formed on the luminal surface of the plumbing connected

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to hospital hand wash sinks. However factors that drive persistence and prosperity of these organisms in a hospital sink drain biofilm remains unknown (Carling, 2018; Gordon et al., 2017; Parkes and Hota, 2018). In a single observation study on hospital room sink usage, handwashing was found to be a mere 4% of all the events (224/5614), and occurrence of nutrients disposal events at an average daily frequency was recorded (Grabowski et al., 2018). Other reports have also highlighted the potential risks of disposal of nutrients into the patient sinks in the form of beverages, IV fluids, body fluids and other liquid wastes (De Geyter et al., 2017; Leitner et al., 2015; Lowe et al., 2012; Roux et al., 2013; Smolders et al., 2018).

While a variety of conventional and unconventional interventions implemented were able to reduce the transmissions or contain the outbreak, none of these interventions completely eliminated drain reservoirs of highly resistant nosocomial pathogens (Carling, 2018; French et al., 2017; Gordon et al., 2017; Parkes and Hota, 2018). A fundamental challenge around understanding the persistence of pathogens in the sink environment and/or investigating the effectiveness of targeted interventions can be attributed to the lack of a controlled infrastructure.

We sought to further examine the impact of timing and type of nutrient exposure on the hospital CPE isolates *in vitro* and within real-world hospital sink biofilms. Hospital sinks were transplanted into a controlled unique lab setting for this testing. The findings may help accentuate the importance of nutrient limitation as a targeted intervention strategy for decreasing highly-resistant, potentially pathogenic bacteria in a sink drain reservoir.

2. Materials and Methods

2.1. Sink lab and sink design

A Biosafety Level 2 certified sink lab accommodates nine modular sinks (Fig. 1A), each module comprising a Corian 831 Sink bowl (dimensions- 22" × 13 ½" × 6") integrated with countertop (Mid-Atlantic Manufacturing, Oilville, VA). Over each sink are fitted 8-inch Centerset 2-handle Gooseneck faucets (Model LKD232SBH5C, ELKAY®, Oak Brook, IL). U-shaped Splatter shields made of 24″ high Plexiglas sheet were installed over the edge of each sink countertop to contain splatter and prevent cross contamination between the sinks. Each sink bowl and the integrated counter was supported on a custom built stainless-steel rig (Fig. 1A and B). The drain line under each sink comprised of cast grid drain (P/N 760-1 Dearborn Brass®-Oatey, Cleveland, Ohio), a chrome coated over brass 6-inch tailpipe, 1¼-inch P-trap, trap-arm (P/N 701-1, Dearborn Brass®-Oatey, Cleveland, Ohio) and 2-inch PVC pipe (Charlotte Pipe Charlotte, NC) leading into 60 gallon high-density polyethylene resin (HDPE) holding water tank (Ronco Plastics, Tustin, CA The PVC included a sanitary T which was fitted with a 2 inch vent to prevent air locks and back up of water in the sink bowl. The tailpipes and trap-arms were modified with holes and butyl rubber stoppers to allow sampling (Fig. 1C).

2.2. Sink operation and automation

A ½" Brass Electric solenoid valve (JFSV00006, US Solid) with 4 mm orifice was installed on cold water faucet connectors. A peristaltic pump (MasterFlex C/L, Cole-Parmer, Vernon Hills, IL) that supplied soap (Kleenex® Foam Skin Cleanser, Kimberly-Clark Professional, Roswell, GA) from a 1L refill container connected using a silicone tubing (Puri-Flex tubing, Cole-Parmer, Vernon Hills, IL). The silicone tubing for soap was attached to an 11 ½ GA 316 stainless steel tube, which was clamped to the faucet such that the tip of the stainless steel tube was directly in the stream of water emerging from the faucet. This facilitated even mixture of soap and water. Controlling the solenoid valve and the peristaltic pump was an Arduino Uno microcontroller (Sparkfun, Niwot, CO) with a custom-built shield accommodating two MOSFETs. These together activated water in each sink and the flow of soap for 15 s, once every 2 h. This translated to 1275 ml of water (at 5.1 L/min flow rate) and 2 ml of soap every faucet event. The wastewater was collected into holding tanks, disinfected using chlorine tabs before discharging into common floor drain. On weekly basis faucet water samples were collected to measure the temperature, pH, water flowrate and residual chlorine (data not included).

2.3. Sink transplant and sampling

Patient intensive care unit sink drains and/or P-traps in a University hospital, which consistently tested positive for *Klebsiella pneumoniae* producing Enterobacterales (KPCE), were harvested and installed in the sink lab (within 24h of harvest). Sink transplant events occurred in two phases of testing. In the first phase, eight P-traps were transplanted into the sink rigs by installing them with new unused plumbing (drain, tailpipe and the trap arms). In the latter phase, 18 complete sink drain assemblies (drain, tailpipe, P-trap and trap arm) were harvested and transplanted for testing.

Fig. 1. A) Sink lab infrastructure with an array of nine modular sink rigs operated using indigenously developed automation; B) schematic of a single sink module; C) Sink plumbing depicting the sampling ports [*] for P-trap water and biofilm sampling.

Table 1
Media composition of the various nutrients tested. Values in g/L and cefepime was spiked at final concentration 0.1 μg/mL wherever mentioned. *R2A was not tested; listed for comparison.

	TSB	TSB-D	R2A*	modR2A	remodR2A	Water	NaCl
Tryptone	17	17	0	0	0	0	0
Peptone	0	0	0.5	0.5	5	0	0
Yeast Extract	0	0	0.5	0.5	0.5	0	0
Soy	3	3	0	0	0	0	0
Casamino acids	0	0	0.5	0.5	0.5	0	0
Dextrose	2.5	0	0.5	1.1	1.1	0	0
Starch	0	0	0.5	0	0	0	0
NaCl	5	5	0	5	5	0	8.5
K_2HPO_4	2.5	2.5	0.3	0.3	0.3	0	0
KH_2PO_4	2.5	2.5	0	0	0	0	0
$MgSO_4$	0	0	0.05	0	0	0	0
Na-Pyruvate	0	0	0.3	0	0	0	0

One sink in the sink lab during each phase of testing was designated and operated as a negative control with a set of previously unused plumbing fittings to monitor cross contamination. Drain biofilm and P-trap water were sampled and processed as described previously (Mathers et al., 2018). In case of experiments with nutrients supplementation, 25 ml TSB was added to the sinks on a daily basis by pouring over the drain with an average 2h dwell time.

2.4. Growth and biofilm assay

Four strains of KPCE, *Citrobacter freundii* (CAV1857), *Enterobacter hormaechei* (CAV1983), *Serratia marcescens* (CAV1976), and *Klebsiella quasipneumoniae* (CAV2018), previously isolated from the hospital sink drain or P-trap were considered for the growth and biofilm assay experiments. Prior to experiments, each strain was revived from −80 °C frozen stocks by streaking onto tryptic soy agar, single isolated colony was inoculated into 5 mL of tryptic soy broth (TSB; BD™ Bacto™), incubated overnight at 37 °C. The cells were harvested by centrifugation at 12,000×*g* for 10 min at 4 °C, washed and suspended into 0.85% NaCl solution. The primary bacterial concentration was adjusted to OD_{600} = 0.15 using a microplate reader (VersaMax, Molecular Devices Company, CA, US) and an aliquot (20 μl) of each strain was inoculated into 180 μl of each of the different nutrient media listed in Table 1. Media without bacterial inoculum served as a negative control. For growth assay, absorbance of the liquid cultures at intervals was measured at 600 nm. All isolates and media were tested in triplicate.

Biofilm assay were performed in 96-well polystyrene microplates. The plates with listed media were inoculated as above with KPCE isolates and incubated without shaking at 37 °C. Following incubation, media was decanted and rinsed three times with sterile de-ionized water. One replicate set of wells were air-dried for 30 min, and stained with 200 μL of 0.25% (w/v) crystal violet for 30 min. The staining solution was then removed, and rinsed three times with sterile de-ionized water. The crystal violet bound to the biofilm was then solubilized with 200 μL of 95% ethanol for 30 min. The absorbance was measured at 570 nm. Another replicate set of wells were processed for quantification of KPCE within the biofilm by adding 200 μL of sterile phosphate buffer saline with 0.02% TWEEN 80 (PBST) and set on shaking platform for 30 min to allow resuspension of cells. The PBST cell suspension was subsequently dilution plated on tryptic soy agar plates and incubated at 37 °C overnight. Colony forming units (CFUs) from each set of replicates were enumerated to determine bacterial abundance within the biofilm.

2.5. Metagenomic sequencing

DNA from sink drain biofilm samples was extracted using DNeasy PowerSoil® Kit (Qiagen) following manufacturer's protocol. After quantification using picogreen, the extracted DNA was used to construct shotgun metagenomic libraries using the Nextera XT kit following the manufacturer's protocols. Sequencing was performed using the Illumina HiSeq 4000, multiplexing six samples per lane to generate approximately 40 million paired-end reads

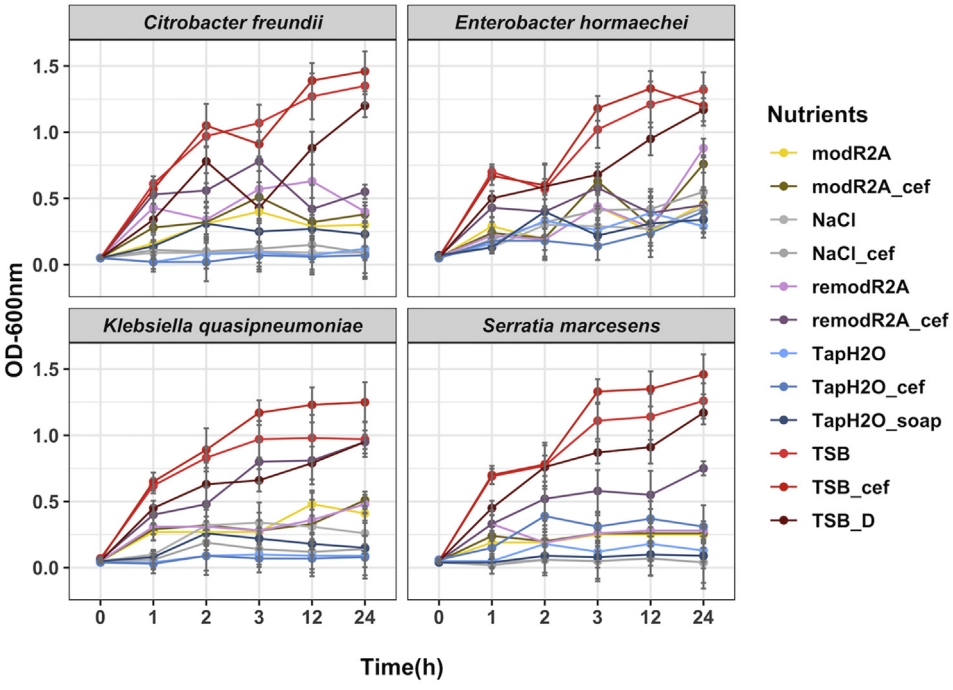

Fig. 2. Growth profile of the four KPCE species across various nutrients. Absorbance measured at OD_{600}.

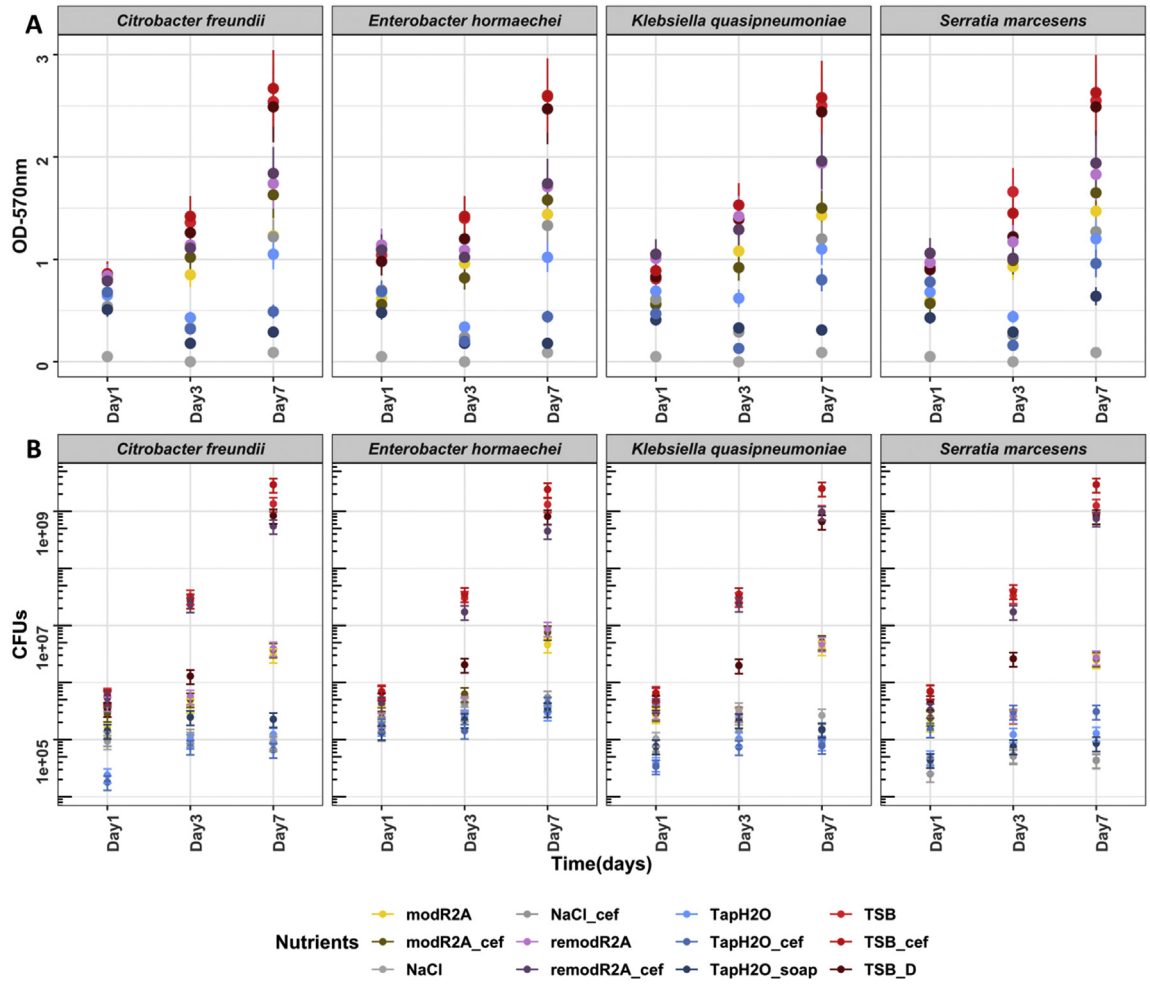

Fig. 3. Histogram depicting A) biofilm formation ability (Crystal violet staining-absorbance measured at OD570) and B) KPCE population (CFUs) in the biofilms over time using various nutrients and four KPCE species. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

(150 bp) per sample.

2.6. Bioinformatics analysis

Low-quality portions of sequences were trimmed using TrimGalore (https://github.com/FelixKrueger/TrimGalore) (Krueger, 2015). Specifically, we discarded reads below average Phred score of Q25 and a length cut-off of 75 bp, and the first 13 bp of Illumina adapters. Taxonomic composition of the metagenomics samples was estimated using Kraken (Wood and Salzberg, 2014) against a reference database composed of NCBI's RefSeq bacterial and viral genomes (accessed in February 2018). For each taxonomic level, relative abundances were estimated using Bracken (Lu et al., 2017). To remove spurious and low-abundance assignments, only taxa with >0.01% relative abundance were considered for downstream diversity and statistical analysis. For AMR gene abundance estimation, the filtered metagenomics reads were mapped using BBMAP (Bushnell, 2014), against the Comprehensive Antibiotic Resistance Database (CARD v2.0.1, Jia et al., 2017). As short reads do not provide sufficient resolution to distinguish highly similar AMR gene homologs, we pre-clustered the CARD database (n = 2,448 sequences) at 90% identity and only retained one representative sequence for each cluster (n = 1,074 sequences). The aligned BAM files were used for gene counting, normalization and abundance estimation using the approach implemented within ResPipe (Gweon et al., 2019). Briefly, gene cluster counts were generated for reads that mapped with 100% sequence identity to representative sequence, followed by pseudo-abundance estimation by normalizing using the gene length as well as *lateral* coverage of the gene sequence by reads.

2.7. Statistical analysis

Within-sample alpha diversity was measured at each taxonomic-level by counting the distinct taxa (community richness) and calculating the Shannon index (community evenness) that accounts for relative abundances of taxa. Compositional dissimilarities between samples was calculated using the Bray-Curtis index and visualized by Principal Coordinates Analysis. Taxonomic relative abundance profiles were converted to arcsine square-root transformed proportions to calculate random effects model estimates, using sinks as random effects to account for within-sink heterogeneity. *P* values obtained from the random effects models were corrected for multiple comparisons using "FDR" method, with q-values < 0.1 considered statistically significant. Similarly, resistance gene counts were analysed using random effects models directly on the pseudo-counts.

2.8. Data availability

The metagenomic sequences are available at NCBI's Sequence Read Archive (SRA) under the BioProject ID: PRJNA574189.

3. Results

3.1. Effect of nutrient profiles on growth of KPCE

The effect of twelve nutrient combinations (Table 1) on the growth of four KPCE species (*Citrobacter freundii, Enterobacter hormaechei, Serratia marcescens,* and *Klebsiella qausipneumoniae*) predominantly detected in the hospital sinks was tested. Across all the four KPCE, TSB that provided substantially higher protein concentration in the form of tryptone and soy, was clearly a better growth promoter followed by remodified R2A (remodR2A) and modified R2A (modR2A) (Fig. 2). TSB spiked with 0.1 μg/mL cefepime (TSB_cef), was found to be most growth promoting across the four species. TSB and TSB without dextrose (TSB_D) had growth profiles comparable to TSB with cefepime. Dextrose appeared to have no or marginal effect in promoting the growth. Attributing to relatively higher peptone concentration, remodR2A on an average was found to be a better growth promoter than modR2A. NaCl, tap water or tap water spiked with soap that comprised negligible carbon (C) and nitrogen (N), were not found to promote growth of the KPCE species tested.

3.2. Effect of nutrient profiles on biofilm formation of KPCE

Similar trend was observed with the effect of nutrient categories on biofilm formation ability of KPCE (Fig. 3A). Higher biofilm was quantified in case of TSB followed by remodified-R2A and modified-R2A. Relatively lesser biofilm was recorded in case NaCl or tap water. The average biofilm buildup (OD_{570} Day1 to Day7) was significantly higher in nutrients containing TSB compared to that in nutrients containing remodifiedR2A and modifiedR2A ($p = 0.00026$ and $p = 0.000032$ respectively). This was true in case of all the four KPCE species tested. Bacterial abundance within the biofilm was also found to be significantly higher in nutrients containing TSB ($p = 0.0002$) (Fig. 3B). In summary biofilm buildup and bacterial population in the biofilms was higher in case of carbon and nitrogen rich nutrients than without. NaCl or tap water supported relatively lower biofilm buildup and 4–5 log lesser bacterial abundance. Biofilm formation ability and cell abundance was marginally higher in presence of cefepime. Dextrose or soap had no effect on either indices.

3.3. Microbiology in the initial sink transplants

Initially 8 sink P-traps tested positive for KPCE at the time or before the harvest were transplanted into the sink lab for testing. This was done with the presumption that P-traps were the key reservoirs of KPCE and would allow for colonization of the previously unused plumbing. Once installed supplying water and soap alone (2h interval), three of the seven sinks that were KPCE positive at the time of harvest had no detectable KPCE in the P-trap water sampled the next day (Fig. 4). By Day 7, none of the P-traps had detectable KPCE in P-trap water samples screened using enriched culture method. When 25 ml TSB was added to these sinks on a daily basis by pouring over the drain with 3h dwell time before flushing; KPCE was again detected in all the P-traps within one to two days. Average time between KPCE becoming undetectable and

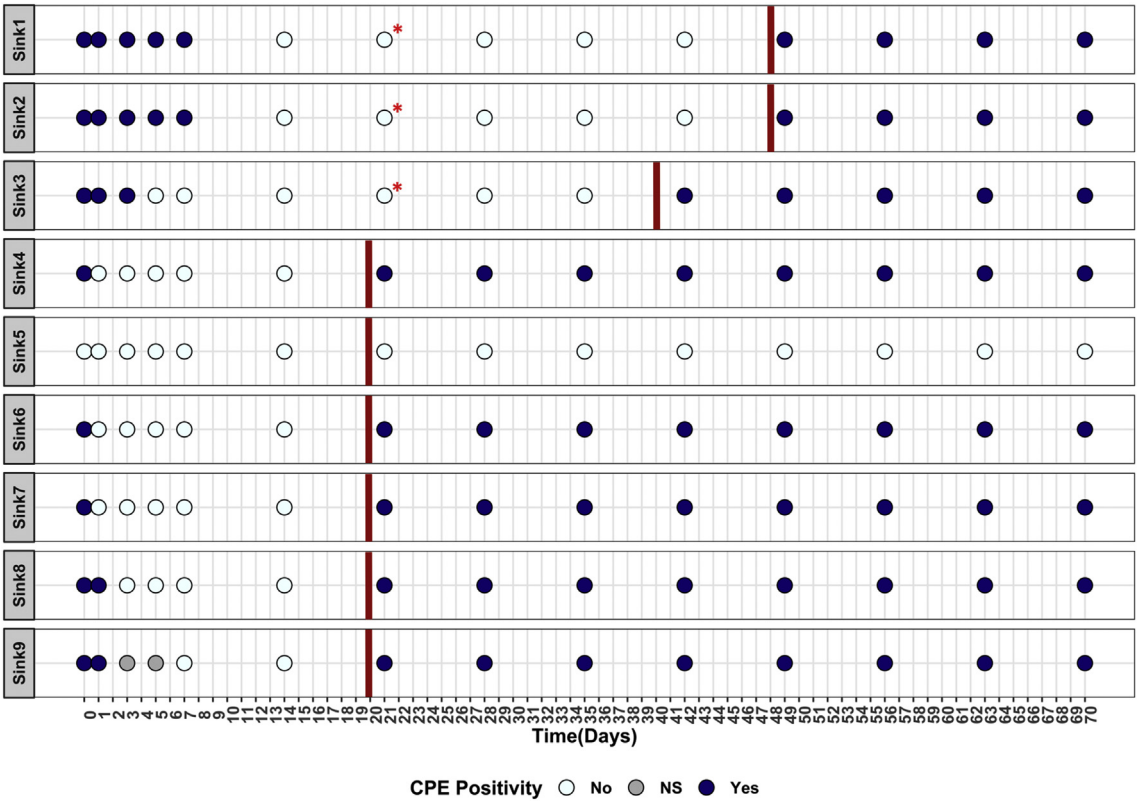

Fig. 4. CPE positivity in sink P-traps across time. –No = not detected, NS = not sampled and Yes = detected. Vertical brown bars represents day of nutrient introduction. Sink #5 was operated as a negative control (no transplant). *represents the P-trap biofilm sampling time points. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

then again detectable with nutrient exposure was 29 days (range 19–40 days). Detectable levels of KPCE in P-trap water using an enriched culture method was maintained only with the exposure to nutrients. After installation but prior to the introduction of nutrients KPCE were detected in the biofilms formed on the luminal surface of the P-traps (Fig. 4 - Day 21 Sinks#1, 2, 3) suggesting KPCE could survive in the biofilm under nutrient deficit conditions and upon exposure to nutrients KPCE was detected in the P-trap water. Five unique KPCE species (*Enterobacter* spp., *Klebsiella oxytoca, Pantoea* spp., *Citrobacter freundii, Enterobacter asburiae*-data not shown) were found to be colonized in these sinks and interestingly, all of these species showed a similar trend of revival in their populations upon exposure to nutrients. KPCE was not detected in the negative control sink at any point of time.

3.4. Effect of delayed introduction of nutrients on sink microbiology

In this phase of testing 18 sinks that were previously tested positive for KPCE were transplanted into the sink lab. Sinks were randomly grouped for varying intervals of delay in introduction of nutrients (TSB) - 0, 1, 2 and 3 months (Fig. 5). When sinks were operated with soap and water alone following the install, KPCE were not detected either at the drain or P-trap level in five of the six sinks (Fig. 5- Sink# 1, 2, 3, 5 and 6). In contrast, all 12 sinks to which nutrients (25 ml TSB every day) were supplied beginning Day 0 of the transplant, KPCE were detected for 7–28 days (Fig. 5-Group 4). In two sinks (Fig. 5-Group 2) that had KPCE below detectable limits for 3 months while operating with soap and water, KPCE were detected immediately after introduction of nutrients and were consistently detectable for 28 days. Interestingly both these sinks had *Kluyvera intermedia/Pantoea* spp. as the dominant KPCE species before transplant and after introduction of nutrients. In one sink however (Sink #7) KPCE were detectable before and after introduction of nutrients.

3.5. Effect of nutrients on sink microbiome

Streamlined with the culture data, whole shotgun metagenomic sequencing dataset from drain biofilms of six transplanted sinks - 1, 2, 3, 5, 6 and 7 was collected and analysed. Three representative time points each before (no nutrients) and after (with nutrients) nutrient introduction in each sink were considered. Fig. 6A and B shows differentially abundant taxa at family-level and genus-level between samples collected before and after the introduction of nutrients. There were no significant longitudinal changes in community richness and evenness seen across our samples (data not shown). Ordination plots of between-sample dissimilarity distances revealed community structure shifts in two of the 6 sinks (Fig. 6C- Sinks 1 and 6). Interestingly, the longitudinal dynamics of microbial relative abundances are not in concordance with the upsurge in KPCE population following introduction of nutrients as detected in the culture data. Relative abundance of Enterobacteriaceae remained relatively constant (data not shown). There was an evident decrease in antimicrobial resistance gene carriage in the sink drain biofilms before introduction of nutrients, but did not reach significance (Fig. S1). Decrease in abundance estimations of specific resistance gene clusters was seen in samples before and after nutrient introduction, with genes conferring resistance to aminoglycosides, sulfonamides and beta-lactams contributing to the overall decrease (Fig. S2).

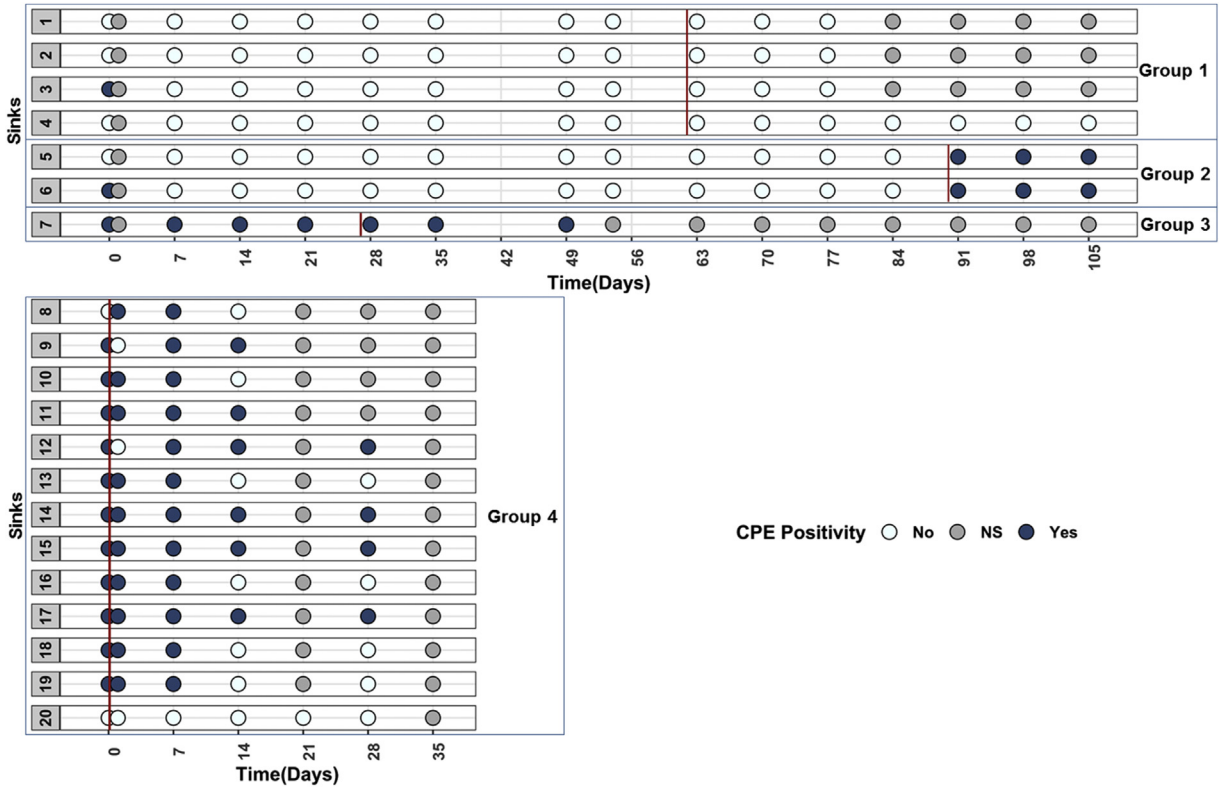

Fig. 5. KPCE positivity at the drain level in the transplanted sinks. Vertical brown bars represents day of nutrient introduction. Grouped 1 through 4 by delay in nutrient introduction (2, 3, 1 and 0 months respectively). Sink #4 and Sink #20 were operated as a negative controls (no transplant). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

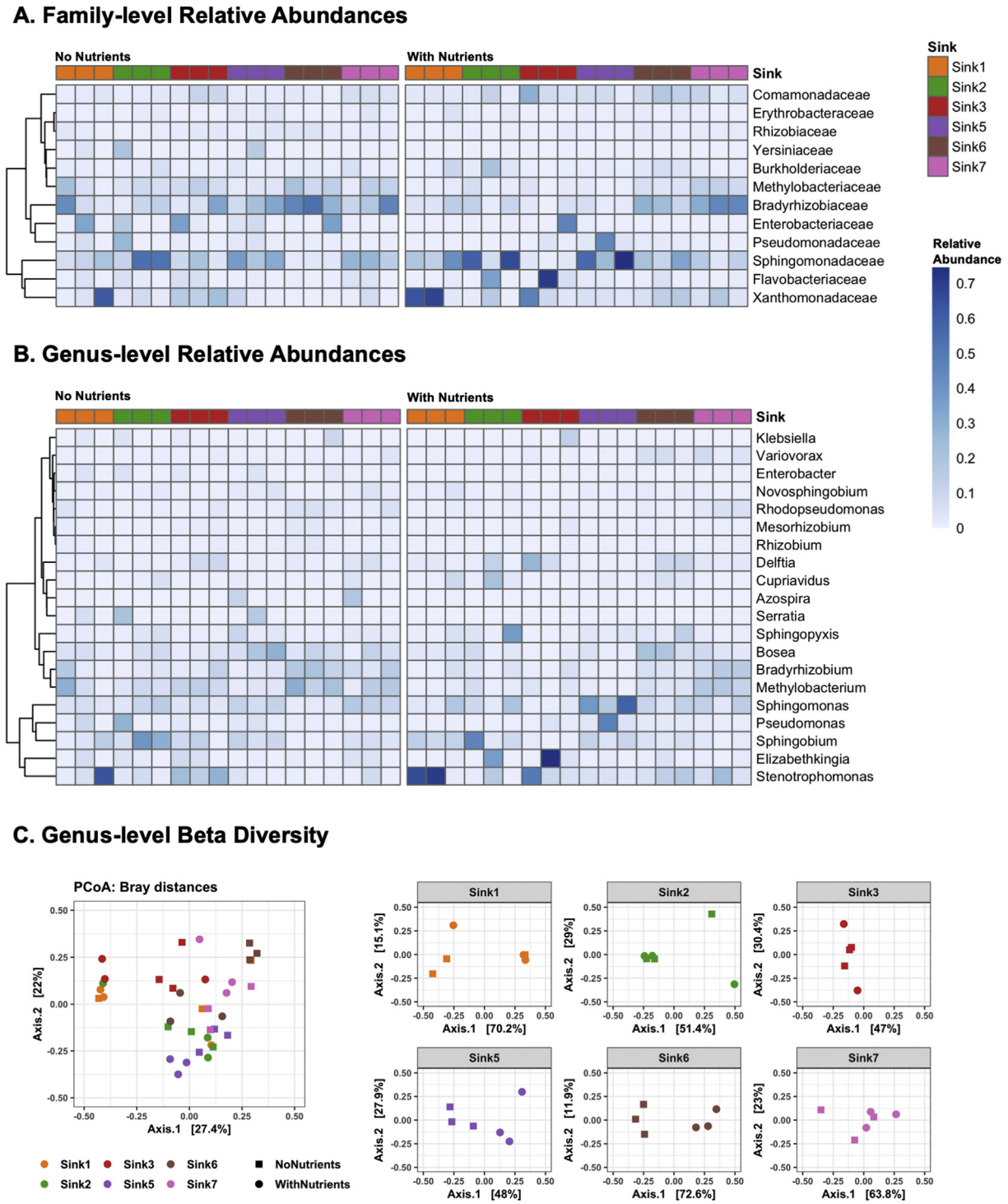

Fig. 6. Microbial community composition of sink drain biofilm. Heat map showing relative abundance of differentially abundant A) family and B) genera in sink biofilms before and after nutrient introduction, as determined by linear mixed effects model. Taxa (shown in rows) are arranged using supervised hierarchical clustering. (See Supplementary Table S1 for estimates, 95% CI, p-values and q-values for each taxa). Samples (shown in columns) collected from various sinks are grouped together, indicated using a colored annotation bar at the top. C) Principal Coordinates Analysis (PCoA) of beta-diversities between all samples, measured using bray-curtis dissimilarity index. Although no overall significant clustering was observed, ordination of dissimilarities at individual sink level suggest community compositional shifts with and without nutrients, especially Sink5 and Sink6.

4. Discussion

In the current study, we found that nutrients appear to play a critical role in KPCE abundance in real world hospital drain biofilms once transplanted into a sink lab. We took advantage of having established a BSL-2 sink lab along with the enhanced understanding of hospital behaviors which may contribute to CPE persisting in hospital drains (Grabowski et al., 2018). Common interventions including chemical or complete exchange of the premise plumbing were proven to be temporary or ineffective (Carling, 2018; Decraene et al., 2018; Gordon et al., 2017). It will be imperative that we seek sustainable solutions which decrease the

bioburden of problematic pathogens in the drain biofilm which once established are challenging to eliminate. While we demonstrate that with just soap and water the burden of KPCE decreased to undetectable levels in transplanted sinks, we do not know the threshold number of bacteria on a sink drain that are needed to cause a dispersion with a faucet event. We speculate that a decrease in KPCE would likely lead to less dissemination.

We also demonstrate that addition of nutrients results in a rapid increase in KPCE to detectable levels even after a long period of undetected KPCE. This suggests that biofilms rather than the wastewater in the P-traps act as a dormant reservoir allowing these drug-resistant bacteria to integrate and survive in a viable state. Upon exposure to nutrients the KPCE can regrow back to detectable levels plausibly as a result of detached KPCE shed from the biofilm growing under nutrient rich conditions. This would be consistent with the concept that once a drain biofilm is a reservoir it perpetually carries a greater risk for increased bacterial burden of Enterobacterales. This will require that hospital staff and patients remain vigilant about not disposing nutrient containing wastes into handwash sinks. It may also require regulations on nutrient disposal into sinks intended for hand washing should be added to guidelines. Keeping in mind the potential risk that KPCE could surge after a single exposure of nutrient solutions, new sink redesign may be necessary that take into account drain position, drainage rate, and other dynamics which promote droplet dispersion from a colonized drain (Aranega-Bou et al., 2018; Kotay et al., 2019).

Retrospectively, initial transplants in our sink lab involved only the P-traps with an incomplete understanding that P-traps were the sole source and reservoir of KPCE contamination in the sinks. The findings from first phase of transplant has redirected our focus on the biofilm as the potential reservoirs of these bacteria which can form and extended rapidly along the luminal surfaces of the plumbing and simultaneously may help them persist longer period while remaining undetectable.

Protein rich nutrient was apparently the most preferred for growth of KPCE species. TSB is a commonly used nutrient media for culturing non-fastidious bacteria and is a rich source of N and C. R2A broth is primarily used for cultivation and maintenance of heterotrophic bacteria from potable waters by providing essential growth factors required for the metabolism. Even with the variations, by supplementing additional protein (remodR2A) and/or dextrose (modR2A), it was not sufficient to enhance growth of KPCE. Rationale for the addition of 1.1 g/L dextrose was to simulate its concentration in typical dialysis solution, a commonly discarded liquid waste in ICU sinks (De Geyter et al., 2017; Leitner et al., 2015; Lowe et al., 2012; Roux et al., 2013; Smolders et al., 2018). In a recent study, residues of antibiotics were regularly detected in sink P-traps and drains at varying concentrations (Voigt et al., 2019). Cefepime is a commonly used broad-spectrum cephalosporin. We used 0.1 μg/mL cefepime which is several dilutions below the mean inhibitory concentration (MIC), allow growth and mimic potential selection pressure which may be encountered in a hospital environment. A carbapenem was not selected for this experiment because of instability in solution.

This study is the first attempt to investigate sink drain biofilms using shotgun metagenomics to understand the dynamics of bacterial community and resistance gene compositions. A closer inspection of the data did not reveal significant associations in response to nutrient introduction. In the light of our paired KPCE culture results, it indicates that addition of nutrients possibly had a larger positive impact on the growth of other bacterial groups thereby resulting in a decreased relative abundance of KPCE as well as total resistance load. We observed a clear trend in the decrease in relative abundance of Enterobacterales along with overall decrease in aggregate antimicrobial gene estimations, in samples collected from transplanted sinks with no nutrients. However, due to the zero-inflated nature of the compositional data combined with our limited sample size, the observed trends did not reach statistical significance. Further research on the community dynamics on a longer term basis would be needed to understand how KPCE responds to the changes in the sink microbiome and the implications it has on the overall resistance load.

The sink lab is an *ex situ* model which may not reflect the *in situ* dynamics of the actual premise plumbing even though we try to mimic the hospital environment as closely as possible. This is an obvious limitation of the study. Due to the concern of nutrients promoting KPCE in hospital sinks the ethics of performing the study *in situ* were prohibitive. The sample size in this study was relatively small and therefore this work should be considered in that context. Lastly, the biofilm and culture response to nutrients were based on mono-microbial selective cultures and the behavior and response to nutrients may not be the same when considered in a mixed biofilm community.

5. Conclusions

The study illuminates the role that nutrients play on the growth of CPE in real-world *ex situ* harvested hospital wastewater premise plumbing transplanted into a sink lab. Further we demonstrate that drug-resistant bacteria can persist with nutrient deficit conditions within the biofilms formed on the inside lumen of a sink P-trap and can rapidly recover, mobilize and spread via the wastewater up to sink drain or downstream. This highlights the necessary diligence on avoidance of nutrient disposal and some of the likely challenges in elimination of KPCE from wastewater premise plumbing entirely.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: AJM has conflict of interest as Consultant for Antimicrobial Resistance (AMR) Services, Inc. SMK, HIP, KEB, & JC have conflict of interest as having conducted independent research contracted by Antimicrobial Resistance Services, Inc. None of the work presented in this manuscript was funded by Antimicrobial Resistance Services, Inc. All authors contributed significantly to the work and will transfer all copyright ownership of the manuscript in the event the work is accepted for publication. The manuscript has not been previously published nor is not being considered for publication elsewhere. A portion of this work was presented at the American Society of Microbiology Annual Meeting (ASM Microbe) in New Orleans, LA in 2017 as an oral presentation.

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Appendix A. Supplementary data

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