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Pathogens and pharmaceuticals in source-separated urine in eThekwini, South Africa

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Abstract

In eThekwini, South Africa, the production of agricultural fertilizers from human urine collected from urine-diverting dry toilets is being evaluated at a municipality scale as a way to help finance a decentralized, dry sanitation system. The present study aimed to assess a range of human and environmental health hazards in source-separated urine, which was presumed to be contaminated with feces, by evaluating the presence of human pathogens, pharmaceuticals, and an antibiotic resistance gene. Composite urine samples from households enrolled in a urine collection trial were obtained from urine storage tanks installed in three regions of eThekwini. Polymerase chain reaction (PCR) assays targeted 9 viral and 10 bacterial human pathogens transmitted by the fecal-oral route. The most frequently detected viral pathogens were JC polyomavirus, rotavirus, and human adenovirus in 100%, 34% and 31% of samples, respectively. *Aeromonas* spp. and *Shigella* spp. were frequently detected gram negative bacteria, in 94% and 61% of samples, respectively. The gram positive bacterium, *Clostridium perfringens*, which is known to survive for extended times in urine, was found in 72% of samples. A screening of 41 trace organic compounds in the urine facilitated selection of 12 priority pharmaceuticals for further evaluation. The antibiotics sulfamethoxazole and trimethoprim, which are frequently prescribed as prophylaxis for HIV-positive patients, were detected in 95% and 85% of samples, reaching maximum concentrations of 6800 µg/L and 1280 µg/L, respectively. The

antiretroviral drug emtricitabine was also detected in 40% of urine samples. A sulfonamide antibiotic resistance gene (*sul1*) was detected in 100% of urine samples. By coupling analysis of pathogens and pharmaceuticals in geographically dispersed samples in eThekwini, this study reveals a range of human and environmental health hazards in urine intended for fertilizer production. Collection of urine offers the benefit of sequestering contaminants from environmental release and allows for targeted treatment of potential health hazards prior to agricultural application. The efficacy of pathogen and pharmaceutical inactivation, transformation or removal during urine nutrient recovery processes is thus briefly reviewed.

Keywords: urine nutrient recovery, health hazards, risk, sustainable sanitation

1 Introduction

Decentralized sanitation technologies that separate human urine from feces at the toilet user interface have been implemented in low-, middle- and high-income countries, though primarily at pilot scales. Source-separation of urine has the advantage over traditional toilets of sequestering nutrients primarily excreted in urine, namely nitrogen, potassium, and phosphorus, while enhancing the dehydration of separately stored feces in dry toilets. Collected urine can be used directly as a fertilizer in agriculture after a period of storage or processed into concentrated powder or liquid products. The use or sale of urine-derived fertilizers could offset the purchase of costly synthetic fertilizers or generate a source of revenue for sanitation service providers. Such a sanitation system would also reduce nutrient loads to treatment facilities or other urine discharge locations (Larsen et al., 2013).

The eThekwini Water and Sanitation (EWS) municipality in the KwaZulu-Natal province of South Africa serves the greater eThekwini region including Durban. EWS installed over 80,000 urine diverting dry toilets (UDDTs) in historically underserved rural and peri-urban areas of eThekwini, providing an ideal test case for the scale-up of urine collection and use. Currently in this system, source-separated urine is diverted to soak pits, and feces are collected in vaults for drying. EWS would like to incorporate nutrient recovery from urine into the system. The VUNA (Valorisation of Urine Nutrients in Africa) project is designing, testing and optimizing urine collection and nutrient recovery technologies to produce a commercially viable fertilizer. Pilot-scale technologies have been developed to produce a solid phosphate-based fertilizer (struvite) as well as a concentrated liquid fertilizer via biological nitrification and distillation (Udert et al., 2015). Urine-derived fertilizers, and their production processes, need to be safe with respect to environmental and public health concerns. This study thus aims to identify potential human and environmental health hazards in source-separated urine, targeting microbial and chemical contaminants. The ultimate goal is to promote the hygienic handling of urine and to design fertilizer production technologies that allow for its safe end use as a fertilizer.

The most pressing human health concerns associated with source-separated urine stem from the presence of human pathogens. While enteric pathogens are primarily excreted in feces rather than urine, the cross-contamination of source-separated urine with feces is well established (Schönning et al., 2002). Diseases transmitted by the fecal-oral route are therefore relevant in the design of urine treatment and recycling systems. Pathogen reduction can be partially achieved through storage, which allows inactivation by heat or ammonia (Decrey et al., 2015; Nordin et al., 2013). The World Health Organization (WHO) recommends storage for >6 months at >20˚C to achieve sufficient pathogen inactivation in urine intended for agricultural use for unprocessed food crops (WHO, 2006). While feasible in rural areas with ample space, this storage time can be prohibitive for urine collection at scale in dense urban environments due to storage volume requirements. Long term storage can also cause odor problems and lead to loss of recoverable nutrients by ammonia volatilization. These challenges highlight the need for alternative urine treatment technologies. Urine-derived fertilizers can be produced after short-term urine storage and provide a treatment benefit. However, implementation of this strategy necessitates investigation of the fate of locally relevant pathogens in the fertilizer production processes.

While pathogens are primarily human health hazards, the primary concerns for the presence of trace organic compounds (TrOCs) in the environment stem from their potential ecosystem health hazards (Kolpin et al., 2002). A meta-analysis of 212 pharmaceuticals revealed that an average of 64% of the consumed mass is excreted in urine (Lienert et al., 2007). The use and excretion of antiretroviral (ARV) drugs may be of particular interest in South Africa where HIV prevalence is high, particularly in the KwaZulu-Natal province (16.9% of the population in 2012; Shisana et al., 2014). South Africa has rolled out the largest ARV treatment program in the world – in 2012, 31.2% of eligible people living with human immunodeficiency virus (HIV) or Acquired Immune Deficiency Syndrome (AIDS) had been exposed to ARV treatment (Shisana et al., 2014). While the benefits of this program are clearly demonstrated through the reduction of AIDS deaths in the country, antiviral pharmaceuticals were predicted to be amongst the most hazardous therapeutic drug classes for several ecotoxicological end-points (Sanderson et al., 2004). Commonly prescribed antibacterial

compounds with high excretion rates may also be prevalent in source-separated urine. Sulfamethoxazole and trimethoprim, for example, are frequently prescribed together as a broad-spectrum prophylaxis against bacterial pathogens and protozoa for HIV-positive patients (Zachariah et al., 2007). One potential human health consequence of such widespread use of antibiotics for infectious disease therapy is the accelerated propagation of antibiotic resistance, especially in low-income countries (Okeke et al., 2005).

The objective of this study was to evaluate the presence of pathogenic organisms that are causative agents of diarrheal disease as well as pharmaceuticals in source-separated urine collected from throughout eThekwini. Based on a review of enteric pathogens present in South Africa, polymerase chain reaction (PCR) assays targeting 9 viral and 10 bacterial pathogens transmitted by the fecal-oral route were selected for a screening of human pathogens in the collected urine. An initial screening of 41 TrOCs in the urine facilitated selection of 12 priority pharmaceuticals for further quantitative evaluation. The presence of a sulfonamide antibiotic resistance gene (ARG) in bacteria from urine was also evaluated. By coupling analysis of pathogens and pharmaceuticals, this study seeks to identify a range of priority human and environmental health risk determinants in urine that is intended for fertilizer production and agricultural applications.

2 Materials and Methods

2.1 Sampling

Samples were collected at four different times between 2010 and 2013. Samples from 2010 and 2011 were used for TrOC method validation and to select priority compounds for further monitoring. These initial urine samples were collected from two UDDTs on 10 November 2010, and four composite samples consisting of urine from 29 UDDTs were obtained between 28 April and 25 May 2011. The composite samples were prepared by adding urine collected daily over one week to a 1500 L black polyethylene tank. At the end of

121 the week, the tank was stirred and sampled into triplicate 50 mL centrifuge tubes. The tank was then
122 emptied in preparation for the subsequent composite sample.
123
124 Samples collected in 2012 and 2013 were geographically distributed in the EWS service area. From
125 December 2012 to May 2013, nine urine storage tanks of 1000 to 1500 L capacity were temporarily installed
126 in three distinct regions of the EWS service area in peri-urban Durban as part of an economic study on the
127 collection of urine using incentives (Tilley, 2015). Three urine tanks were installed per region, and each tank
128 was accessible for urine deposits by approximately 90 to 150 nearby households that were provided with
129 urine collection jerry cans. Tanks received deposits of urine carried by individuals from their homes to the
130 tanks during opening hours one day per week. Urine tanks were emptied by the municipality when full. The
131 nine storage tanks were sampled on December 19, 2012 and subsequently on several days from April 12-29,
132 2013 for combined TrOC and microbiological assessments. In December, two additional urine tanks (UT)
133 located at the University of KwaZulu-Natal (UKZN) were also sampled. The study plan was approved by the
134 UKZN Biomedical Research Ethics Committee (BE147/13). Specific locations of the sampling sites are omitted
135 as stipulated in the project protocol.
136
137 **2.2 Chemical and physical urine characteristics**
138
139 The urine pH, temperature, conductivity, total ammonia and orthophosphate concentrations were measured
140 as described in the Supporting Information.
141
142 **2.3 Analytes**
143
144 **2.3.1 Bacteria**
145 The presence of bacterial pathogens was monitored by detection of bacterial DNA. Bacteria targets in this
146 study were: *Clostridium perfringens*, *Yersinia enterocolitica*, *Eschericia coli* O157:H7, verocytotoxin producing

Eschericia coli (VTEC), *Aeromonas* spp. (*A. salmonicida*, *A. sobria*, *A. bivalvium*, *A. hydrophila*), *Vibrio* spp. (*V. cholera*, *V. parahamolyticus*, and *V. vulnificus*), *Clostridium difficile* toxin B, *Salmonella* spp. (*S. bongori* and *S. enterica*), *Shigella* spp. (*S. flexneri*, *S. boydii*, *S. sonnei* and *S. dysenteriae*) and *Campylobacter* spp. (*C. jejuni* and *C. coli*). Multiplex end-point PCR was conducted using two bacterial Seegene ACE Diarrheal detection kits (BÜHLMANN Laboratories AG, Schönenbuch, Switzerland). Details of sample processing procedures are available in the Supporting Information. Each of the urine tanks were also evaluated for total viable heterotrophic bacteria (April 2013) as an indicator of the total bacteria concentrations in source-separated urine. One ml of each of three dilutions of sampled urine (at least 100-fold dilution in phosphate buffered saline, 5mM PO_4, 10mM NaCl, pH 7.4) from each urine tank was aliquoted on a 3M Petrifilm dryplate (3M, St. Paul, MN, USA) and handled as per manufacturer instructions. Plates were incubated for 48 hrs at 35˚C prior to enumeration, and results are presented as colony forming units per ml urine sampled (CFU/ml).

2.3.2 Viruses

The presence of viruses was monitored by detection of viral RNA or DNA. The viral targets were human adenovirus (HAdV), rotavirus (RoV), norovirus (NoV) GI and GII, human astrovirus (HAstV), enteroviruses (EV), hepatitis A Virus (HAV) and JC Polyomavirus (JCPyV). While not typically associated with fecal contamination, JCPyV was included herein because it has been detected consistently in wastewater worldwide (Bofill-Mas et al., 2000). Unlike the other viruses evaluated, JCPyV is excreted indefinitely in the urine of healthy individuals following infection. As such, this virus was expected to be ubiquitously present in urine, and served as an internal process control for virus extraction and detection. Bacteriophage MS2 (DSMZ 13767) was used for optimization of the nucleic acid extraction procedure and PCR method. HAdV, RoV, NoV GI and GII, and HAstV were analyzed by viral Seegene ACE Diarrheal detection kits (BÜHLMANN Laboratories AG, Schönenbuch, Switzerland), which included positive controls. EV and MS2 were analyzed by one-step reverse-transcriptase (RT)-PCR with SYBR Green using a Rotor-Gene 3000 thermocycler. HAV and

JCPyV were analyzed by RT-PCR with selective Taqman® probes. Further method details are available in the Supporting Information.

2.3.3 Trace Organics

A method initially developed to analyze pharmaceuticals in hospital wastewater (Kovalova et al., 2012) was validated on 2010 and 2011 samples. Samples were screened for a group of 41 TrOCs (see Supporting Information) using liquid chromatography tandem mass spectrometry (LC-MS/MS). A group of 12 priority compounds were selected for further monitoring in the 2012 and 2013 sampling campaign. Concentrations are reported as averages with 95% confidence intervals (CI) unless otherwise noted. Details of the analytical method as well as the process for selecting priority pharmaceutical analytes are available in the Supporting Information. Methods applied for the analysis of the sulphonamide antibiotic resistance gene *sul1* are described in the Supporting Information.

3 Results

3.1 Urine tank conditions

Characteristics of urine in the storage tanks during 2012 and 2013 sampling campaigns are presented in

Table 1. The average temperature for all urine tank samples in December 2012 was 25.9 ± 0.7 ˚C, ranging from 24.8 to 34.3˚C at the time of sampling, and the pH was 8.72 ± 0.10. The average temperature for all tank samples in April 2013 was 20.4 ± 2.0 ˚C, ranging from 15.5 to 27.1 ˚C at the time of sampling, with pH 8.90 ± 0.09. Average ammonia measured in urine tanks was 2240 ± 1180 mg/L NH_4-N (range of 400 – 8550 mg/L NH_4-N) and average phosphate was 240 ± 20 mg/l PO_4-P (range of 170-290 mg/l PO_4-P).

197 **Table 1.** Characteristics of urine in storage tanks.

Region	Sample Collection	Ammonia (mg/L NH_4-N) Avg. ± 95% CI (range, n)	Conductivity (mS/cm) Avg. ± 95% CI (range, n)	Temperature (°C)[1] Avg. ± 95% CI (range, n)	pH Avg. ± 95% CI (range, n)
1	December 2012	-	30.3 ± 3.4 (25-34.3, 6)	27.6 ± 0.3 (27.2-28.1, 6)	8.65 ± 0.06 (8.58-8.75, 6)
	April 2013	1500 ± 530 (400-2100, 6)	-	20.3 ± 0.5 (19.8-20.8, 3)	9.02 ± 0.08 (8.95-9.08, 3)
2	December 2012	-	25.7 ± 0.7 (24.8-26.8, 6)	24.5 ± 0.5 (23.7-24.9, 6)	8.88 ± 0.04 (8.81-8.9, 6)
	April 2013	2250 ± 640 (1600-2650, 3)	-	19.2 ± 3.8 (15.5-27.1, 6)	8.91 ± 0.13 (8.68-9.08, 6)
3	December 2012	-	27.5 ± 2.3 (25.5-31.3, 6)	25.6 ± 1.0 (24.1-26.5, 6)	8.63 ± 0.24 (8.26-8.90, 6)
	April 2013	3650 ± 4820 (800-8550, 3)	-	22.8 ± 1.6 (21.3-24.2, 3)	8.75 ± 0.15 (8.60-8.86, 3)

198

199 **3.2 Pathogen detection in source-separated urine**

200

201 The detection frequencies of pathogenic bacteria and viruses in the urine storage tanks during the 2012 and
202 2013 sampling campaigns are presented in Table 2. Up to six pathogens were detected in a single urine
203 sample.

204

205 **3.2.1 Bacteria occurrence**

206 The following bacteria were detected in at least two urine tank samples: *C. perfringens*, *Y. enterocolitica*,
207 *E.coli 0157:H7*, verocytotoxin-producing *E.coli* (VTEC), *Aeromonas spp*., *Vibrio spp*., and *Shigella spp*. At least
208 one diarrheal bacterium species was detected in each of the 18 samples (100%), and an average of three
209 bacteria species were detected in each sample. Two samples contained five bacteria species each, the
210 maximum number of diarrheal bacteria species detected in a single sample. Region 2 had the most total
211 detects followed by Region 3 and Region 1 (Table 2). The highest frequency detected bacteria were
212 *Aeromonas spp.* (94%); *C. perfringens* (72%); and *Shigella spp*. (61%). Three of the bacteria were not

[1] In Durban, the average low temperature is 20˚C in December and 18˚C in April; the average high temperature is 28˚C in December and 27˚C in April.

213 detected in any samples: *C. difficile*, *Salmonella spp*., and *Campylobacter spp*. Heterotrophic plate counts
214 ranged from 1×10^5 to 7×10^6 CFU/ml across all urine tanks sampled, with an average concentration of 2×10^6
215 CFU/ml.
216
217 **3.2.2 Virus occurrence**
218 The following viruses were detected in at least one urine sample from Durban: JC polyomavirus, human
219 adenovirus, rotavirus, hepatitis A virus, and norovirus GII. Three to five virus species were detected in each
220 of the sampling regions, and up to three virus species were detected in a single sample. The three most
221 frequently detected viruses were JCPyV, HAdV and RoV detected in 100%, 34% and 31% of samples,
222 respectively. In the present study, NoV GII was detected in only one sample, which was collected in April
223 2013. HAstV and NoV GI were not detected.
224
225 **3.3 Pharmaceuticals in source-separated urine**
226
227 Concentrations of pharmaceuticals from the priority list for samples taken from urine storage tanks in
228 December 2012 and April 2013 are shown by region in Table 2 and discussed below. Up to 10 of 12
229 pharmaceuticals assessed were detected in a single urine sample. Results from the 2010 and 2011 screening
230 are available in the Supporting Information.
231
232 **3.3.1 Antibacterial detection**
233 The antibacterial pharmaceuticals tested in this study were: sulfamethoxazole (SMX), trimethoprim (TMP),
234 and clarithromycin. N4-acetylsulfamethoxazole (N4-AcSMX), a human metabolite of SMX, was also tested.
235 SMX and its conjugate N4-AcSMX as well as TMP were frequently detected at high concentrations. SMX, N4-
236 AcSMX and TMP were detected in 95%, 90% and 85% of samples, respectively, with concentrations in urine
237 reaching 6800, 3500 and 1300 µg/L, respectively. In contrast, clarithromycin was detected in only 20% of
238 samples and at relatively lower concentrations in this study (17 ± 29 µg/L). The sum of SMX and N4-AcSMX

(∑SMX) concentrations varied widely from less than 2 µg/L to 8500 µg/L, with an average of 2700 ± 1100 µg/L. A weak correlation was observed between TMP concentrations and ∑SMX (R^2 = 0.34), such that when ∑SMX was found in very high concentrations, TMP was also typically measured at elevated concentrations.

3.3.2 Antiviral detection

Among the four analyzed antivirals, only emtricitabine and rintonavir were detected, in 40% and 70% of samples, respectively. The concentration of emtricitabine averaged to 100 ± 97 µg/L but was highly variable among storage tanks with a maximum concentration of more than 900 µg/L. Ritonavir concentrations were typically low, with an average concentration of 1.5 ± 0.5 µg/L and a maximum concentration of 5 µg/L.

3.3.3 Other pharmaceuticals

The anti-inflammatory drug diclofenac was detected consistently in 100% of the samples, though typically at low concentrations (30 ± 10 µg/L; max = 72 µg/L). Hydrochlorothiazide, a diuretic drug often prescribed to treat high blood pressure, measured on average 42 ± 18 µg/L and was found in 80% of samples. The beta-blocker atenolol and its metabolite atenolol acid were detected in 55% and 75% of samples, respectively, with average concentrations of 31 ± 33 and 98 ± 110 µg/L. Atenolol acid is a human metabolite of both atenolol and metoprolol, neither of which were detected in the initial 2010 and 2011 screening.

3.4 Antibiotic Resistance

The *sul1* antibiotic resistance gene was present in 100% of bacterial extracts (n = 18) and not detected in the two field blanks analyzed. A two-tailed pairwise t-test indicates that duplicate analyses of the same samples were not significantly different ($p>0.3$). Of 36 samples tested, six were above the highest standard (10^6 gc/reaction); one of these was excluded as an outlier because it was several orders of magnitude higher in concentration than replicate analyses of the sample using the same DNA extraction method (Method A, see

264 SI), as well as a different extraction method (Method B, see SI). The number of *sul1* genome copies detected

265 per mL of urine sample ranged from $1x10^{8}$ to $4x10^{10}$.

Table 2. Detection of pathogens, pharmaceuticals, and antibiotic resistance genes in source-separated urine.

	Region 1		Region 2		Region 3		UT[1]	All Samples
	Dec. '12	Apr. '13	Dec. '12	Apr. '13	Dec. '12	Apr. '13	Dec. '12	
Virus	**Detection Frequency**		**Detection Frequency**		**Detection Frequency**		**Det. Freq.**	**Freq. % (n = 29)**
JC polyomavirus	3	6	3	6	3	6	2	100%
Adenovirus	2	2	1	0	2	3	0	34%
Rotavirus	3	0	3	1	0	0	2	31%
Hepatitis A virus	0	0	0	1	0	1	0	7%
Norovirus GII	0	0	0	1	0	0	0	3%
Astrovirus	0	0	0	0	0	0	0	0%
Echovirus	0	0	0	0	0	0	0	0%
Enterovirus	0	0	0	0	0	0	0	0%
Norovirus GI	0	0	0	0	0	0	0	0%
Bacterium	**Detection Frequency**		**Detection Frequency**		**Detection Frequency**			**Freq. % (n= 18)**
Aeromonas spp.	-	5	-	6	-	6	-	94%
C. perfringens	-	2	-	6	-	5	-	72%
Shigella spp.	-	2	-	5	-	4	-	61%
Vibrio spp.	-	2	-	2	-	0	-	22%
Y. enterocolitica	-	0	-	0	-	2	-	11%
E.coli O157:H7	-	1	-	1	-	0	-	11%
VTEC	-	0	-	2	-	0	-	11%
Campylobacter spp.	-	0	-	0	-	0	-	0%
C. difficile toxin B	-	0	-	0	-	0	-	0%
Salmonella spp.	-	0	-	0	-	0	-	0%
Pharmaceutical	**Concentration Range (µg/L)**		**Concentration Range (µg/L)**		**Concentration Range (µg/L)**		**Conc. Range (µg/L)**	**Freq. % >LOQ (n=20)**
Diclofenac	3.2 – 72	20 – 62	1.6 – 45	16 – 44	13 – 56	16 – 44	27 – 30	100%
Sulfamethoxazole	<2 – 300	320–5200	800–5000	1000–6800	560–4800	1600–6400	5 – 12	95%
N4 Acetyl-SMX	<1 – 560	280 – 720	60 – 3500	9.6 – 280	8 – 520	34 – 174	<1 – 2	90%
Trimethoprim	<2 – 3.6	22 – 380	4.3 – 56	260–1300	<2 – 300	100 – 440	<2	85%
Hydrochlorothiazide	<3 – 19	52 – 94	17 – 120	18 – 64	20 – 134	52 – 94	<3	80%
Atenolol acid	4.2 – 100	<4 – 100	<4 – 11	<4	8.0–1100	20 – 98	130 – 175	75%
Ritonavir	<1 – 2.8	<1 – 1.5	<1 – 1.7	<1 – 1.4	1.9 – 4.6	<1 – 1.0	2.8	70%
Atenolol	<1 – 2.9	<1 – 300	<1	<1	1.2 – 4.3	20 – 184	26 – 29	55%
Emtricitabine	<6	<6	<6 – 920	<6 – 240	<6 – 16	<6 – 380	26 – 120	40%
Clarithromycin	<1	<1	<1 – 1.1	<1	<1	4.4 – 300	<1	20%
Atazanavir	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	0%
Darunavir	<1	<1	<1	<1	<1	<1	<1	0%
Antibiotic Resistance Gene	**Detection Frequency**		**Detection Frequency**		**Detection Frequency**			**Freq. % (n = 18)**
sul1	-	6	-	6	-	6	-	100%

[1] UT refers to the two urine collection tanks at the University of KwaZuluNatal (UKZN).

4 Discussion

4.1 Pathogens in source-separated urine and potential health risks

The present study is the first of its kind to assess a broad diversity of fecal pathogens in source-separated urine. The urine storage tanks represent a composite of urine from potentially hundreds of individuals. Any urine handled downstream of this set-up is likely to contain a mixture of pathogens originating from fecal contamination. This assumption is corroborated by our findings that demonstrate the ubiquitous presence of a wide spectrum of fecal pathogens in source-separated urine in Durban. The pathogen load and diversity will depend on the extent of fecal contamination of the urine, which in turn depends on the toilet user interface design, usage and maintenance patterns, and disposal processes, in addition to local cultural practices. For example, whether or not children use the UDDTs, especially during an active gastrointestinal infection, will influence the pathogen profile of the collected waste. The detection of rotavirus, a common infection in children, indicates that children likely use the UDDTs in eThekwini.

The results presented here are based on presence of the pathogen DNA or RNA measured by PCR, and thus do not yield data on the infectivity of detected pathogens. However, the application of PCR to identify pathogens in this study is a conservative approach for the identification of potential health hazards in urine by, for example, addressing the well-known issue of viable but non-culturable pathogens. A safe assumption for the human pathogens detected is that they are infective in fresh feces and therefore also in freshly contaminated urine. Additionally, pathogen monitoring by PCR avoids exclusion of pathogens that were inactivated at the time of sampling but may pose a health risk to individuals in contact with the material prior to inactivation. Finally, several studies have evaluated the inactivation of organisms in source-separated urine, confirming that some bacteria and viruses may remain infective in stored urine for months (Höglund et al., 1998; Vinneras et al., 2008). It is thus reasonable to assume that the detected pathogens were at least in part infective, and thus constitute a potential health risk. A health-risk assessment would

benefit from a culture-based analysis of human pathogens to assess the viable and culturable status of detected organisms relative to their total abundance at important exposure points in the urine collection and handling chain.

When considering the health risks of using source-separated urine for fertilizer, viruses are of particular relevance due to their low infective dose and resistance to inactivation in stored urine (Höglund et al., 2002a). Each of the viruses detected in this study have been identified in various human and environmental matrices in South Africa. Notably, rotavirus was the most frequently detected virus in a 2008 survey of fecal specimens from patients with gastroenteritis in the Pretoria region, followed by adenovirus and norovirus (Mans et al., 2010). Rotavirus may pose a risk in agricultural applications of urine (Höglund et al., 2002b) and continues to be a major contributor to childhood diarrheal disease in South Africa, though the benefits of a vaccine implemented in 2009 are beginning to be demonstrated (Seheri et al., 2012). In contrast to RoV, JCPyV is not expected to increase the public health concerns of urine collection and agricultural application, though it was universally detected in the samples. JCPyV already infects most people asymptomatically during childhood, though its pathogenicity emerges in HIV-infected individuals (Major et al., 1992). This consideration demonstrates the importance of considering the risk factors of each viral target, in addition to evaluating virus prevalence, to assess its relative importance to public health.

Several other viruses that are prevalent in South Africa and transmitted by the fecal-oral route were not detected in the present study. These include enterovirus (Ehlers et al., 2005) and HAstV (Mans et al., 2010; Taylor et al., 1997). The prevalence of these viruses could be low, as was the case for HAstV in South African patients with gastroenteritis (Taylor et al., 1997), yielding concentrations below the detection limit. Alternatively, limited detection could be a result of degradation of the viral genome, an inactivating process expected for single stranded (ss)RNA viruses in stored urine (Decrey et al., 2015). ssRNA viruses are less stable than DNA viruses and double-stranded (ds)RNA viruses, which are expected to retain their infectivity and genome integrity in urine over long periods of time (Decrey, 2015). Correspondingly, the three most

frequently detected viruses in this study (JCPyV, HAdV and RoV) are dsDNA or dsRNA viruses. In contrast, even ssRNA viruses with high seroprevalence in South Africa, including HAV and NoV GII (Mans et al., 2010), were detected less frequently. Their limited detection in the present study may be indicative of genome degradation during urine storage.

Similar to viral pathogens, the bacterial pathogen targets assessed in this study have each been detected in various human and environmental matrices collected in South Africa. For example, *Shigella* spp. was commonly identified in drinking water consumed by individuals with HIV/AIDS in rural households (Samie et al., 2012) and may contribute to the chronic diarrhea commonly experienced by HIV/AIDS patients (Obi and Bessong, 2002). The etiological agent of cholera, *Vibrio cholerae*, which was also detected in our samples, has been regarded as endemic in South Africa and is a common cause of illness and death in the country (Momba et al., 2010). While not detected in this study, *Campylobacter* spp. and *Salmonella* spp., along with other diarrhea-causing bacterial pathogens, are known to circulate in South Africa (Obi and Bessong, 2002). These results illustrate that while country-specific prevalence studies are useful to identify a range of potential pathogen hazards, the local relevance of specific pathogens varies greatly.

In addition to yielding an overview of the health status of the sample population at the time of measurement, the results indicate potentially problematic levels of fecal contamination in terms of health risk to humans during downstream handling and use of the urine. The actual health risk to humans from this diversity of pathogens will depend on the influent composition, pathogen inactivation rates as well as human exposure routes during urine handling and processing, which requires further evaluation. JCPyV, which is excreted in urine, may be useful as an indicator for tracking the fate of pathogens in source-separated urine or as a treatment process indicator for viruses because of its consistent detection. JCPyV has been proposed as a sewage indicator organism due to its high excretion titer, frequent detection in urban sewage, stability, and absence of animal host (Bofill-Mas et al., 2000).

346 **4.2 Pharmaceutical excretion in urine and potential health risks**

347 Little public information is available concerning specific pharmaceutical usage in South Africa, rendering
348 estimation of consumption, and excreted concentrations, difficult. Therefore, average measured urine
349 concentrations in South Africa were compared to predicted urine concentrations using pharmaceutical
350 consumption data from the USA, France, Germany and Switzerland in order to identify outliers (Table 3). For
351 example, low to no detection of the antibacterial clarithromycin indicates limited prescription in the study
352 region, contrary to high-income countries (Michael et al., 2013). Similarly, concentrations of the diuretic,
353 hydrochlorothiazide, were relatively low in eThekwini. However, average measured concentrations of SMX
354 and TMP in urine were elevated as compared to predicted concentrations. Concentrations of SMX in
355 particular were markedly high, more than an order of magnitude above the range predicted.
356
357 In addition to being widely used for the treatment of bacterial infections, SMX and TMP are frequently
358 prescribed as prophylaxis to prevent opportunistic infections in patients with compromised immune systems
359 (e.g., for HIV-positive individuals) (Madhi et al., 2002). In a preventative approach, patients receive such
360 pharmaceuticals regularly over an extended period of time, in contrast to targeted antibiotic treatment for
361 acute infections. If 4 – 11% of all HIV infected persons in the KwaZulu-Natal province were taking SMX
362 prophylactic (see SI for estimation), this could explain the observed concentrations of SMX. Therefore, the
363 surprisingly high SMX concentrations detected are reasonable considering the high prevalence of HIV-
364 positive people in eThekwini. Additionally, SMX and TMP prescriptions are often administered together as
365 co-trimoxazole in a 5:1 ratio. Because N4-AcSMX is a metabolite of SMX produced in the human body and
366 deconjugates to SMX under environmental conditions (Göbel et al., 2005), the sum of SMX and N4-AcSMX
367 was expected to correlate with TMP. Correspondingly, TMP was usually found in high concentrations when
368 ∑SMX also occurred in high concentrations, though this prescription ratio was not preserved in the samples.
369
370 In addition to widespread use of antibacterial pharmaceuticals in South Africa, nationally, just over 2.0 of 6.4
371 million estimated people living with HIV in 2012 received anti-retroviral treatment (Shisana et al., 2014). Of

four ARV pharmaceuticals tested, emtricitabine and ritonavir were detected. Target antivirals detected at low concentrations or not detected in storage tanks may reflect the low consumption and/or low excretion rates of those compounds. Emtricitabine exhibits a urine excretion rate of 75%, whereas less than 8% of consumed atazanavir, darunavir, and ritonavir are excreted in urine (Table 3). Therefore, low concentrations or lack of detection of antivirals other than emtricitabine in the urine is not necessarily indicative of their low consumption. The presence of feces in urine may also have contributed to the observed ritanovir, as 41% of the consumed drug is excreted in feces. Antivirals not evaluated in this study, such as those recommended for public sector ARV treatment in South Africa (WHO, 2005), may also be present in the urine from eThekwini.

393 **Table 3.** Predicted and measured pharmaceutical concentrations in 2012 and 2013 urine samples, and

394 predicted environmental concentrations in soil following a single application of urine as a fertilizer.

Compounds	Usage	Excretion rate[%][1]	Predicted concentration in urine (µg/L)[2]		Measured concentration in urine (µg/L) (Avg ± 95% CI; n = 20)	PEC_{soil} (µg/kg)[3]	
			min	max		min	max
Atazanavir	Antiviral	6	1	-	<2.5	0	0.3
Atenolol	Beta-blocker	37	74	246	31 ± 33	0	7.5
Atenolol acid	(Human) Metabolite of atenolol and metoprolol	ND[4]	ND	ND	98 ± 110	0	25
Clarithromycin	Macrolide antibacterial	25	99	137	17 ± 29	0	5.4
Darunavir	Antiviral	7.7	2	-	<1	0	0.1
Diclofenac	Analgestic: antiinflammaotry / antirheumatic	1	0.7	25	30 ± 10	0.2	4.7
Emtricitabine	Antiviral	75	20	-	101 ± 97	0	23
Hydrochlorothiazide	Diuretic	82	526	1118	42 ± 18	0.2	7.1
N^4Acetyl-sulfamethoxazole	(Human) Metabolite of sulfamethoxazole	50	303	468	360 ± 340	0.2	82
Ritonavir	Antiviral	3.5	0.9	2.1	1.6 ± 0.5	0	0.2
Sulfamethoxazole	Sulfonamide antibacterial	20	121	187	2300 ± 1000	11	390
Trimethoprim	Antibacterial	60	2	120	190 ± 140	0.4	39

395

396 One consequence of the increased prescription and consumption of antibiotics is the increased prevalence

397 of antibiotic resistance determinants. For example, a diversity of multidrug resistant diarrheal bacteria,

[1] Excretion rates from Swiss Compendium of Medicines by Documed: www.compendium.ch, last accessed March 10, 2015.

[2] Predicted minimum and maximum concentrations in urine calculated based on consumption in four countries (CH, DE, FR, USA); see Supporting Information Table S4.

[3] Minimum and maximum predicted environmental concentrations in soil (PEC_{soil}) calculated based on measured urine concentrations for a single application of urine as fertilizer for agriculture; see Supporting Information.

[4] ND = Not Determined.

notably including *Shigella* spp., were isolated from household water stored for use by HIV-positive patients in Limpopo province (Samie et al., 2012). In the present study, the sulfonamide antibiotic resistance gene, *sul1*, was detected in 100% of urine samples. While the *sul1* resistance gene is known to be widespread in the environment (e.g., (Czekalski et al., 2014)) and is not broadly representative of other antibiotic resistance determinants, it is typically detected at lower rates in urine samples than observed in the present study. For example, in a 16-country study of urine collected from patients with urinary tract infections, *sul1* was detected in 31% of 350 uropathogenic *E.coli* isolates (as compared to the sulfonamide resistance gene *sul2* in 48% of isolates). Co-trimoxazole is a common prescription for this infection (Blahna et al., 2006). Further, the concentration of *sul1* gene copies was high, from 10^8 to 10^{10} gc/ml, compared to less than 10^7 gc/ml in hospital and municipal wastewater effluents evaluated in Switzerland (Czekalski et al., 2012), while heterotrophic bacteria concentrations were similar (~10^6 CFU/ml) in the source-separated urine to the levels in wastewater evaluated by Czekalski et al. (2012). The ubiquitous detection of *sul1* in the present study thus encourages further evaluation of the prevalence and health risks associated with other clinically relevant ARGs in source-separated urine, and especially those harbored within human pathogens. Despite the potential health challenges from the spread of antibiotic resistance, HIV-infected African adults receiving co-trimoxazole currently still garner consistent benefits in survival even in areas where co-trimoxazole resistance is common (Zachariah et al., 2007).

Beyond the human health concerns associated with antibiotic resistance determinants, pharmaceutical usage and environmental release pose potential ecotoxicological risks. Urine discharged from UDDTs or urine collected and applied directly as fertilizer may contribute to such risks. However, recommendations regarding environmentally safe concentrations of pharmaceuticals are limited, especially for soil applications of human-waste derived fertilizers (e.g., wastewater sludge, manure, or urine). Predicted no effect concentrations in soil ($PNEC_{soil}$) were previously estimated for SMX, TMP, and diclofenac using aquatic PNECs and compound physiochemical properties (Martín et al., 2012). For comparison to these values, we estimated a range of predicted environmental concentrations (PEC_{soil}) and risk quotients (RQ = PEC_{soil}/

424 $PNEC_{soil}$) based on urine application as fertilizer in agriculture (Table 3 and Table S5). The estimated soil
425 concentrations were based on a one-time direct urine application to achieve 150 kg N/ha (see SI for
426 calculation), assuming no degradation of the target compound in the soil. While the RQs for TMP and
427 diclofenac (RQ<<1) indicated no significant environmental risk, the RQ for SMX for a range of scenarios
428 evaluated (RQ>1) suggests a potential ecotoxicological risk from this compound due to its high concentration
429 in urine and relatively low $PNEC_{soil}$. The actual SMX concentration in soil, and associated risk quotients,
430 would depend on urine and water application rates, the soil characteristics, the degradation of SMX in soil,
431 and measured $PNEC_{soil}$ values. However, the results presented herein suggest that further detailed study on
432 SMX ecotoxicity in these scenarios is warranted.
433
434 The breadth, high frequency and sometimes high concentrations of pharmaceuticals detected in source-
435 separated urine in this study highlight the importance of evaluating pharmaceutical fate during urine
436 collection and fertilizer production. Sequestering these compounds via urine diversion and collection
437 prevents their release into the environment and provides an opportunity to remove or transform the
438 compounds via urine treatment.
439
440 **4.3 Fate of pathogens and pharmaceuticals in treatment processes for urine-derived fertilizer production**

441 Due to the widespread detection of pathogenic viruses and bacteria as well as high concentrations of several
442 pharmaceuticals in source-separated urine in eThekwini, thorough consideration of the downstream urine
443 treatment processes is imperative for the protection of individuals handling stored urine and for its use as an
444 agricultural fertilizer. In eThekwini, the primary urine treatment and fertilizer production technologies under
445 development for potential scale up are: (1) urine storage for pathogen inactivation; (2) struvite fertilizer
446 production for phosphorus recovery; and (3) urine nitrification for ammonia stabilization followed by
447 distillation to recover all nutrients in a concentrated liquid product (Udert et al., 2015). In each of these
448 technologies, processes promoting the degradation, inactivation, or removal of pathogens and TrOCs may
449 occur. Below, available data from literature is interpreted in the context of eThekwini to evaluate the

450 treatment benefits of nutrient recovery processes in reducing environmental loads of human and
451 environmental health risk determinants from the application of source-separated urine, or fertilizers
452 produced from that urine, at scale.
453
454 **4.3.1 Urine storage**
455 The efficiency of pathogen indicator organism inactivation during the storage of urine under a range of
456 conditions has been extensively evaluated (Höglund et al., 2002a; Nordin et al., 2013; Vinneras et al., 2008).
457 Uncharged ammonia (NH_3), a volatile compound produced during biological urea hydrolysis, acts as an *in situ*
458 sanitizer in stored urine (Decrey et al., 2015; Vinneras et al., 2008). Measured ammonia concentrations in
459 this study were similar to those reported in urine storage tanks in Sweden (Höglund et al., 2000). To
460 maximize its biocidal effect, NH_3 losses from stored urine should be minimized. Therefore, dilution of urine
461 or the use of unsealed tanks or aeration during pumping of urine to transport facilities, which could lead to
462 NH_3 volatilization, should be limited. Ammonia losses should also be avoided to limit occupational inhalation
463 or dermal exposure to ammonia, by which deleterious health effects may be experienced (ATSDR, 2004), as
464 well as potential detrimental environmental effects (Galloway and Cowling, 2002).
465
466 Besides NH_3, high storage temperature is conducive to pathogen inactivation in urine. Measured urine tank
467 temperatures depend on the external temperature, sun exposure, tank color, and time of day that samples
468 were taken. Fluctuating temperatures within urine storage tanks due to diurnal heating from the sun may
469 further enhance inactivation (Nordin et al., 2013). Durban is characterized by a mild subtropical climate, with
470 average maximum temperatures of 28 or 29˚C occurring between January and March and 23 or 24˚C in
471 winters from June to August.[1] Given these high temperatures year-round, urine storage in Durban may
472 achieve significant inactivation of a range of important pathogens. Specifically, of the most frequently
473 detected bacteria in the present study, the gram negative bacteria *Aeromonas spp.* and *Shigella spp*. are

[1] Durban Yearly Weather Summary : http://www.worldweatheronline.com/Durban-weather-averages/Kwazulu-Natal/ZA.aspx, accessed January 28, 2014.

474 expected to be inactivated rapidly in undiluted, stored urine with a temperature above 20˚C. *Aeromonas*
475 *hydrophila*, for example, loses greater than 90% of infectivity per day in urine at 20˚C (Höglund et al., 1998).
476 This is commonly observed for other gram negative bacterial pathogens and fecal indicator organisms. *C.*
477 *perfringens*, in contrast, is likely to remain infective in stored urine for extended periods of time (Höglund
478 and Stenström, 1999).
479
480 Viruses in stored urine exhibit a wide range of inactivation rates that are typically slower than those of gram
481 negative bacteria. As discussed above, dsDNA viruses are the most persistent in urine at urine storage
482 temperatures expected in Durban (Decrey, 2015). The dsDNA viruses most frequently detected in this study,
483 JCPyV and HAdV, are thus likely to remain infective in undiluted or diluted stored urine for several weeks or
484 longer. In contrast, ssRNA viruses such as HAV and NoV are expected to inactivate more rapidly than dsRNA
485 or dsDNA viruses due to susceptibility of the genome to degradation.
486
487 While the efficiency of pathogen and surrogate inactivation during the storage of urine under a range of
488 conditions has been extensively evaluated, data is lacking regarding the behavior of pharmaceuticals during
489 urine storage. Forthcoming results (Özel Duygan et al., 2015) indicate little benefit of urine storage for the
490 transformation of pharmaceuticals detected in eThekwini, as 11 out of 12 compounds were very stable; only
491 hydrochlorothiazide was degraded substantially. Nevertheless, source-separation coupled with urine
492 collection has the potential to sequester excreted drugs and reduce the diffuse discharge of pharmaceuticals
493 to the environment.
494
495 **4.3.2 Struvite fertilizer production and drying**
496 Struvite ($MgNH_4PO_4 \cdot 6H_2O$) can be precipitated from stored urine by adding a magnesium source such as
497 MgO, $MgCl_2$ or bittern (Etter et al., 2011). The process is completed by filtration and drying of the struvite
498 precipitate, which can be applied directly as a fertilizer.
499

In the production of struvite, heterotrophic bacteria in urine accumulate in the solid during struvite precipitation and filtration and are inactivated during drying with decreasing struvite moisture content (Schoger, 2011). However, concentrations of the *in situ* bacteria in struvite may stabilize under mild drying conditions (e.g., 20˚C, 80% relative humidity). The inactivation kinetics of pathogenic bacteria during struvite drying has not been considered specifically, though achieving only partial inactivation could be problematic for attaining high fertilizer end-product quality. Increased desiccation of struvite at elevated temperatures would likely mitigate this challenge, though struvite degrades rapidly above 55˚C due to ammonia loss (Bhuiyan et al., 2008). Thorough dessication of struvite at temperatures above ambient conditions is also important to inactivate more resistant pathogens (e.g., helminths), which are also retained in the precipitate during filtration (Decrey et al., 2011). In contrast to bacteria, viruses present in the urine are retained in struvite pore spaces but are not preferentially accumulated (Decrey et al., 2011). Virus inactivation was also found to increase linearly with decreasing moisture content of the struvite.

Similar to viruses, pharmaceuticals in struvite were contained in the residual urine before struvite drying. For seven pharmaceuticals evaluated, an average of 98% of the analytes remained in urine following struvite precipitation (Ronteltap et al., 2007). Washing struvite with water prior to drying was found to further reduce pharmaceutical concentrations in the end-product by removing the residual urine. Such a procedure would likely reduce pathogen concentrations in struvite as well.

With proper washing and drying procedures, the production of struvite can thus promote the inactivation or removal of bacterial and viral pathogens in fertilizers produced from source-separated urine and reduce end-product pharmaceutical concentrations. These procedures facilitate the production of urine-based fertilizers with shortened urine storage times. However, pathogens and pharmaceuticals remaining in the urine after struvite precipitation are not treated during this procedure and remain a potential human and environmental health hazard. Longer-term field scale assessments as well as analysis of additional pathogens

and TrOC targets are also required in order to fully assess the fate and transport of contaminants during struvite production.

4.3.3 Urine nitrification for nutrient stabilization

An alternative to struvite production for urine nutrient recovery is the application of biological nitrification to stabilize nutrients in urine (Udert et al., 2003). Recent evaluation of the inactivation of bacterial and viral surrogates during urine nitrification indicates that nitrification is insufficient as a stand-alone technology for pathogen inactivation (Bischel et al., 2015). A relatively short nitrification reactor hydraulic retention time would likely yield only 90% or less inactivation. Viruses are likely to remain infective through nitrification treatment. However, because volatile ammonia is converted to nitrate during nitrification, the nitrified urine product can be distilled to yield a concentrated fertilizer and a pathogen treatment benefit. In a pilot reactor (Udert et al. 2015), nitrified urine is distilled for several hours at 80°C. This is a harsher treatment than recommended for sanitization of compost (70°C for 30 min; US EPA, 2003), and can be expected to yield a microbially safe product.

Of the 12 pharmaceuticals studied herein, only four were found to degrade substantially during urine nitrification (Özel Duygan et al., 2015). This is in accordance to the behavior of pharmaceuticals in municipal wastewater treatment, as many are still detected in considerable concentrations after wastewater treatment (Michael et al., 2013).

Nitrification of urine alone thus does not provide a significant treatment benefit for pathogens and pharmaceuticals in urine. However, coupling biological nutrient stabilization with the post-nitrification steps of distillation and advanced treatment can improve the quality of the liquid fertilizer by inactivating pathogens and removing pharmaceuticals, respectively. Several treatment processes have been successfully tested for the removal of pharmaceuticals from stored urine: ozonation (Dodd et al., 2008), electrodialysis (Pronk et al., 2006), and nanofiltration (Pronk et al., 2006). Treatment with activated carbon was shown to

be an effective process for pharmaceutical removal from nitrified urine (Özel Duygan et al., 2015). Nitrification with distillation provides the added benefit over struvite production of complete nutrient recovery for enhanced fertilizer product quality.

4.4 Conclusion

UDDTs provide increased access to sanitation, especially when coupled with resource recovery and reuse, which further creates a financial driver for the sanitation system (Diener et al., 2014). With a large network of dry, urine-diverting toilets in place, the opportunity is ripe in eThekwini to move up the "sanitation ladder" (Mara et al., 2010) by eliminating the practice of urine discharge via soak-away pits. The presence of pathogens, pharmaceuticals and antibiotic resistance determinants in the source-separated urine reveals an important opportunity to reduce discharge of these contaminants to the environment via urine collection. However, the implications of such human and environmental health hazards also require further evaluation. In particular, the results may be contextualized in eThekwini via a quantitative microbial risk assessment of local urine collection practices, when infective pathogen concentrations are expected to be highest and opportunities for accidental human-urine contact are likely greatest.

Beyond urine collection, urine nutrient recovery processes must also yield high-quality products that are protective of human and environmental health and that ensure adequate fertilizer quality for optimal market value. The high content of such unwanted substances in the fertilizer source material may impact the quality, or perceived quality, of end-products. Therefore, this study also highlights how potential environmental and human health concerns could be reduced by incorporating a urine treatment step. Processes to produce urine-derived fertilizers can be designed or modified to inactivate pathogens and transform or remove pharmaceuticals in source-separated urine. The production of the solid fertilizer, struvite, can inactivate pathogens and remove pharmaceuticals from the end-product when implemented with proper washing and drying procedures. Biological nitrification for urine nutrient stabilization can be

coupled with the post-nitrification steps of distillation and advanced treatment to improve the quality of the liquid fertilizer in terms of pathogen and pharmaceutical concentrations.

Further evaluation and optimization of such urine treatment and fertilizer production technologies at field-scales is needed to reduce occupational hazards to staff involved in urine processing and minimize environmental risks associated with urine management. Nevertheless the prevention of pathogen and pharmaceutical discharge to the environment via urine source separation and collection is a considerable advantage to this sanitation technology.

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References

ATSDR, 2004. Toxicological profile for ammonia. Atlanta, Georgia.

Bhuiyan, M.I.H., Mavinic, D.S., Koch, F.A., 2008. Thermal decomposition of struvite and its phase transition. Chemosphere 70, 1347–1356.

Bischel, H.N., Schertenleib, A., Fumasoli, A., Udert, K.M., Kohn, T., 2015. Inactivation kinetics and mechanisms of viral and bacterial pathogen surrogates during urine nitrification. Environ. Sci. Water Res. Technol. 1, 65–76.

Blahna, M.T., Zalewski, C.A., Reuer, J., Kahlmeter, G., Foxman, B., Marrs, C.F., 2006. The role of horizontal gene transfer in the spread of trimethoprim-sulfamethoxazole resistance among uropathogenic Escherichia coli in Europe and Canada. J. Antimicrob. Chemother. 57, 666–72.

Bofill-Mas, S., Pina, S., Girones, R., Bofill-mas, L., 2000. Documenting the epidemiologic patterns of polyomaviruses in human populations by studying their presence in urban sewage. Appl. Environ. Microbiol. 66, 238–245.

Czekalski, N., Berthold, T., Caucci, S., Egli, A., Bürgmann, H., 2012. Increased levels of multiresistant bacteria and resistance genes after wastewater treatment and their dissemination into lake geneva, Switzerland. Front. Microbiol. 3, 106.

Czekalski, N., Gascón Díez, E., Bürgmann, H., 2014. Wastewater as a point source of antibiotic-resistance genes in the sediment of a freshwater lake. ISME J. 8, 1381–90.

Decrey, L., 2015. Virus inactivation in human excreta and animal manure. PhD Thesis, EPFL.

Decrey, L., Kazama, S., Udert, K.M., Kohn, T., 2015. Ammonia as an in-situ sanitizer: inactivation kinetics and mechanisms of the ssRNA virus MS2 by NH3. Environ. Sci. Technol. 49, 1060–1067.

Decrey, L., Udert, K.M., Tilley, E., Pecson, B.M., Kohn, T., 2011. Fate of the pathogen indicators phage ΦX174 and Ascaris suum eggs during the production of struvite fertilizer from source-separated urine. Water Res. 45, 4960–4972.

Diener, S., Semiyaga, S., Niwagaba, C.B., Muspratt, A.M., Gning, J.B., Mbéguéré, M., Ennin, J.E., Zurbrugg, C., Strande, L., 2014. A value proposition: Resource recovery from faecal sludge—Can it be the driver for improved sanitation? Resour. Conserv. Recycl. 88, 32–38.

Dodd, M.C., Zuleeg, S., Gunten, U., Pronk, W., 2008. Ozonation of source-separated urine for resource recovery and waste minimization: Process modeling, reaction chemistry, and operational considerations. Environ. Sci. Technol. 42, 9329–9337.

Ehlers, M.M., Grabow, W.O.K., Pavlov, D.N., 2005. Detection of enteroviruses in untreated and treated drinking water supplies in South Africa. Water Res. 39, 2253–8.

Galloway, J.N., Cowling, E.B., 2002. Reactive Nitrogen and The World : 200 Years of Change. Ambio 31, 64–71.

Göbel, A., Thomsen, A., McArdell, C.S., Joss, A., Giger, W., 2005. Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. Environ. Sci. Technol. 39, 3981–9.

Höglund, C., Ashbolt, N., Stenström, T.A., Svensson, L., 2002a. Viral persistence in source-separated human urine. Adv. Environ. Res. 6, 265–275.

Höglund, C., Stenström, T.A., Ashbolt, N., 2002b. Microbial risk assessment of source-separated urine used in agriculture. Waste Manag. Res. 20, 150–161.

Höglund, C., Stenström, T.A., Jönsson, H., Sundin, A., 1998. Evaluation of faecal contamination and microbial die-off in urine separating sewage systems. Water Sci. Technol. 38, 17–25.

Höglund, C., Vinnerås, B., Stenström, T. a., Jönsson, H., 2000. Variation of chemical and microbial parameters in collection and storage tanks for source separated human urine. J. Environ. Sci. Heal. Part A 35, 1463–1475.

Höglund, C.E., Stenström, T.A.B., 1999. Survival of Cryptosporidium parvum oocysts in source separated human urine. Can. J. Microbiol. 45, 740–746.

Kolpin, D., Furlong, E., Zaugg, S., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. Streams, 1999-2000 : A national reconnaissance 1999–2000.

Kovalova, L., Siegrist, H., Singer, H., Wittmer, A., McArdell, C.S., 2012. Hospital wastewater treatment by membrane bioreactor: performance and efficiency for organic micropollutant elimination. Environ. Sci. Technol. 46, 1536–45.

Larsen, T.A., Udert, K.M., Lienert, J., 2013. Source separation and decentralization for wastewater management. IWA Publishing, London, UK.

Lienert, J., Bürki, T., Escher, B.I., 2007. Reducing micropollutants with source control: substance flow analysis of 212 pharmaceuticals in faeces and urine. Water Sci. Technol. a J. Int. Assoc. Water Pollut. Res. 56, 87.

Madhi, S. a, Cutland, C., Ismail, K., O'Reilly, C., Mancha, A., Klugman, K.P., 2002. Ineffectiveness of trimethoprim-sulfamethoxazole prophylaxis and the importance of bacterial and viral coinfections in African children with Pneumocystis carinii pneumonia. Clin. Infect. Dis. 35, 1120–6.

Major, E.O., Amemiya, K., Tornatore, C.S., Houff, S.A., Berger, J.R., 1992. Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clin. Microbiol. Rev. 5, 49–73.

Mans, J., De Villiers, J.C., Du Plessis, N.M., Avenant, T., Taylor, M.B., 2010. Emerging norovirus GII. 4 2008 variant detected in hospitalised paediatric patients in South Africa. J. Clin. Virol. 49, 258–264.

Mara, D., Lane, J., Scott, B., Trouba, D., 2010. Sanitation and health. PLoS Med. 7, e1000363.

Martín, J., Camacho-Muñoz, D., Santos, J.L., Aparicio, I., Alonso, E., 2012. Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: removal and ecotoxicological impact of wastewater discharges and sludge disposal. J. Hazard. Mater. 239-240, 40–7.

Michael, I., Rizzo, L., McArdell, C.S., Manaia, C.M., Merlin, C., Schwartz, T., Dagot, C., Fatta-Kassinos, D., 2013. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review. Water Res. 47, 957–95.

Momba, M.N.B., Madoroba, E., Obi, C.L., 2010. Apparent impact of enteric pathogens in drinking water and implications for the relentless saga of HIV/AIDS in South Africa, in: Méndez-Vilas, A. (Ed.), Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. Formatex, pp. 615–625.

Nordin, A., Niwagaba, C., Jönsson, H., Vinnerås, B., 2013. Pathogen and indicator inactivation in source-separated human urine heated by the sun. J. Water, Sanit. Hyg. Dev. 3, 181.

Obi, C.L., Bessong, P.O., 2002. Diarrhoeagenic Bacterial Pathogens in HIV-positive Patients with Diarrhoea in Rural Communities of Limpopo Province , South Africa. J. Heal. Popul. Nutr. 20, 230–234.

Okeke, I.N., Laxminarayan, R., Bhutta, Z. a, Duse, A.G., Jenkins, P., O'Brien, T.F., Pablos-Mendez, A., Klugman, K.P., 2005. Antimicrobial resistance in developing countries. Part I: recent trends and current status. Lancet. Infect. Dis. 5, 481–93.

Özel Duygan, B.D., Udert, K.M., Remmele, A., Strande, L., McArdell, C.S., 2015. Fate of pharmaceuticals during storage, biological and powdered activated carbon treatment of source-separated urine. Prep.

Pronk, W., Biebow, M., Boller, M., 2006a. Electrodialysis for recovering salts from a urine solution containing micropollutants. Environ. Sci. Technol. 40, 2414–2420.

Pronk, W., Palmquist, H., Biebow, M., Boller, M., 2006b. Nanofiltration for the separation of pharmaceuticals from nutrients in source-separated urine. Water Res. 40, 1405–1412.

Ronteltap, M., Maurer, M., Gujer, W., 2007. The behaviour of pharmaceuticals and heavy metals during struvite precipitation in urine. Water Res. 41, 1859–1868.

Samie, A., Mashao, M.B., Bessong, P.O., Nkgau, T.F., Momba, M.N.B., Obi, C.L., 2012. Diversity and antibiograms of bacterial organisms isolated from samples of household drinking-water consumed by HIV-positive individuals in rural settings, South Africa. J. Heal. Popul. Nutr. 30, 241–249.

Sanderson, H., Johnson, D.J., Reitsma, T., Brain, R. a, Wilson, C.J., Solomon, K.R., 2004. Ranking and prioritization of environmental risks of pharmaceuticals in surface waters. Regul. Toxicol. Pharmacol. 39, 158–83.

Schoger, M., 2011. Bacterial inactivation in struvite recovered from urine in South Africa. Master Thesis, EPFL.

Schönning, C., Leeming, R., Stenström, T.A., 2002. Faecal contamination of source-separated human urine based on the content of faecal sterols. Water Res. 36, 1965–1972.

Seheri, L.M., Page, N. a, Mawela, M.P.B., Mphahlele, M.J., Steele, a D., 2012. Rotavirus vaccination within the South African Expanded Programme on Immunisation. Vaccine 30 Suppl 3, C14–20.

Shisana, O., Rehle, T., Simbayi, L., Zuma, K., Jooste, S., Zunga, N., Labadarios, D., Onoya, D., Al., E., 2014. South African national HIV prevalence, incidence and behaviour survey, 2012. HSRC Press, Cape Town.

Taylor, M.B., Marx, F.E., Grabow, W.O.K., 1997. Rotavirus, astrovirus and adenovirus associated with an outbreak of gastroenteritis in a South African child care centre. Epidemiol. Infect. 119, 227–230.

Tilley, E., 2015. Acceptance, impact and feasibility of incentives for increasing toilet use: A case study in eThekwini, South Africa. PhD Thesis, ETH ZURICH.

Udert, K.M., Buckley, C.A., Wächter, M., McArdell, C.S., Kohn, T., Strande, L., Zöllig, H., Hug, A., Oberson, A., Etter, B., 2015. Technologies for the treatment of source-separated urine in the eThekwini municipality. Water SA 41, 212–221.

Udert, K.M., Fux, C., Münster, M., Larsen, T.A., Siegrist, H., Gujer, W., 2003. Nitrification and autotrophic denitrification of source-separated urine. Water Sci. Technol. 48, 119–130.

US EPA, 2003. Control of pathogens and vector attraction in sewage sludge. Cincinnati, OH.

Vinneras, B., Nordin, A., Niwagaba, C., Nyberg, K., 2008. Inactivation of bacteria and viruses in human urine depending on temperature and dilution rate. Water Res. 42, 4067–4074.

WHO, 2005. South Africa: Summary country profile for HIV/AIDS treatment scale-up.

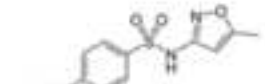
WHO, 2006. Guidelines for the safe use of wastewater, excreta and greywater. Volume 4 excreta and greywater use in Agriculture. World Health Organization.

Zachariah, R., Harries, A.D., Luo, C., Bachman, G., Graham, S.M., 2007. Scaling-up co-trimoxazole prophylaxis in HIV-exposed and HIV-infected children in high HIV-prevalence countries. Lancet Infect. Dis. 7, 686–

Graphical Abstract

Urine Diversion

Urine Collection



Pharmaceuticals

Pathogens

Antibiotic Resistance

Health Hazards Assessment