

Supplementary File: A review on occurrence and spread of antibiotic resistance in wastewaters and in wastewater treatment plants: Mechanisms and perspectives

- 1 Cansu Uluseker^{1†}, Krista Michelle Kaster^{1†}, Kristian Thorsen², Daniel Basiry¹, Sutha
- 2 Shobana³, Monika Jain⁴, Gopalakrishnan Kumar¹, Roald Kommedal¹, Ilke Pala-Ozkok^{1*}

3

- 4 Department of Chemistry, Bioscience and Environmental Engineering, Faculty of Science and
- 5 Technology, University of Stavanger, Box 8600 Forus, 4036 Stavanger, Norway
- 6 ² Department of Electrical Engineering and Computer Science, Faculty of Science and Technology,
- 7 University of Stavanger, Box 8600 Forus, 4036 Stavanger, Norway
- 8 ³ Department of Chemistry and Research Centre, Aditanar College of Arts and Science,
- 9 Virapandianpatnam, Tiruchendur, Tamil Nadu, India
- 10 ⁴ Department of Natural Resource Management, College of Forestry, Banda University of
- 11 Agricultural and Technology, Banda 210001, India
- 12 † These authors have contributed equally to this work
- 13 * Correspondence:
- 14 Corresponding Author
- 15 ilke.palaozkok@uis.no
- 16 Keywords: antibiotics, antibiotic resistance genes, antibiotic resistant bacteria, spread
- 17 mechanisms, wastewater treatment plant

Supplementary Table 1: Bacteria, resistant bacteria, and resistance genes remaining in biosolids after different treatment methods

Sludge/biosolids Resistant bacteria treatment, Wastewater plant		(to	Resistance otal cellular +		Wastewater treatment process, source of biosolids, sludge treatment, measurement methods and other notes	Ref.
Belt press dewatering						
East Lansing, Michigan, USA (cake 18.05% solids)	Total CFU/g 10 ^{10.0} -	(16S r	ŕ	10 ^{11.5} – 10 ^{12.1}	Activated sludge treatment, biosolids from a mix of primary sludge and activated sludge dewatered by belt press only. Bacteria numbers by heterotrophic plate count on R2A media	#: (Munir et al.,
*	tet resistant CFU/g 10 ^{7.4} – (16 ug/mL tet)	10 ^{8.3} tetW tetO	copies/g copies/g	$10^{8.0} - 10^{8.5} \\ 10^{8.8} - 10^{9.3}$	with antifungal cyclohexamide (200 ug/ml), gene copy numbers from qPCR, numbers per gram of biosolids (wet or	2011) PT&TP:
	sul resistant CFU/g 10 ^{8.7} – (50.4 ug/ml sul)	10 ^{9.1} sulI	copies/g	$10^{6.9} - 10^{9.2}$	dry weight not specified), absolute ranges from $n=3$ samples taken at different times of the year.	(City of East Lansing
					Biosolids disposed to landfill.	Webpages, 2009; Munir et al., 2011)
Gravity thickening						
Imlay, Michigan, USA (slurry 1.49% solids)	Total CFU/g 10 ^{9.5} –	0 ^{10.0} Total (16S r	copies/g RNA)	$10^{12.6} - 10^{12.7}$	Oxidation ditch treatment, biosolids from unknown sludge mix, gravity thickening only. Bacteria numbers by	#: (Munir et al.,
*	tet resistant CFU/g 10 ^{7.0} – (16 ug/mL tet)	tetW tetO	copies/g copies/g	$10^{8.7} - 10^{8.9} \\ 10^{8.7} - 10^{8.9}$	heterotrophic plate count on R2A media with antifungal cyclohexamide (200 ug/ml), gene copy numbers from qPCR, numbers per gram of biosolids (wet or dry weight not	2011) PT&TP: (Imlay City
	sul resistant CFU/g 10 ^{8.0} – (50.4 ug/ml sul)	10 ^{9.3} sulI	copies/g	$10^{7.5} - 10^{9.4}$	specified), absolute ranges from $n=2$ samples taken at different times of the year.	Webpages, 2020)
					Biosolids used as fertilizer on agricultural land.	
Anaerobic digestion						
Romeo, Michigan, USA (slurry 7.98% solids) Traverse city, Michigan,	Total CFU/g $10^{7.3}$ - tet resistant CFU/g $10^{4.6}$ -	(16S r	ŕ	$10^{12.0} - 10^{12.6}$ $10^{7.8} - 10^{9.6}$	Combined data from the Romeo and the Traverse city plants. Rotating biological contactors (Romeo) with biosolids from unknown sludge mix. Aeration basin and membrane	(Munir et al.,
USA (slurry 4.85% solids)	tet resistant CFU/g 10 ^{4.6} – (16 ug/mL tet)	10 ^{5.7} tetW tetO	copies/g copies/g	$10^{7.0} - 10^{7.0}$ $10^{7.1} - 10^{8.4}$	biological reactor (Traverse city) with biosolids from a mix of primary sludge and activated sludge dewatered by a gravity	2011) PT&TP:

*	sul resistant CFU/g (50.4 ug/ml Sul)	10 ^{6.7} – 10 ^{7.2}	sulI	copies/g	10 ^{7.6} – 10 ^{8.2}	belt concentrator before digestion. Bacteria numbers by heterotrophic plate count on R2A media with antifungal cyclohexamide (200 ug/ml), gene copy numbers from qPCR, numbers per gram of biosolids (wet or dry weight not specified), interquartile ranges from n = 7 samples in total taken from different times of the year (unspecified how many samples from each site). Temperature during anaerobic digestion unspecified.	(Munir et al., 2011; Traverse City/Jacobs, 2018)
Guadarrama Medio, Brunete, Madrid, Spain (cake 16.5% solids)	Total CFU/g amp resistant CFU/g (32 ug/mL Amp) Fecal coliforms CFU/g	$10^{11.2}$ $10^{8.8}$ $10^{8.6}$				Biosolids used as fertilizer on agricultural land. Activated sludge treatment, biosolids from a mix of primary sludge and activated sludge. Gravity thickening of the sludge before digestion. Two stage digestion with heating. Mechanical dewatering of biosolids by centrifuges and thermal drying. Bacteria numbers by heterotrophic plate count on R2A media with antifungal cyclohexamide (200 ug/ml), fecal coliforms numbers by plate count on TTC medium. Numbers per gram dry weight of biosolids, average numbers from unknown number of samples. Temperature during anaerobic digestion unspecified.	#: (Gondim-Porto et al., 2016) PT&TP: (Gondim-Porto et al., 2016; Canal de Isabel II S.A., 2017)
Galt, Ontario, Canada (slurry 4.9% solids)	Total coliforms CFU/g Fecal coliforms CFU/g <i>E. coli</i>		Total (rrnS) sulI strA strB	copies/g copies/g copies/g	$10^{10.1}$ $10^{9.9}$ $10^{8.5}$ $10^{7.4}$	Biosolids used as fertilizer on agricultural land. Activated sludge treatment, biosolids from a mix of primary sludge and mechanically thickened activated sludge. Total coliforms, fecal coliforms, and <i>E. coli</i> numbers by plate count on mEndo-LES medium, mFC medium, and MFC-BCIG medium respectively. Gene copy numbers from qPCR. Numbers per gram dry weight of biosolids. Average numbers from n = 3 samples for bacteria count and n = 4 samples for gene numbers. Temperature during anaerobic digestion unspecified.	#: (Lau et al., 2017) PT&TP: (Lau et al., 2017; Region of Waterloo, 2018)
Galt, Ontario, Canada (cake 30.1% solids)	CFU/g		Total (rrnS)	copies/g	10 ^{8.4}	Biosolids used as fertilizer on agricultural land and for remediation of mine tailing ponds from mining industry. Landfill as contingency option. Activated sludge treatment, biosolids from a mix of primary sludge and mechanically thickened activated sludge. Mechanical dewatering of biosolids by centrifuges. Total coliforms, fecal coliforms, and <i>E. coli</i> numbers by plate count	#: (Lau et al., 2017)

Aerobic digestion	Total coliforms CFU/g Fecal coliforms CFU/g E. coli CFU/g	$10^{6.6} \\ 10^{6.0} \\ 10^{5.8}$	sulI strA strB	copies/g copies/g copies/g	10 ^{7.4} 10 ^{6.7} 10 ^{5.6}	on mEndo-LES medium, mFC medium, and MFC-BCIG medium respectively. Gene copy numbers from qPCR. Numbers per gram dry weight of biosolids. Average numbers from $n=3$ samples for bacteria count and $n=4$ samples for gene numbers. Temperature during anaerobic digestion unspecified. Biosolids used as fertilizer on agricultural land and for remediation of mine tailing ponds from mining industry. Landfill as contingency option.	PT&TP: (Lau et al., 2017; Region of Waterloo, 2018)
	Total CFU/g	1011.2				Activated sludge treatment, biosolids from unknown sludge	-
Campo Real, Madrid, Spain (cake 14.2% solids) **	amp resistant CFU/g (32 ug/mL Amp) Fecal coliforms CFU/g	10 ^{9.1}				Activated studge treatment, biosolids from unknown studge mix. Gravity thickening of the studge before digestion. Biosolids are air-dried after digestion. Bacteria numbers by heterotrophic plate count on R2A media with antifungal cyclohexamide (200 ug/ml), fecal coliforms numbers by plate count on TTC medium. Numbers per gram dry weight of biosolids, average numbers from unknown number of samples. Temperature during aerobic digestion unspecified.	#: (Gondim-Porto et al., 2016) PT&TP: (Gondim-Porto et al., 2016; Canal de Isabel II S.A.,
	r cear comornis er o/g	10				compress remperature assume assessed assessed ameperature	2017)
Tillsonburg, Ontario, Canada (slurry 1.6% solids) ***	Total coliforms CFU/g Fecal coliforms CFU/g E. coli CFU/g §	$10^{6.6} \\ 10^{5.4} \\ < 10^{3.6}$	Total (rrnS) sulI strA strB	copies/g copies/g copies/g copies/g	$10^{10.2}$ $10^{10.6}$ $10^{8.9}$ $10^{7.8}$	Biosolids used as fertilizer on agricultural land. Activated sludge treatment, biosolids from unknown sludge mix. Aerobic digestion. Total coliforms, fecal coliforms, and $E.\ coli$ numbers by plate count on mEndo-LES medium, mFC medium, and MFC-BCIG medium respectively. Gene copy numbers from qPCR. Numbers per gram dry weight of biosolids. Average numbers from $n=3$ samples for bacteria count and $n=4$ samples for gene numbers. Temperature during aerobic digestion unspecified.	#: (Lau et al., 2017) PT&TP: (Oxford County; Lau et al., 2017; Oxford Country, 2017)
Tillsonburg, Ontario, Canada (cake 17.7 % solids) ***			Total (rrnS) sull strA	copies/g copies/g copies/g	10 ^{8.8} 10 ^{9.3} 10 ^{7.9}	Biosolids used as fertilizer on agricultural land. Activated sludge treatment, biosolids from unknown sludge mix. Aerobic digestion followed by dewatering by centrifuges. Total coliforms, fecal coliforms, and <i>E. coli</i> numbers by plate count on mEndo-LES medium, mFC medium, and MFC-BCIG medium respectively. Gene copy	#: (Lau et al., 2017) PT&TP: (Oxford County; Lau et al., 2017;
	Total coliforms CFU/g	$10^{6.7}$	strB	copies/g	$10^{6.7}$		

	Fecal coliforms CFU/g E. coli CFU/g	10 ^{6.3} 10 ^{6.2}				numbers from qPCR. Numbers per gram dry weight of biosolids. Average numbers from n = 3 samples for bacteria count and n = 4 samples for gene numbers. Temperature during aerobic digestion unspecified. Biosolids used as fertilizer on agricultural land.	Oxford Country, 2017)
Lime stabilization							
Lansing, Michigan, USA Lime stabilization only	Total CFU/g	$10^{9.0} - 10^{9.4}$	Total (16S rR	copies/g RNA)	$10^{11.5} - 10^{12.3}$	Activated sludge treatment, biosolids from unknown sludge mix. Bacteria numbers by heterotrophic plate count on R2A	#:
(slurry 9.2% solids) (pH unknown)	tet resistant CFU/g (16 ug/mL Tet)	$10^{5.4} - 10^{5.6}$	tetW tetO	copies/g copies/g	$10^{7.3} - 10^{8.3} \\ 10^{7.4} - 10^{7.6}$	media with antifungal cyclohexamide (200 ug/ml), gene copy numbers from qPCR, numbers per gram of biosolids (wet or dry weight not specified), absolute ranges from	(Munir et al., 2011)
*	sul resistant CFU/g (50.4 ug/ml Sul)	$10^{7.2} - 10^{8.0}$	sulI	copies/g	$10^{6.8}\!-10^{7.0}$	n = 2 samples taken at different times of the year.	PT&TP: (Munir et al., 2011)
						Biosolids used as fertilizer on agricultural land.	
Advanced lime stabilization						g	
St. Marys, Ontario, Canada			Total (rrnS)	copies/g	$10^{8.4}$	Activated sludge anoxic/oxic biological treatment. Biosolids	#•
Lystek proprietary process alkali + heat + high shear mixing			sulI	copies/g	$10^{8.0}$	from gravity thickened wasted activated sludge. The thickened sludge is dosed with polymer and dewatered by centrifuges. Dewatered sludge is run through the proprietary	#: (Murray et al., 2019)
(LysteGro) (slurry 10.8% soilds)			strA strB	copies/g copies/g	$10^{8.2} \\ 10^{6.8}$	Lystek process which involves a combination of heat, alkali, and high shear mixing. Total coliforms, fecal coliforms, and	PT&TP: (Lystek
(pH 7.9 in final product)	Total coliforms CFU/g Fecal coliforms CFU/g E. coli CFU/g			1 3		<i>E. coli</i> numbers by plate count on mEndo-LES medium, mFC medium, and MFC-BCIG medium respectively. Gene copy numbers from qPCR. Numbers per gram dry weight of biosolids. Average numbers from $n=3$ samples for bacteria count and $n=3$ samples for gene numbers.	International; Hornick and Blake, 2019)
Thorold, Ontario, Canada Anaerobic digestion + lime			Total (rrnS)	copies/g	10 ^{5.2}	Biosolids marketed as LysteGro and used as fertilizer on agricultural land. Niagara Biosolids Processing Facility in Thorold, Ontario, which receives dewatered anaerobic digested sludge	#:
stabilization + heat drying (N-Viro/N-Rich)			sulI	copies/g	§§ <≈10 ⁴	(typically 30% solids) from treatment plants in the Niagara Region and from the City of Toronto. The biosolids are mixed with cement kiln dust (primarily calcium oxide), byproduct	(Murray et al., 2019) PT&TP:

(dry granulate 59.3% soilds) (pH 11.3 in final product) Total coliforms CFU/g Fecal coliforms CFU/g E. coli CFU/g E. coli CFU/g TOTAL COLIFORM SS $< 10^{2.3}$ SS $< 10^{2$	walker Industries, 2017)
Windsor, Ontario, Canada Total copies/g 10 ^{9.1} Pellets produced at Windsor Biosolids Processing	
(dry pellets 95.3 % solids) (2014) **** **5 Total coliforms CFU/g Fecal coliforms CFU/g E. coli CFU/g *** * * * * * * * * * * * * * * * * *	tion Control nt. The two n biological lks at Little nd activated pelletized at 1 coliforms, the count on MFC-BCIG tom qPCR. toge numbers samples for
retention time of 20 min.	in a typicar
Windsor, Ontario, Canada (dry pellets 93.2% solids) Biosolids used as fertilizer on agricultural land. Pellets produced at Windsor Biosolids Processing which receives dewatered and centrifuged biosolids.	ids (approx. #:
(2017) sull copies/g 10 ^{8.1} 30% solids) from two WWTPs: Little River Pollut Plant and Lou Romano Water Reclamation Plan	

*5	Total coliforms CFU/g Fecal coliforms CFU/g <i>E. coli</i> CFU/g		strA strB	copies/g copies/g		10 ^{7.8} 10 ^{6.4}	WWTPs uses activated sludge treatment, with biological aerated filters at Lou Romano and aeration tanks at Little River. The biosolids are from a mix of primary and activated sludge. The biosolids are further heat dried and pelletized at the Windsor Biosolids Processing Facility. Total coliforms, fecal coliforms, and <i>E. coli</i> numbers by plate count on mEndo-LES medium, mFC medium, and MFC-BCIG medium respectively. Gene copy numbers from qPCR. Numbers per gram dry weight of biosolids. Average numbers from n = 3 samples for bacteria count and n = 3 samples for gene numbers. Temperature during heat drying 400 – 450 °C with a typical retention time of 20 min.	PT&TP: (City of Windsor; Murray et al., 2019)
Milwaukee, Wisconsin, USA (dry granulate >90% solids)		1	Total (16S rR' tetL tetO sulI	copies/g NA) copies/g copies/g		$10^{11.0}$ $10^{6.0}$ $10^{7.0}$ $10^{8.0}$	Biosolids used as fertilizer on agricultural land. Heat-dried biosolids from a blend of wasted activated sludge and anaerobically digested primary sludge from an unspecified municipal WWTP. Gene copy numbers from qPCR. Numbers are reported per gram dry weight. Temperature during heat drying unspecified.	#: (Kimbell et al., 2018) PT&TP: (Kimbell et al., 2018)
				, 0			Use of biosolids unspecified.	,
Heat drying and pyrolysis								
Milwaukee, Wisconsin, USA (dry biochar) (300 °C for 60 min)			Total (16S rR) tetL tetO	copies/g NA) copies/g copies/g	§ § § §	$10^{6.0}$ $< 10^{3.9}$ $< 10^{2.9}$	Lab scale experiment. Pyrolysis of already heat-dried biosolids. The heat-dried biosolids are from a blend of wasted activated sludge and anaerobically digested primary sludge from an unspecified municipal WWTP. Gene copy numbers	#: (Kimbell et al., 2018)
			sulI	copies/g	\$\$ §§	< 10 ^{3.9}	from qPCR. Numbers are reported per gram dry weight of the biochar remains after pyrolysis.	PT&TP: (Kimbell et al., 2018)
Milwaukee, Wisconsin, USA (dry biochar)			Total (16S rR	copies/g NA)		107.2	Lab scale experiment. Pyrolysis of already heat-dried biosolids. The heat-dried biosolids are from a blend of wasted activated sludge and anaerobically digested primary sludge	#: (Kimbell et al.,
(500 °C for 5 min)			tetL tetO	copies/g copies/g	§§ §§	$< 10^{3.9} $ $< 10^{2.9}$	from an unspecified municipal WWTP. Gene copy numbers	2018)

			sulI	copies/g	$\S\S$ < $10^{3.9}$	from qPCR. Numbers are reported per gram dry weight of the biochar remains after pyrolysis.	PT&TP: (Kimbell et al., 2018)
Soil (for comparison)							
Nebraska, USA Ungrazed native prairie	Total CFU/g	$10^{5.0} - 10^{5.9}$				Soil from native prairies where landowners could confirm no grazing by food animals for the last 20 years. Total and	- #:
soil	tet resistant CFU/g (16 ug/mL Tet)	$10^{4.1} - 10^{5.6}$	tetA	copies/g	$10^{4.2} - 10^{5.3}$	resistant bacteria numbers by heterotrophic plate count on R2A media. Coliforms and <i>E.coli</i> enumerated using	(Durso et al., 2016)
*6	Total coliforms CFU/g <i>E. coli</i> CFU/g	$10^{2.3} - 10^{4.2} < 1$	sulI	copies/g	$10^{3.0} - 10^{3.4}$	Quantitray (IDEXX Laboratories). Gene copy numbers from qPCR. Numbers per gram dry weight. 20 different sites/prairies studied. The ranges given are the minimum to maximum of the average numbers for each site. 99 of 100 samples had <i>E. coli</i> levels lower than 1 CFU/g.	
Tartu, Estonia "Eerika" experimental			Total (16S rF	copies/g RNA)	$10^{8.0} - 10^{8.2}$	Experimental field plots with grass. No fertilizer applied for 4 years before the experiment; before that sporadic	#: (Nõlvak et al.,
station Estonian University of Life			tetA	copies/g	$10^{4.8} - 10^{5.1}$	application of mineral fertilizer. Gene copy numbers from qPCR. Numbers per gram dry weight of soil. 25-75% ranges	2016)
Science			sulI	copies/g	$< 10^{3.0}$	from 33 soil samples over a time period 151 days.	
Arganda del Rey, Spain "La Isla" experimental	Total CFU/g	109.0				Experimental field plots. No information about history of previous applications of manure, biosolids, or artificial	#: (Gondim-Porto et
farm	amp resistant CFU/g (32 ug/mL Amp)	108.4				fertilizers. Bacteria numbers by heterotrophic plate count on R2A media with antifungal cyclohexamide (200 ug/ml), fecal coliforms numbers by plate count on TTC medium. Numbers	al., 2016)
	Fecal coliforms CFU/g	$10^{4.4}$				per gram dry weight of soil, average numbers from unknown number of samples.	
London, Ontario, Canada Environmental Science			Total (rrnS)	copies/g	$10^{8.6}$	Experimental field plots previously cropped with mixed grains and with no known history of manure or biosolids	#: (Lau et al., 2017)
Western Field Station			sulI	copies/g	$(n=9)$ $10^{5.7}$ §§ $(n=15) < 10^{3.9}$	applications. Total and fecal coliforms numbers by plate count on mEndo-LES medium and mFC medium, respectively. Gene copy numbers from qPCR. Numbers are	(Tabs. S12 and S14)
	Total coliforms CFU/g	(n=23) 10 ^{3.0} § (n=1) <	strA	copies/g	(n=2) 10 ^{4.1} §§ (n=22) <	per gram wet weight of soil and the average from 3 fields with different crop types (lettuce, radish, and carrot) each sampled at different times throughout the growth season, 8 samples per	
	Fecal coliforms CFU/g	10 ^{1.9} (n=10) 10 ^{2.6} § (n=14) < 10 ^{1.9}	strB	copies/g	10 ^{3.9} (n=1) 10 ^{4.2} §§ (n=23) < 10 ^{3.9}	field. Total of $n=24$ samples. In the measurement of total and fecal coliforms 1 and 14 samples were below the quantification limit of $10^{1.9}$ CFU/(g wet soil) (20 CFU/plate).	

In the measurement of resistance genes 15 samples (Sul-I), 22 samples (Str-A), and 23 samples (Str-B) were below the detection limit of 10^{3.9} copies/(g wet soil).

The table includes levels of resistant bacteria and resistance genes reported in the literature for resistance against four selected antibiotics: *ampicillin (amp), tetracycline (tet), sulfonamide (sul), and streptomycin (str)*. In addition to the numbers reported from the scientific literature (references marked with #), we have also gathered extended information on plant type, treatment processes, sludge sources, and the normal use of biosolids from each plant from public reports and webpages from municipalities and plant operators (references marked with PT&TP). We have designated sludge/biosolids with solids content below 12% as slurry, sludge/biosolids with solids content from 12-55% as cake, and biosolids with solids content above 55% as dry.

Note that the more advanced methods are not used instead of the simple methods listed at the top of the table, but in addition. E.g., thickened/dewatered sludge is used as input for aerobic or anaerobic digestion, and heat drying is done with sludge that has already been digested and/or thickened/dewatered.

Numbers originally reported as ranges are given as ranges with a note detailing whether the range is absolute (min-max), quartiles, or in any other format. Numbers given as averages are reported as a single average number, and variance numbers are disregarded as there is inconsistency on how these are reported (e.g., as variance, as standard deviation (SD), or even as standard error of the mean (SEM)) and from how many samples they are calculated. All numbers are rounded to one decimal digit in the exponent. All gene numbers are total, most studies do not distinguish between cellular DNA and external DNA.

- * These plants showed a significant reduction of resistance genes (copies/100ml) and a significant reduction of resistant bacteria (CFU/100ml) from the raw influent sewage to the treated water effluent, but no significant reduction of resistance genes nor resistant bacteria from the raw influent sewage to the sludge biosolids (Munir et al., 2011).
- ** It is unknown whether the sludge is aerobically digested in a digestor, or if the aerobic digestion happens during or as part of an air-drying process (the sludge is aerobically digested according to (Gondim-Porto et al., 2016), but no specific information about an aerobic digestor is found on the plant fact sheet from the operator (Canal de Isabel II S.A., 2017)).
- *** Note that the gene copy numbers from this study indicate that the aerobic biosolids contains a roughly 3 times higher number of sul1 genes than rrnS genes which were used as a reference for total amount of bacteria. The authors of the study do not comment on this anomality. However, in their Supplementary Material they report that 0.0018 copies of sul1 genes was detected for each copy of the 16S rRNA gene (reference for total amount of bacteria) in a sample of aerobic slurry analyzed by a different method (next generation sequencing) (Lau et al., 2017).
- **** The density of living bacteria in the pelletized biosolids from Windsor are below the detection limits in this study, but the number of genes still seems relatively high. However, the same study reports that the abundance of the same gene targets quantified in agricultural soil directly (2-hours, + 24-48 hours storage at 4° C) after application of the pelletized material was remarkably low compared to digested slurry or cake biosolids from other plants. The authors hypothesize that the DNA are carried in non-viable cells or as free DNA segments with lower than normal stability (due to the heat treatment) in the pellets and that it is rapidly destroyed following contact with soil (Lau et al., 2017).
- *5 The biosolids from the Windsor Biosolids Pelletizing Facility was tested in both 2014 (Lau et al., 2017) and 2017 (Murray et al., 2019). The plant was built in 1999 and operated from 1999-2019 by American Water (formerly Prism-Berlie) (City of Windsor, 2020). The treatments and processing involved to create the pelletized biosolids have to the authors' knowledge not changed from 2014 to 2017 (Lau et al., 2017; Murray et al., 2019; City of Windsor, 2020).

- *6 The Tet-A range is from 18 of 20 of the different prairies, tetA was not detected in 2 of the prairies.
- § Numbers below quantification limit, quantification limit given in the respective table cell.
- §§ Numbers below detection limit, detection limit given in the respective table cell.
- # References for numbers of bacteria and genes.

PT&TP References for plant type and treatment processes.

18

19	References

- 20 Canal de Isabel II S.A. (2017). Factsheet on EDAR Guadarrama Medio.
- 21 https://www.canaldeisabelsegunda.es/documents/2014. Available at:
- 22 https://www.canaldeisabelsegunda.es/documents/20143/638545/Guadarrama+Medio.pdf
- 23 /39a0572c-b1d1-b4a1-d934-72fcdf155f7a [Accessed September 27, 2021].
- 24 City of East Lansing Webpages (2009). City of East Lansing Wastewater Treatment Plant
- 25 Flow Diagram. Available at: https://www.cityofeastlansing.com/248/Water-Resource-
- Recovery-Facility [Accessed November 2, 2020].
- 27 City of Windsor Lou Romano Water Reclamation Plant. Available at:
- 28 https://www.citywindsor.ca/residents/environment/Pollution-
- 29 Control/Laboratory/Pages/Lou-Romano-Water-Reclamation-Plant.aspx [Accessed
- 30 December 5, 2020].
- 31 City of Windsor (2020). Integrated Site Energy Master Plan Study Report for Wastewater
- 32 Treatment Plants. Stantec Consult. Ltd. Available at:
- https://www.citywindsor.ca/Lists/Tenders/EOI 114-20, Organic Waste Management and
- Processing/114-20 Addendum 3 COMPLETE signed.pdf [Accessed December 5,
- 35 2020].
- Durso, L. M., Wedin, D. A., Gilley, J. E., Miller, D. N., and Marx, D. B. (2016). Assessment
- of Selected Antibiotic Resistances in Ungrazed Native Nebraska Prairie Soils. J.
- 38 Environ. Qual. 45, 454–462. doi:10.2134/jeq2015.06.0280.
- 39 Gondim-Porto, C., Platero, L., Nadal, I., and Navarro-García, F. (2016). Fate of classical
- faecal bacterial markers and ampicillin-resistant bacteria in agricultural soils under
- 41 Mediterranean climate after urban sludge amendment. Sci. Total Environ. 565, 200–210.
- 42 doi:10.1016/j.scitotenv.2016.04.160.
- 43 Gunn, E. (2015). A Partnership Creates A Biosolids Facility That Yields A Quality Product.
- 44 Treat. Plant Oper. Mag. Available at:
- https://www.tpomag.com/editorial/2015/03/a partnership creates a biosolids facility t
- hat yields a quality product [Accessed December 5, 2020].
- Hornick, R., and Blake, D. (2019). Town of St. Marys Wastewater Treatment and Collection
- 48 2019 Wastewater Summary Report. St. Marys Ontario Canada, 2019. Available at:
- https://www.townofstmarys.com [Accessed December 5, 2020].
- 50 Imlay City Webpages (2020). Wastewater Treatment. Available at:
- 51 https://www.imlaycity.org/government/wastewater-treatment/ [Accessed October 12,
- 52 2020].
- Kimbell, L. K., Kappell, A. D., and McNamara, P. J. (2018). Effect of pyrolysis on the
- removal of antibiotic resistance genes and class I integrons from municipal wastewater
- 55 biosolids. *Environ. Sci. Water Res. Technol.* 4, 1807–1818. doi:10.1039/c8ew00141c.
- 56 Lau, C. H. F., Li, B., Zhang, T., Tien, Y. C., Scott, A., Murray, R., et al. (2017). Impact of
- 57 pre-application treatment on municipal sludge composition, soil dynamics of antibiotic
- resistance genes, and abundance of antibiotic-resistance genes on vegetables at harvest.
- 59 Sci. Total Environ. 587–588, 214–222. doi:10.1016/j.scitotenv.2017.02.123.

60 61 62	Lystek International Biosolids Processing. Available at: https://web.archive.org/web/20201205154619/https://lystek.com/technology/biosolids-processing/ [Accessed December 5, 2020].
63 64 65	Munir, M., Wong, K., and Xagoraraki, I. (2011). Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan. <i>Water Res.</i> 45, 681–693. doi:10.1016/j.watres.2010.08.033.
66 67 68 69	Murray, R., Tien, Y. C., Scott, A., and Topp, E. (2019). The impact of municipal sewage sludge stabilization processes on the abundance, field persistence, and transmission of antibiotic resistant bacteria and antibiotic resistance genes to vegetables at harvest. <i>Sci. Total Environ.</i> 651, 1680–1687. doi:10.1016/j.scitotenv.2018.10.030.
70 71 72 73	Nõlvak, H., Truu, M., Kanger, K., Tampere, M., Espenberg, M., Loit, E., et al. (2016). Inorganic and organic fertilizers impact the abundance and proportion of antibiotic resistance and integron-integrase genes in agricultural grassland soil. <i>Sci. Total Environ.</i> 562, 678–689. doi:10.1016/j.scitotenv.2016.04.035.
74 75 76	Oxford Country (2017). 2017 Annual Wastewater Treatment System Summary Report. Available at: https://www.oxfordcounty.ca/Services-for-You/Water-Wastewater/Wastewater/Annual-reports [Accessed October 15, 2020].
77 78 79	Oxford County 2017 Annual Biosolids Management Report. 2017. Available at: https://www.oxfordcounty.ca/Services-for-You/Water-Wastewater/Wastewater/Annual-reports, [Accessed October 15, 2020].
80 81 82	Region of Waterloo (2018). Region of Waterloo 2018 Wastewater Treatment Master Plan July 2018. Available at: https://www.regionofwaterloo.ca/en/regional-government/waterand-wastewater.aspx.
83 84 85 86	Traverse City/Jacobs (2018). Traverse City Regional Wastewater Treatment Plant Brochure 2018, Overview of Equipment and Last Installation/Upgrade, Jacobs report number: BI0403181111DEN. Available at: https://www.traversecitymi.gov/wastewaterplant.asp [Accessed November 2, 2020].
87 88 89	Walker Industries (2017). N-Rich Soil Amendment Safety Data Sheet, revision 001. Available at: https://www.walkerind.com/media/2020/01/N-Rich-SDS.pdf [Accessed December 5, 2020].
90	

12