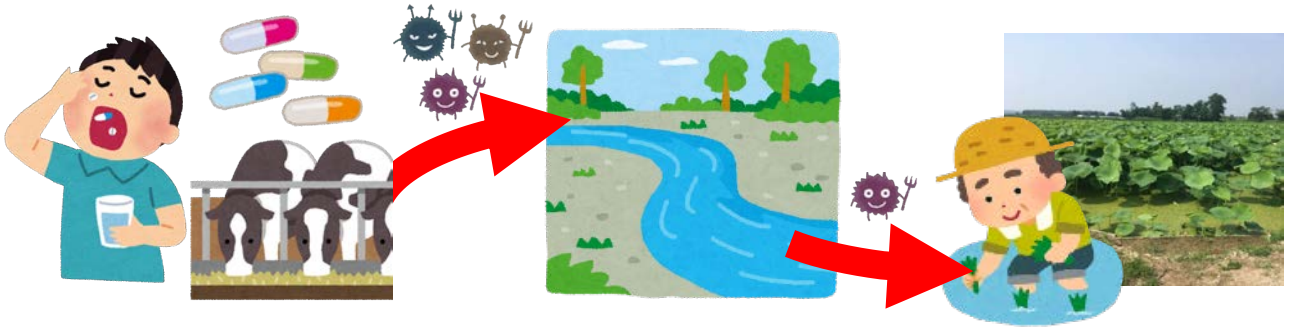
Fate of Antibiotic Resistant Bacteria from Wastewater to Water Environment

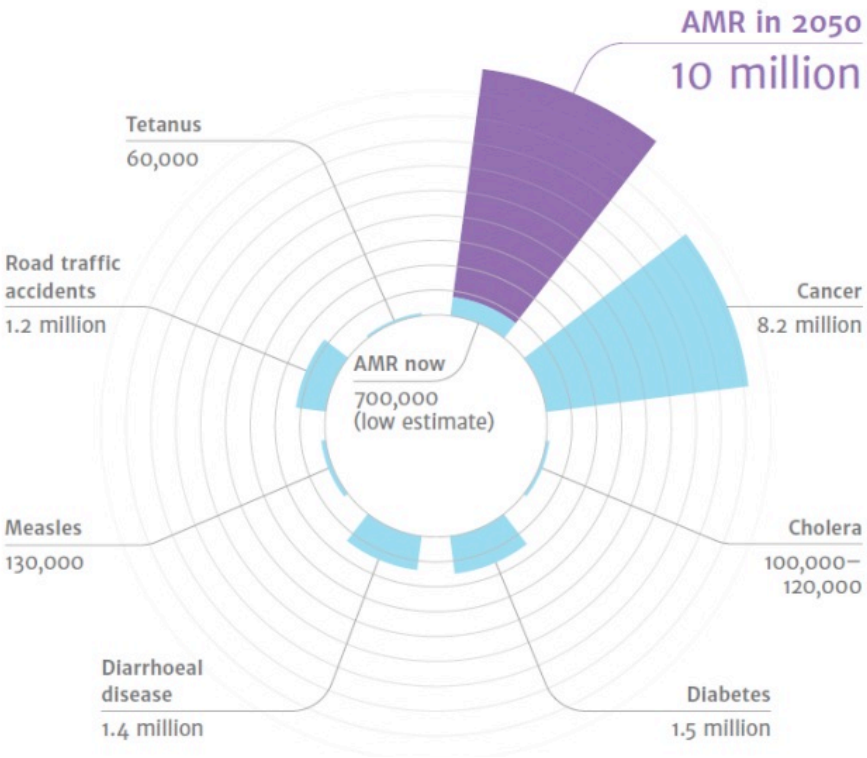
- From Asian Perspectives -



Ryo Honda

Kanazawa University

Antimicrobial Resistance is emerging risk

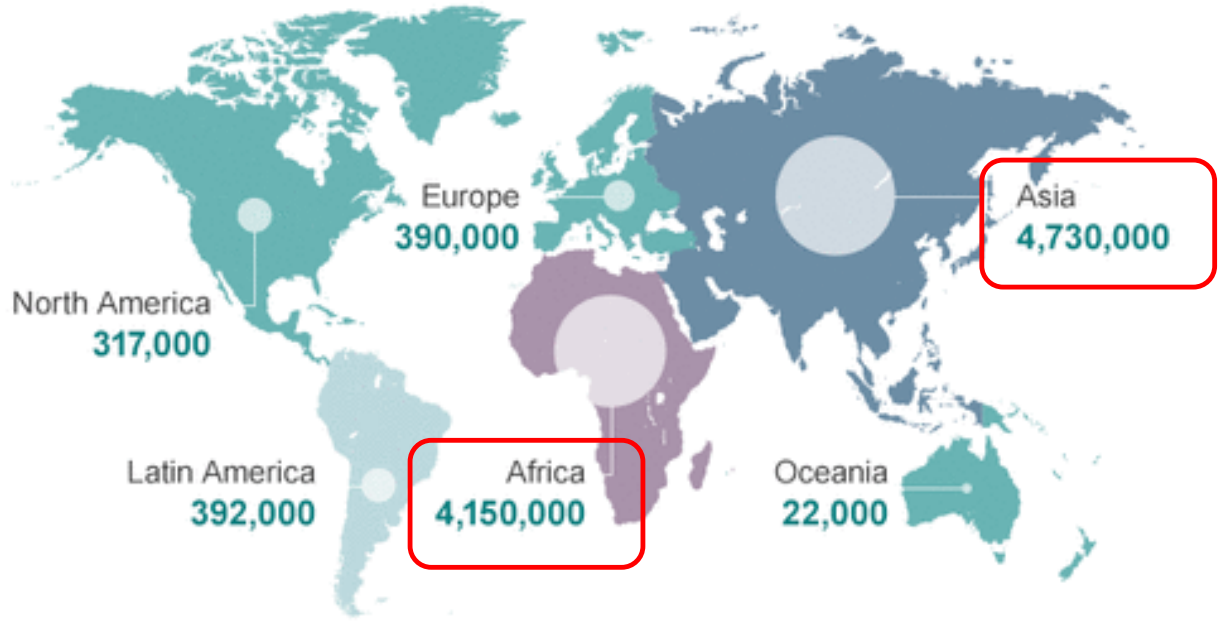


O'Neil Review 2016 (https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf)

Antimicrobial Resistance is emerging risk

Estimated deaths due to AMR: 700,000 (2013) -> 10,000,000 (2050)

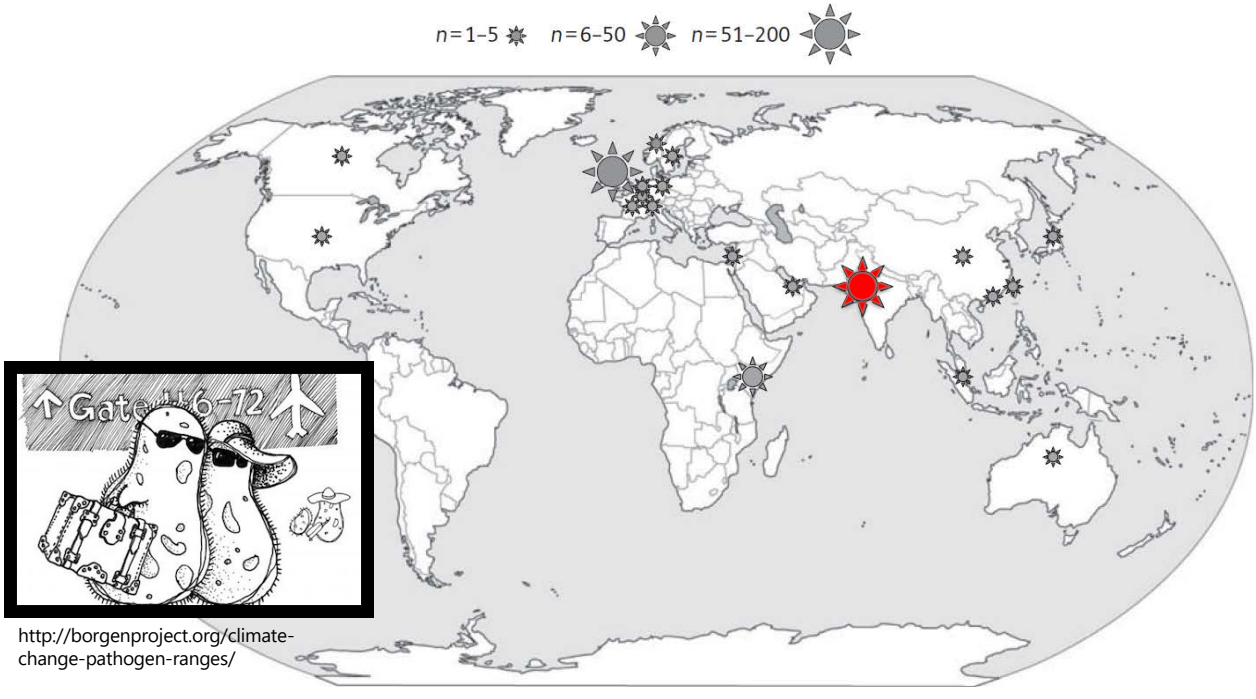
Deaths attributable to antimicrobial resistance every year by 2050



Source: Review on Antimicrobial Resistance 2014

ARB travel around world!

- ✓ India-origin *'Superbug' NDM-1* spread quickly to all continents in a couple of years.
- ✓ Asia and Africa are concerned as potential sites of new AMR emergence.

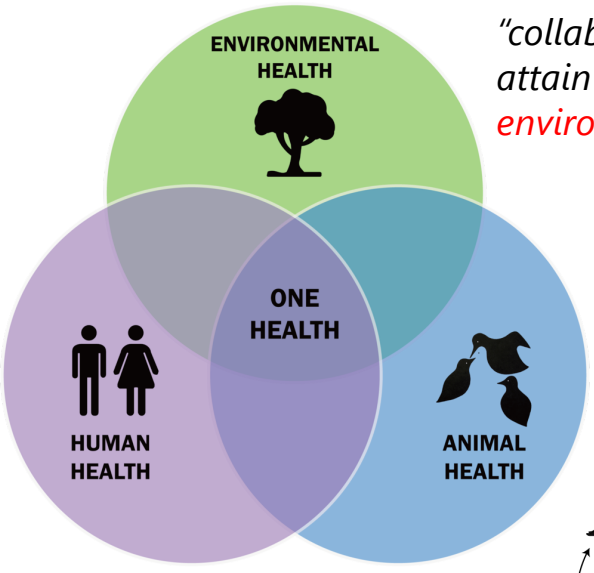


http://borgenproject.org/climate-change-pathogen-ranges/

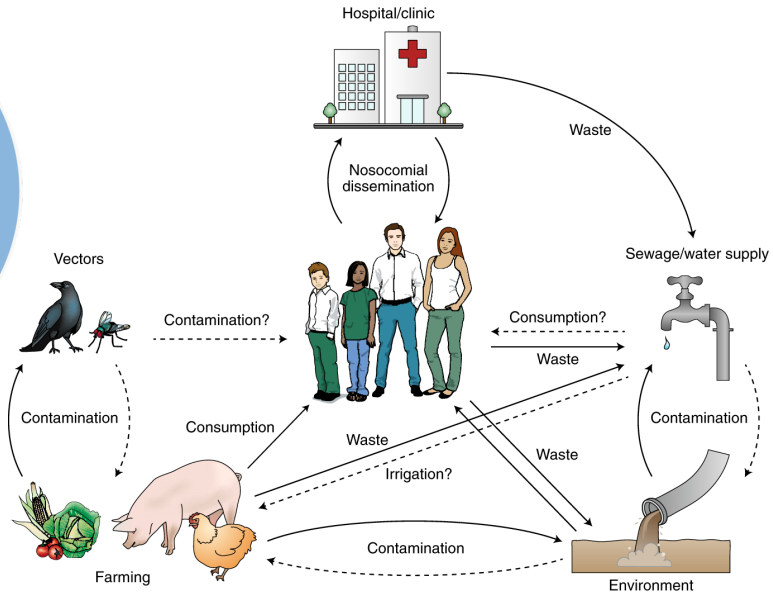
Figure 1. Worldwide distribution of identified cases of NDM-1 producers, 1 December 2010.

(*J. Antimicrob. Chemother.* 66(4):689-692, 2011. doi:10.1093/jac/dkq520)

'*One Health*' approach for AMR mitigation

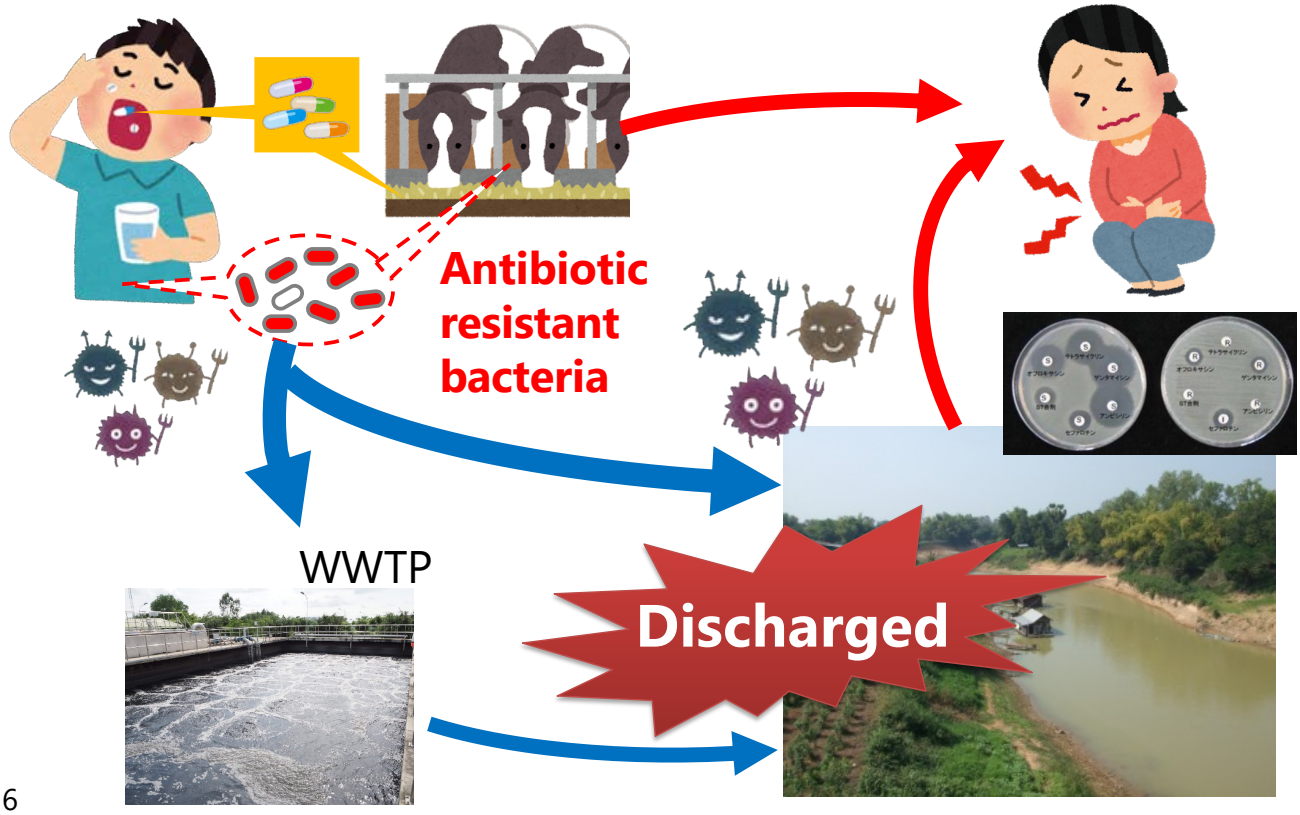


"collaborative efforts of multiple disciplines to attain optimal health for people, animals and our environment"

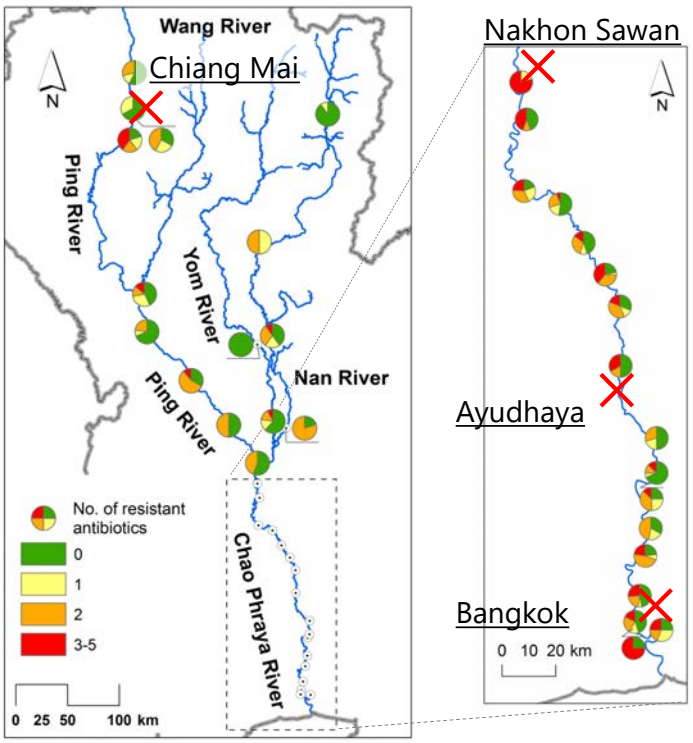


Walsh (2018) Nature Microbiol. 3, 854-855. https://doi.org/10.1038/s41564-018-0208-5

Wastewater is the major source of AMR in environment



Thailand: ARB in Chaophraya River System



Near cities

- ✓ High abundance of newer antibiotics (i.e. quinolones) （%R>0.2） + classic antibiotics
- ✓ Higher abundance of multiple-resistance

Rural areas:

- ✓ High abundance of classic antibiotics （%R>0.5）

Survey period：2011.1.16-28
Target resistance：
Quinolones: CIP, **LVX, NFX, OFX**
Non-quinolones: AMX, **SMZ, TC**, DTC

Honda et al. (2016) *Water Sci. Technol.* 73, 362-374. doi:10.2166/wst.2015.502

Wastewater is the major source of AMR in environment

Sri Lanka: ARB in Kelani River

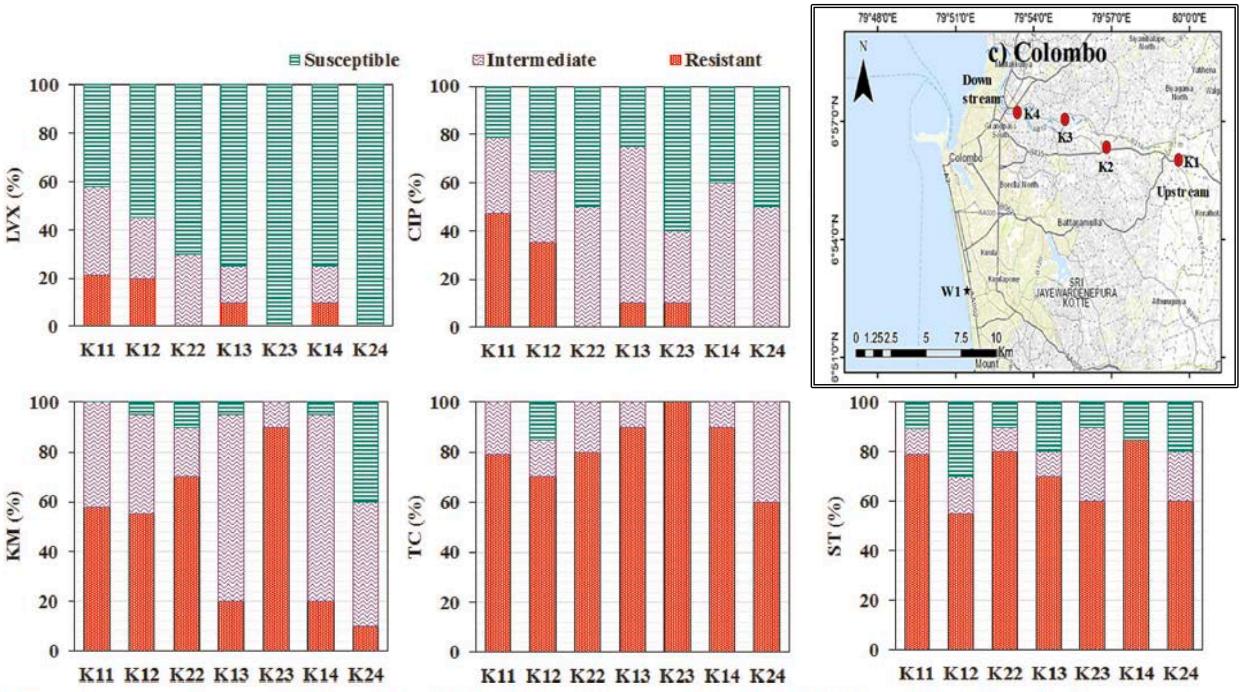


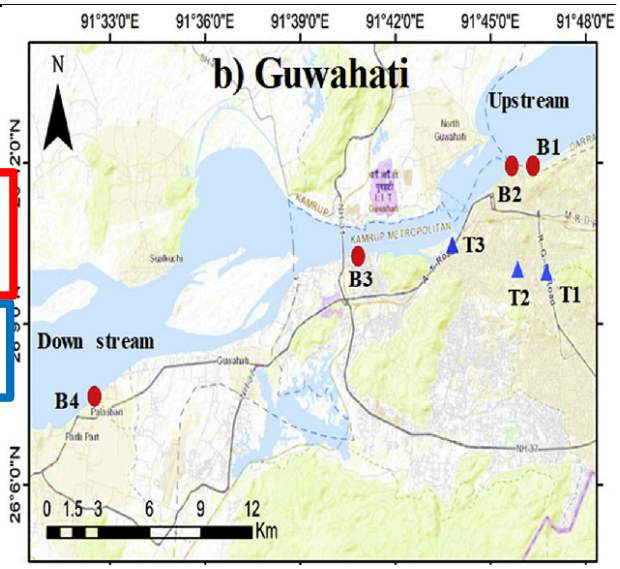
Fig. 2 Percentage Contribution of Different Level of Antibiotic Resistance of *E. coli* isolated from Kelani River. Bar diagram exhibiting percentage of different class of antibiotic resistance (i.e., resistant, intermediate, and susceptible). For **a** levofloxacin (LVX), **b** ciprofloxacin (CIP), **c** norfloxacin (NFX), **d** kanamycin monosulfate (KM), **e** tetracycline (TC), and **f** sulfamethoxazole (ST) in the Kelani River of 2017 representing wet season (K11–K14) and dry season (K22–K24) in 2018.

Kumar et al. (2020) *npj Clean Water* 3, 12. DOI: 10.1038/s41545-020-0058-6

India: ARG in River and Urban Drainage

Sample ID	*qnrB*	*aac-(6')-1b-cr*	*parC*	qnrS	gyrA	tetW	sul1
K1	NA	NA	NA	-	+	+	+
K2				-	+	+	+
K3				-	+	**+	+
K4				-	+	**+	+
B1	-	-	-	-	NA	NA	-
B2	-	-	-	-			+ weak
B3	-	++	+	-			++
B4	-	**+	-	-			+
T1	-	+ weak	-	++			+
T2	-	++	-	++			+
T3	-	**+	-	-			+

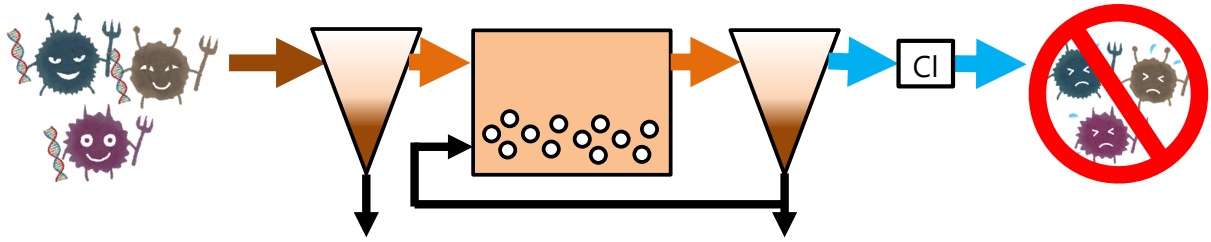
Sample ID	dfr1	vanA	ampC	blaTEM	blaCTX	*blaSHV*
K1	NA	-	+	-	-	NA
K2		-	+	-	-	
K3		-	+	-	-	
K4		-	+	-	-	
B1		-	-	-	NA	-
B2		-	-	-		-
B3		-	+	+weak		-
B4		-	+	-		-
T1		-	-	+		-
T2		-	+	+		-
T3		-	-	++		-



Antibiotic Resistance Genes to clinically important drugs were often detected **in urban drainage.**

Kumar et al. (2020) *Environ. Res.* 188, 109765. DOI: 10.1016/j.envres.2020.109765

Fate of Antibiotic Resistant Bacteria in Wastewater Treatment

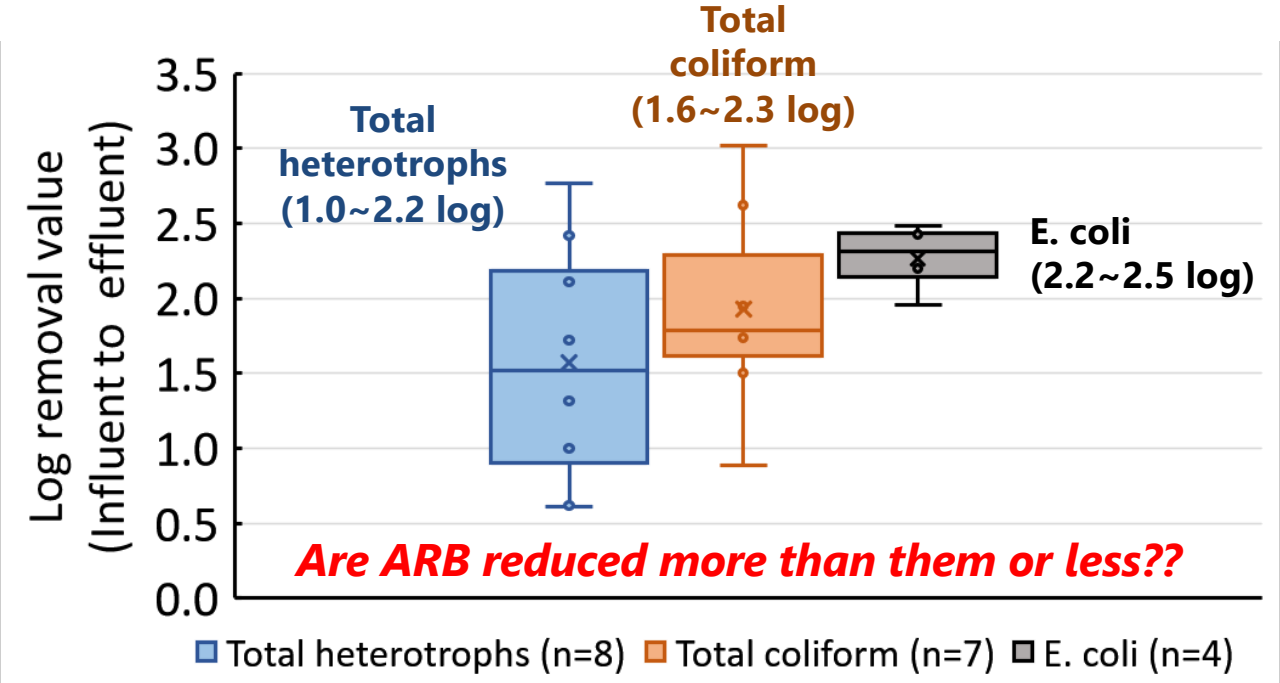


Does a WWTP work as a reservoir or barrier of ARB?

Both.

It works as a barrier, but also reserves ARB in it.

Bacteria population are reduced in WWTP



Created from the data in: [1] Zhang et al. 2015. Chemosphere 135, [2] Novo & Manaia 2010. Appl. Microbiol. Biotechnol. 87, [3] Reinthaler et al. 2013. Water Res. 37, [4] Ferreira da Silva et al. 2007. FEMS Microbiol. Ecol. 60, [5] Tachi et al. 2014. WET2014

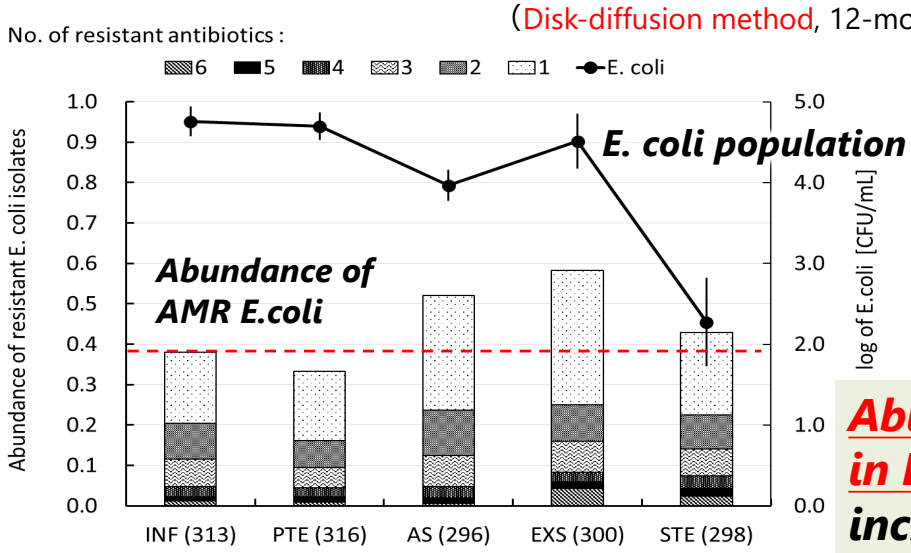
Reduction of ARB = total bacteria

Log removal values of ARB
equal to
LRV of total bacteria

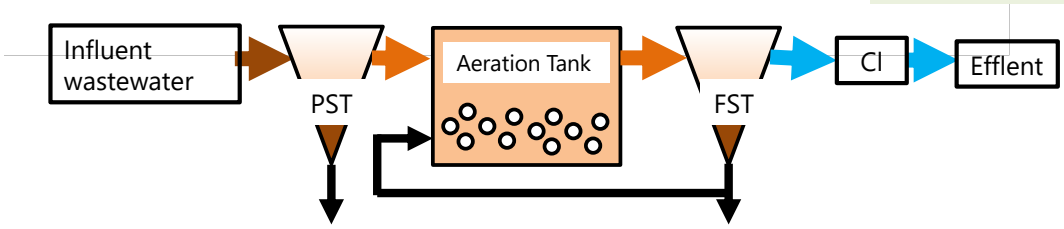
Created from Novo & Manaia 2010. Appl. Microbiol. Biotechnol. 87,

Abundance of antibiotic resistant *E.coli*

(Disk-diffusion method, 12-months monitoring in Japan)



***Abundance of AMR in E.coli* increased in sludge**



Honda et al. (2020) *J. Water Health* 18 (6): 879–889. DOI: 10.2166/wh.2020.013

Abundance of total ARG/16S

(Metagenomic analysis, Japan)

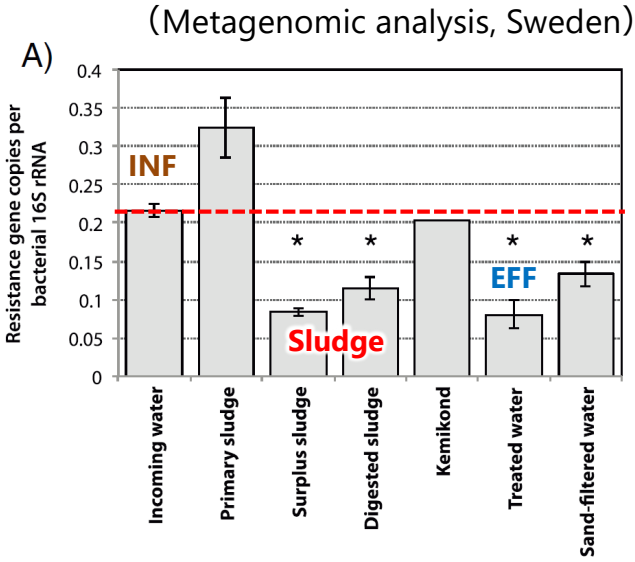
Honda et al. *unpublished*

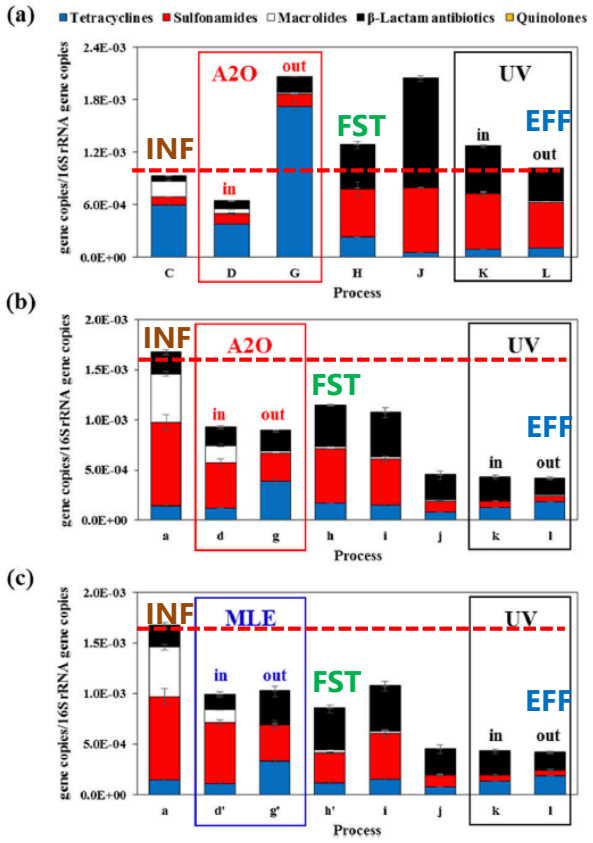
✓ ***Abundance of antibiotic resistance genes decreased in sludge***

Abundance of ARG/16S in other studies

(qPCR, South Korea)

(Metagenomic analysis, Sweden)





✓ ***Abundance of ARG decreases in sludge***

[1] Bengtsson-Palme et al., Sci. Total Environ. 572 (2016) 697–712. doi:10.1016/j.scitotenv.2016.06.228.

[2] Lee et al. Science of the Total Environment 605–606 (2017) 906–914

Culture-based abundance of AMR (in fecal bacteria) does **not change**

Gene-based abundance of AMR (in whole bacteria community) **decreases**

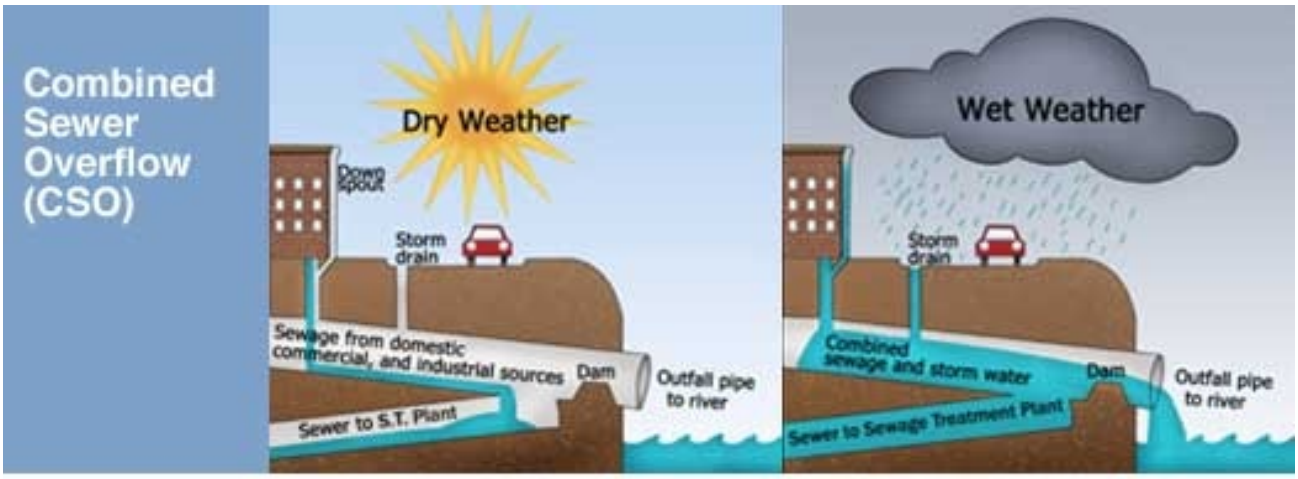
(Honda et al., unpublished)

ARB in wastewater are carried over to effluent

Honda et al. *unpublished*

Discharge of Antibiotic Resistant Bacteria from Stormwater

Wastewater is dumped before treatment



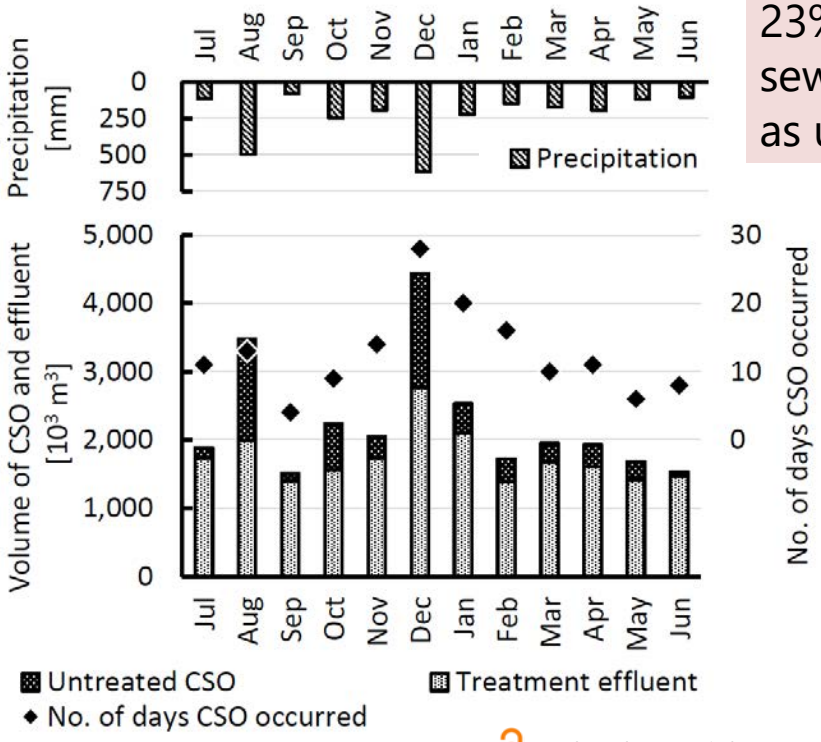
http://www.beachapedia.org/Combined_Sewer_Overflows

In USA, 20% of annual discharge of combined sewage is discharged as untreated CSO.

USEPA. *Report to Congress on Impacts and Control of Combined Sewer Overflows and Sanitary Sewer Overflows*; EPA Publishing, 2004.

Quantity of untreated CSO

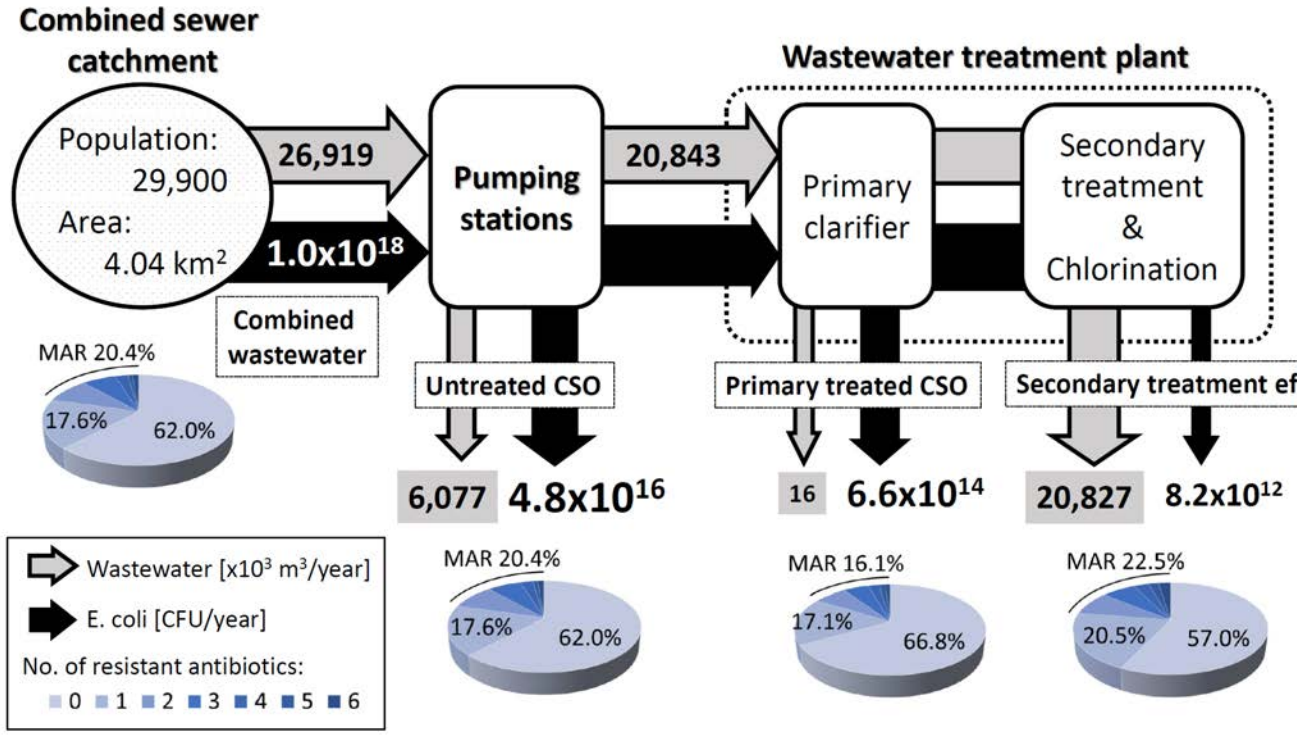
Case in one combined sewer catchment



23% of combined sewage was discharged as untreated CSO

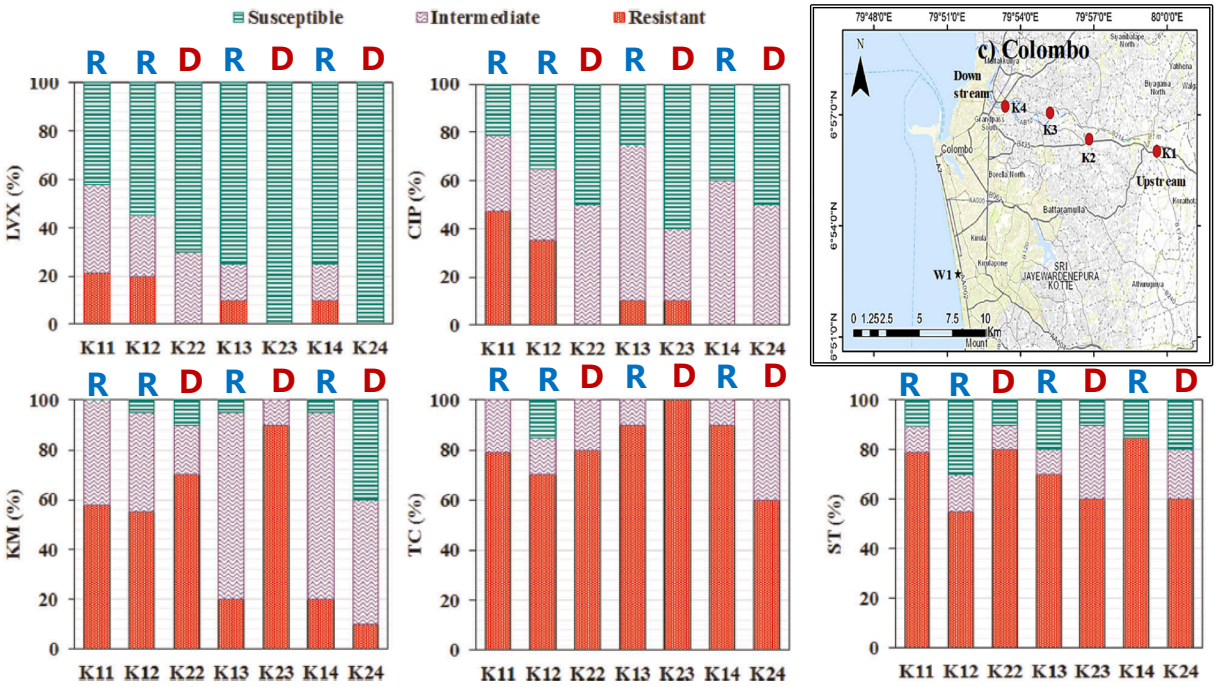
Honda et al. 2020, npj Clean Water 3, 15. https://doi.org/10.1038/s41545-020-0059-5

Annual discharge of ARB from CSO



Honda et al. 2020, npj Clean Water 3, 15. https://doi.org/10.1038/s41545-020-0059-5

Sri Lanka: ARB in Kelani River



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Kumar et al. (2020) *npj Clean Water* 3, 12. DOI: 10.1038/s41545-020-0058-6

AMR discharge affected by Monsoon

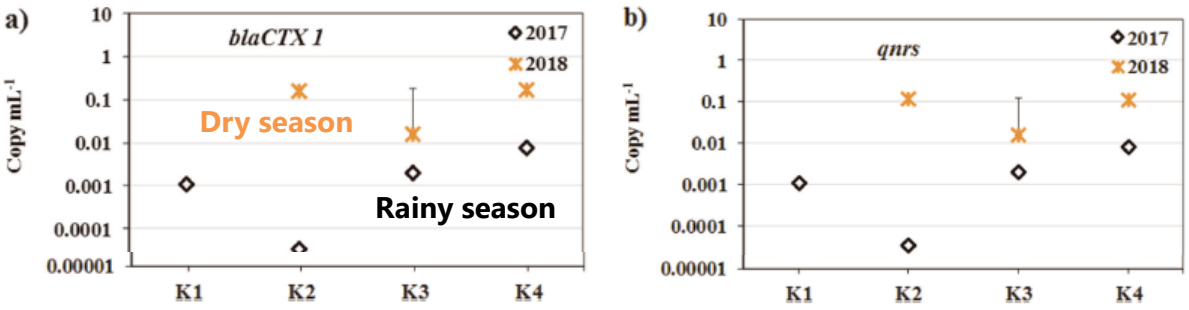

Table 2. Seasonal variation in the antibiotic-resistant gene screening of the Kelani River water.

Sample ID	*aac-(6')-1b-cr*	*parC*	*qnrB*	*gyrA*	*dfr1*	*qnrSm*	*ampC*	*vanA*	*sull*	*blaTEM*
October 2017	**Rainy season**									
K11	NA	NA	NA	+	–	–	+	–	+	–
K12				+	–	–	+	–	+	–
K13				+	–	–	+	–	+	–
K14				+	–	–	+	–	+	–
March 2018	**Dry season**									
K21	–	–	–	NA	NA	–	–	–	+	–
K22	+	–	–			+	–	–	+	–
K23	++	–	–			+	–	–	+weak	–
K24	+	–	–			–	–	–	+	–

+ Detected, – not detected, *NA* not analyzed.
[a]Very weak.

Kumar et al. (2020) *npj Clean Water* 3, 12. DOI: 10.1038/s41545-020-0058-6

Take-home messages

- *Urban wastewater is the major source of AMR* to clinically important antibiotics.
- *WWTP reduces total ARB loads* to environment, but also carry over some ARB to effluent.
- *Stormwater has much higher ARB loads* than WWTP effluent.
- ARB in water environment is *diluted in rainy season in Monsoon area* (although ARB loads can be higher.)

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- Nippon Life Foundation
- Hiramoto-gumi, Inc.

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