

# Antibiotics and Resistance Genes in Biosolids

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# What are Biosolids?

- Organic, *treated* solids and semi-solids recovered during wastewater treatment
- Treated to reduce pathogen and heavy metal loads
- Suitable for soil amendment
- Source of organic matter, plant nutrients, but also heavy metals and salts



# Recycling of Biosolids via land application: Emerging concerns

## Deformities in fish cause concern

Research on Boulder Creek and the South Platte has uncovered a disturbing problem: Some white sucker fish downstream from Denver and Boulder sewage plants have both male and female reproductive tissue. Researchers also found something else: Females far outnumber males. Now they want to know if chemicals that mimic the female hormone estrogen are to blame. Utilities that provide water to more than two dozen communities from the South Platte don't test for the chemicals. Said biologist John Woodling, who discovered the gender-bending fish: "This is the only thing that has ever really scared me."

Denver Post 10/5/2004

- Dioxin?
- Endocrine disruptors?
  - Nonylphenols
  - Flame retardants
- Pharmaceuticals?
  - Prescription drugs
  - Antibiotics

# Therapeutic agents as emerging contaminants in biosolids: persistence and impact on soil microbial communities

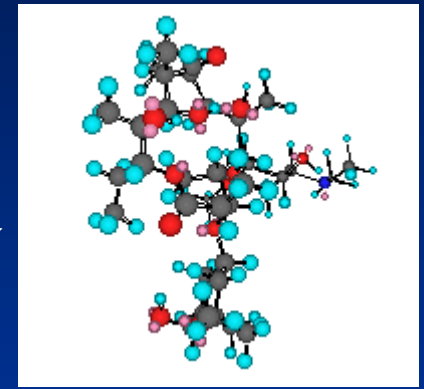
- 2-year project funded by EPA Region 8
- Objectives:
  - Screen biosolids collected from different wastewater treatment facilities for kinds and amounts of antibiotics present
  - Determine the persistence of selected antibiotics, applied alone or along with biosolids, in soils under aerobic conditions
  - Determine the effects of selected antibiotics on microbial activity and microbial antibiotic resistance patterns in soil during aerobic incubation
  - Determine the long-term effects of biosolids, land applied to field soils, on antibiotic resistance patterns of soil microbial communities.



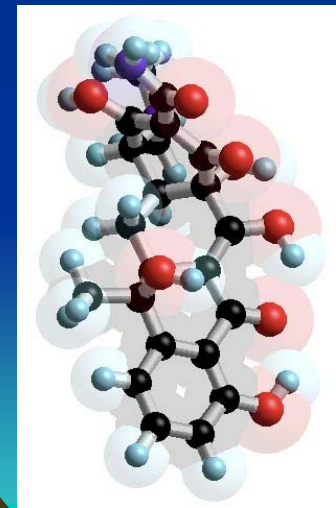
# Are there antibiotics in biosolids?



erythromycin



tetracycline



# Concentration of macrolides in biosolids ( $\mu\text{g kg}^{-1}$ )

	Anaerobic A	Anaerobic B	Anaerobic C	Aerobic A	Aerobic B	Aerobic C
ERY	8.2	BDL	BDL	BDL	74.0	BDL
RTM	10.3	26.5	BDL	BDL	2.9	BDL
TYL	1.1	BDL	BDL	BDL	1.4	1.9

ERY: Erythromycin, RTM: Roxithromycin, TYL: Tylosin

BDL: Below detection limit

# Concentration of sulfonamides in biosolids ( $\mu\text{g kg}^{-1}$ )

	Anaerobic A	Anaerobic B	Anaerobic C	Aerobic A	Aerobic B	Aerobic C
STZ	7.1	10.7	7.0	5.6	5.3	BDL
SMR	BDL	BDL	6.8	6.5	BDL	2.6
SMT	5.2	BDL	BDL	BDL	BDL	4.6
SCP	10.6	6.6	6.2	6.9	3.8	3.2
SMX	9.2	BDL	BDL	9.5	5.1	7.1
SDM	3.2	BDL	3.4	9.9	2.2	5.4

STZ: Sulfathiazole, SMR: Sulfamerazine, SMT: Sulfamethazine,  
SCP: Sulfachlorpyridazine, SMX: Sulfamethoxazole, SDM: Sulfadimethoxine  
BDL: Below detection limit

# Concentration of tetracyclines in biosolids ( $\mu\text{g kg}^{-1}$ )

	Anaerobic A	Anaerobic B	Anaerobic C	Aerobic A	Aerobic B	Aerobic C
TC	843.3	1147.2	1986.6	285.1	1345.6	142.3
OTC	10671.3	386.2	1413.7	6787.0	324.3	26.1
CTC	53.8	BDL	BDL	BDL	112.3	2.6
DMC	16.3	BDL	16.2	98.2	BDL	3.3
MCC	3021.5	389.7	1360.0	3735.9	4975.6	98.7
DXC	441.1	223.5	270.2	505.2	14855.1	61.0

TC: Tetracycline, OTC: Oxytetracycline, CTC: Chlortetracycline  
DMC: Demeclocycline, MCC: Meclocycline, DXC: Doxycycline  
BDL: Below detection limit



# Are there antibiotic-resistant bacteria in biosolids?



# Oxytetracycline-resistant bacteria in Anaerobic A biosolids

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mg OTC L <sup>-1</sup> R2A medium	% OTC-resistant isolates
0	--
0.1	24.1
1.0	17.3
5	0.13
10	0.025
25	0.0003
50	0.00008

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# Oxytetracycline-resistant bacteria in Aerobic A biosolids

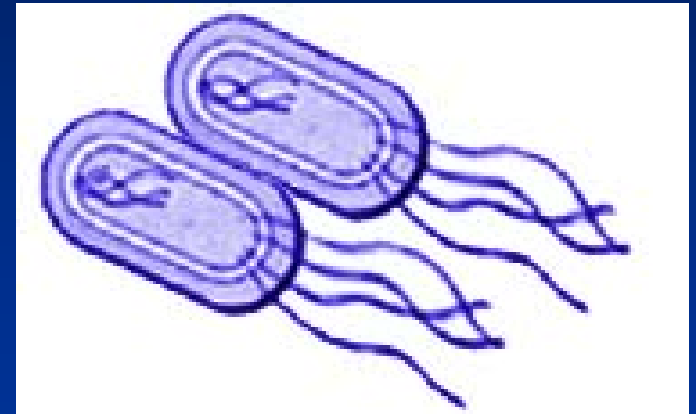
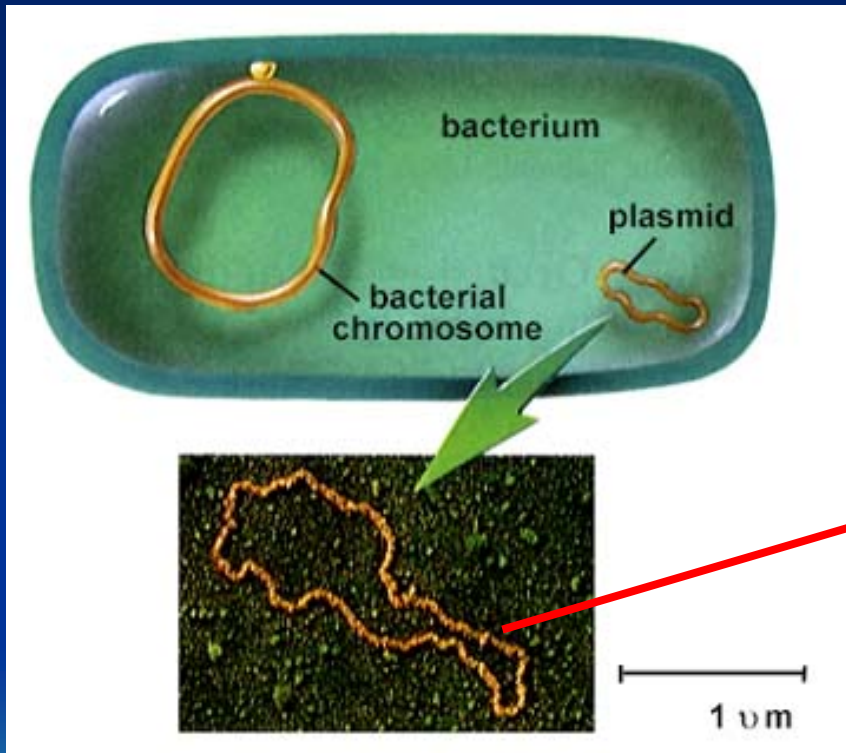
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mg OTC L <sup>-1</sup> R2A medium	% OTC-resistant isolates
0	--
0.1	45.8
1.0	35.4
5	0.068
10	0.049
25	0.026
50	0.022

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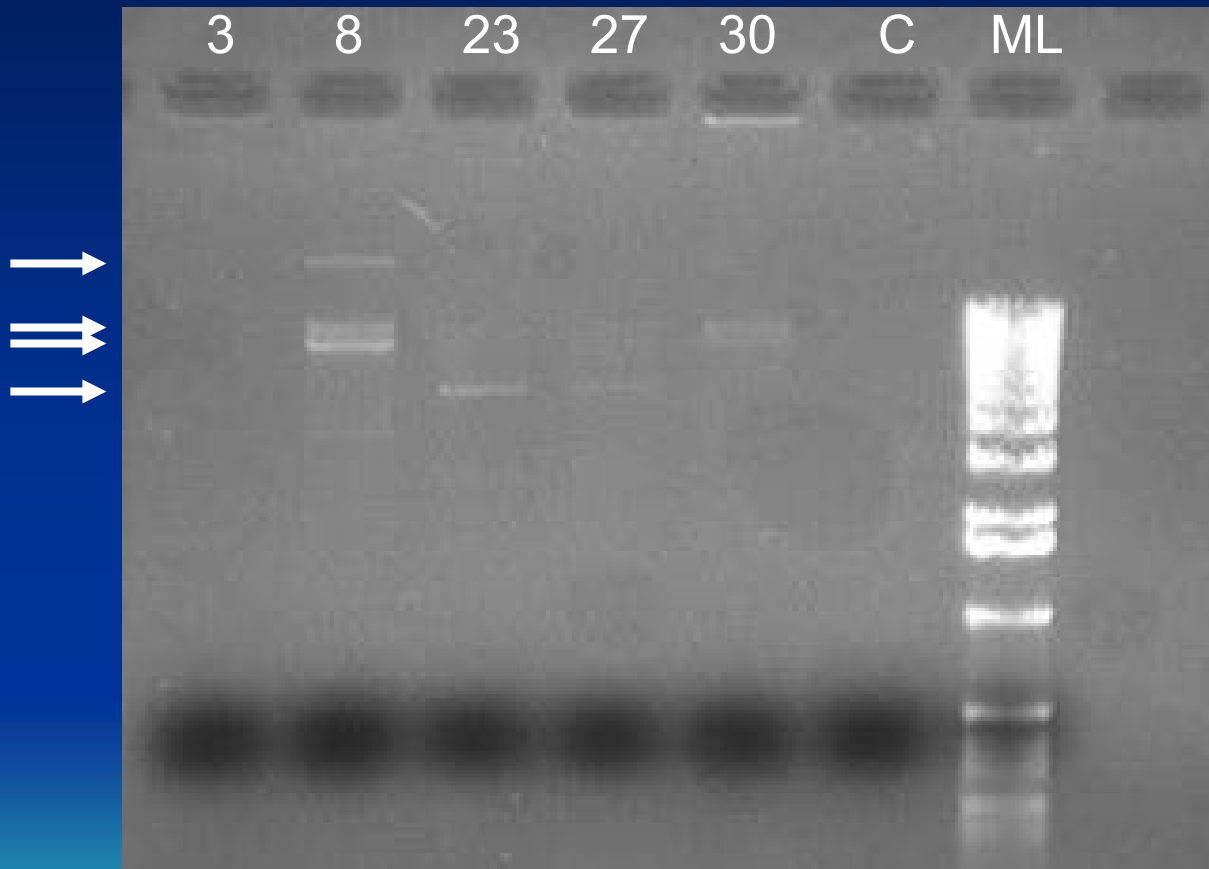


# Are the resistance genes to OTC carried on plasmids?



Horizontal gene transfer

# Plasmids from OTC-resistant Aerobic A bacterial isolates



# Continuing research . . .

- **Laboratory study:** determine how additions of OTC-contaminated biosolids, OTC, or OTC-resistant bacteria to soil affect indigenous soil microbial activities and antibiotic resistance patterns
- **Field study:** determine the antibiotic-resistance patterns of indigenous microbial communities in control soils and soils receiving long-term inputs of biosolids

