
June 29, 2007 District Seminar

ANTIBIOTIC RESISTANT BACTERIA in Wastewater Processed by the Metropolitan Water Reclamation District of Greater Chicago System

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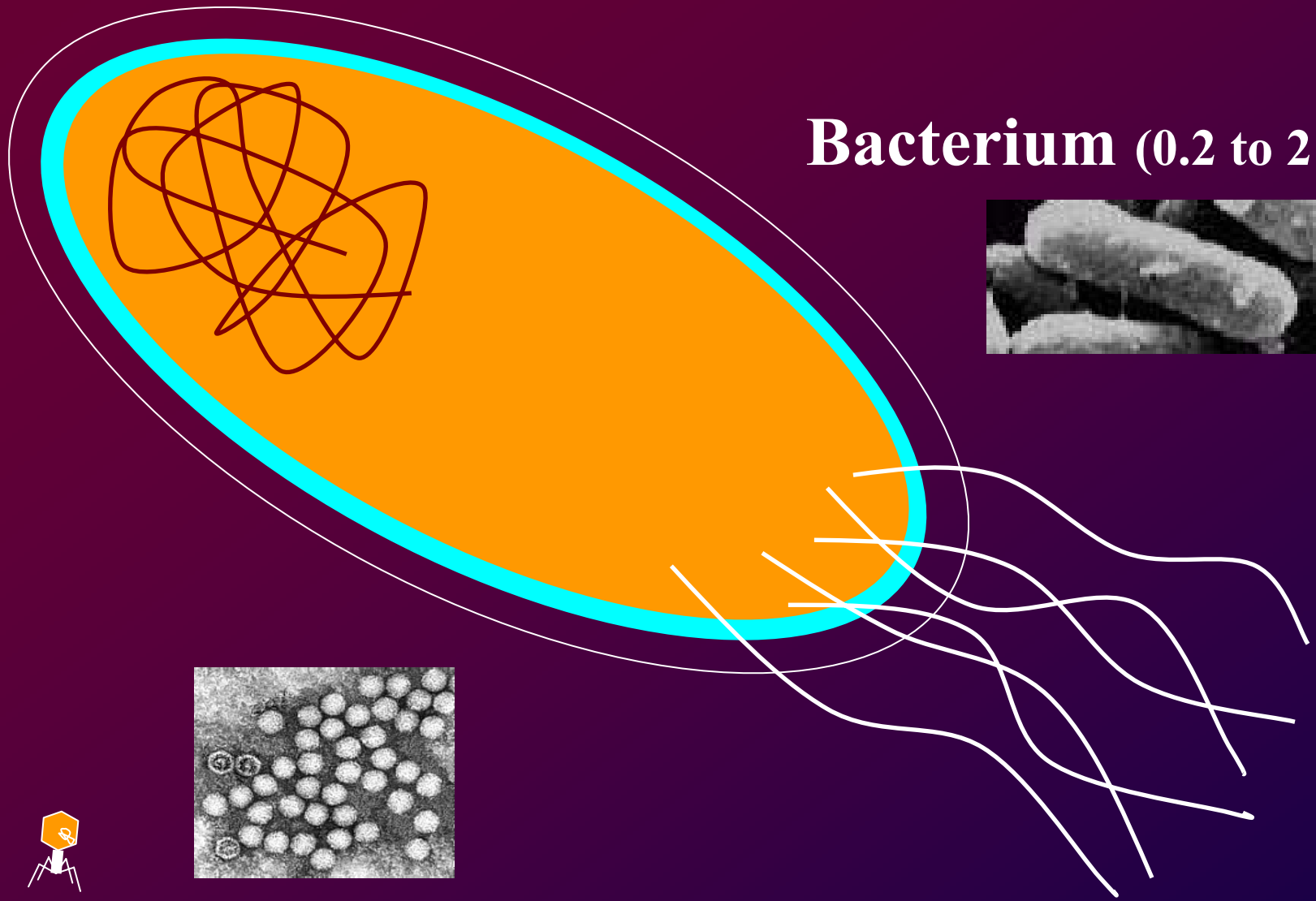
Penicillin

- **British Bacteriologist Alexander Fleming discovered penicillin in 1927.**
- **Penicillin, an important antibiotic derived from mold, is effective against a wide range of disease-causing bacteria.**
- **It acts by killing bacteria directly or inhibiting their growth.**

http://encarta.msn.com/media_461516382/Discovery_of_Penicillin.html

ANTIBIOTICS

- **Antibiotics are compounds given to humans and animals to prevent/treat bacterial infections.**
- **Antibiotics inactivate or kill bacteria by targeting specific important parts of the bacteria's structure or cellular machinery.**
- **Antibiotics kill bacteria, not viruses.**

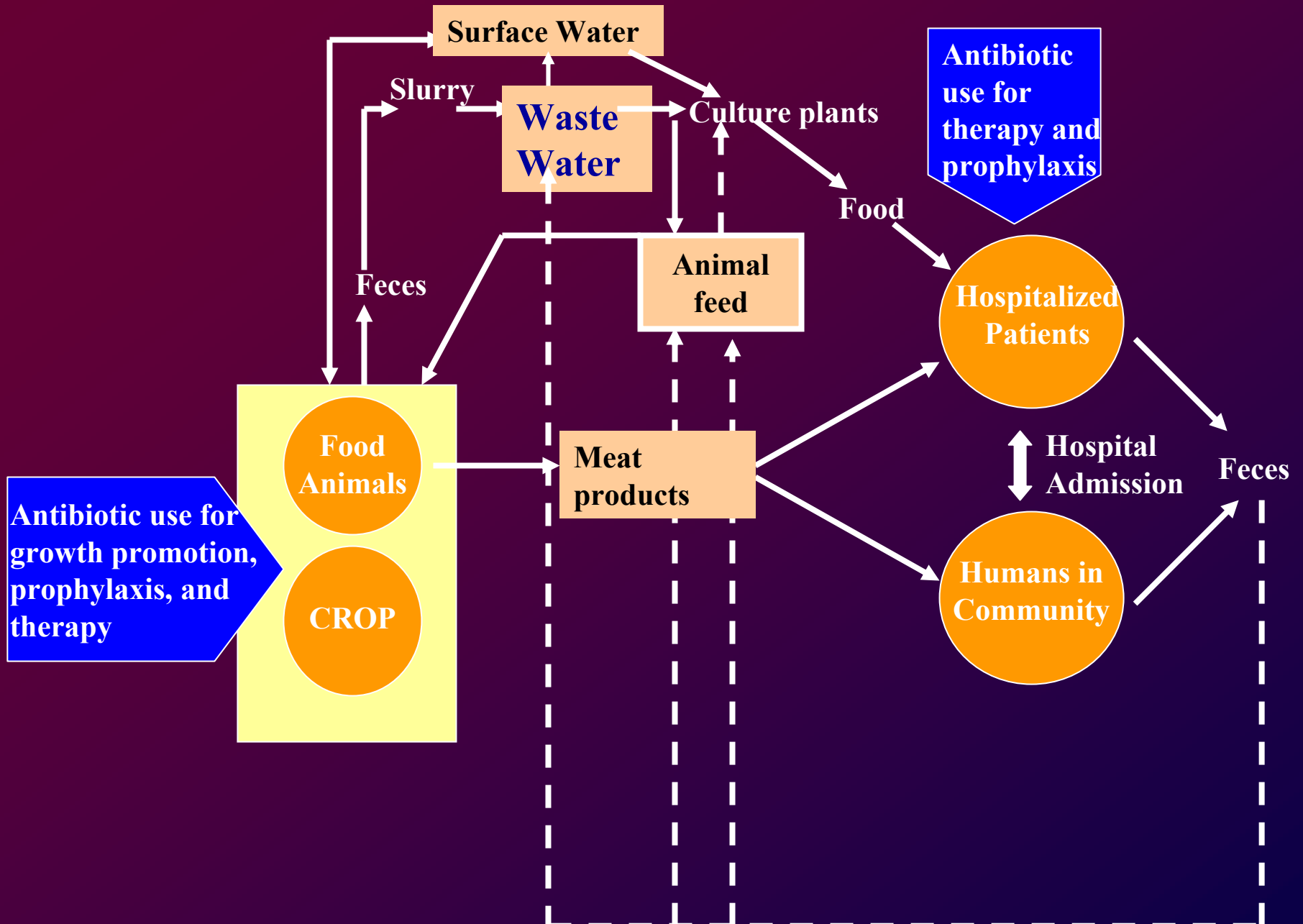


Bacterium (0.2 to 2 μm)

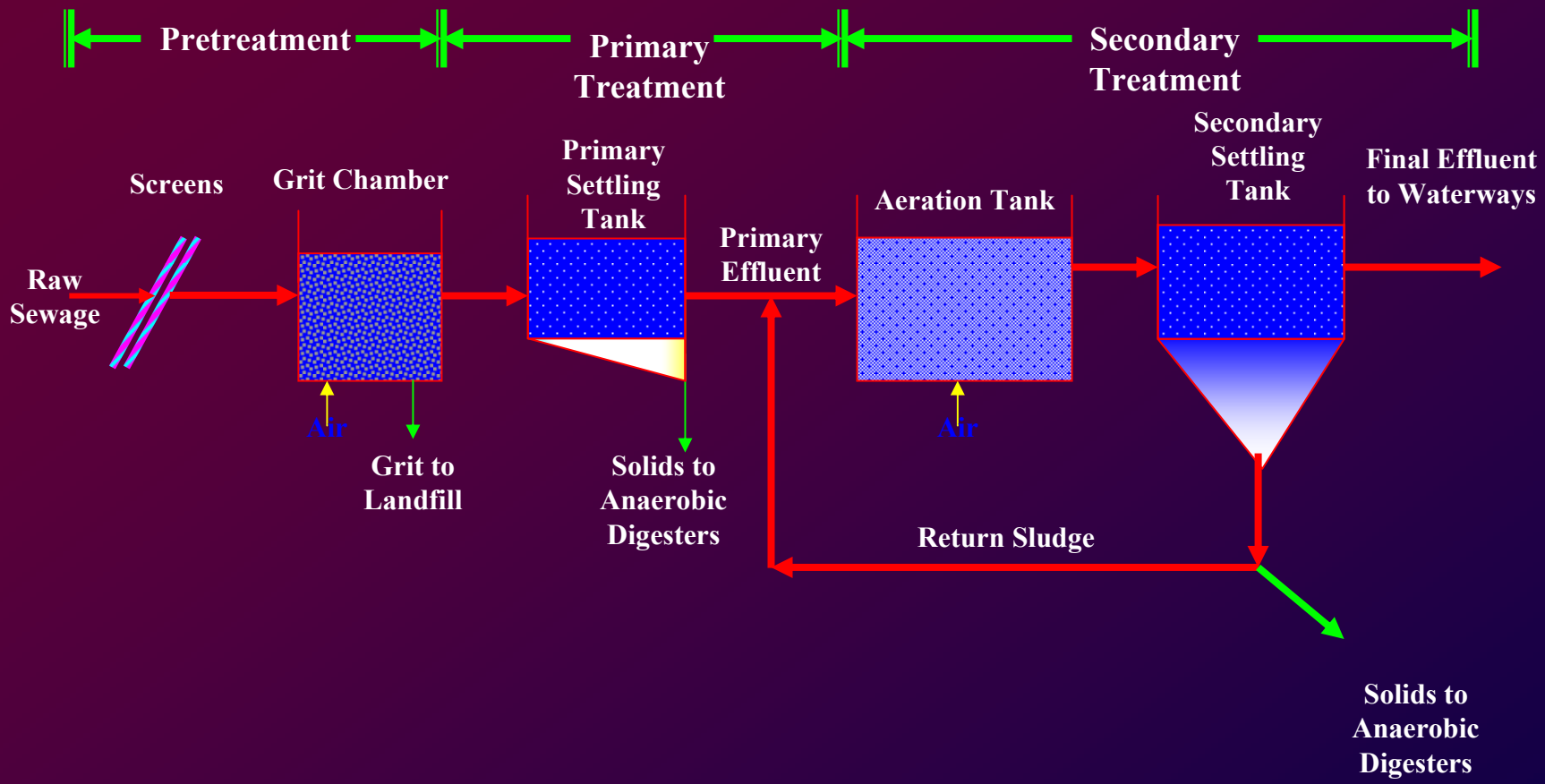
Virus

0.01-0.1 μm

Protein, DNA, Cell Wall, Permeability, Cell membrane


Surface Water
Slurry
Waste Water
Culture plants
Antibiotic use for therapy and prophylaxis
Food
Feces
Animal feed
Hospitalized Patients
Food Animals
Meat products
Hospital Admission
Feces
Antibiotic use for growth promotion, prophylaxis, and therapy
CROP
Humans in Community

WASTEWATER TREATMENT PROCESS



ANTIBIOTICS

- Ampicillin- intestinal, urinary, respiratory, gonorrhea
- Gentamycin-lung, skin, bone, joint, stomach, blood, UTI
- Tetracycline-pneumonia, respiratory,skin, genital, urinary, stomach ulcers
- Medical research has indicated that bacteria have the ability to develop resistance to certain antibiotics if they are misused or overused by people.

How Antibiotic Resistance Happens?

Bacteria faced with a deadly assault

spontaneous change

microbial sex

Free DNA, virus

Vertical transfer: new generations inherit antibiotic resistance genes

Horizontal transfer: bacteria share or exchange sections of genetic material with other bacteria

How Antibiotic Resistance Happens?

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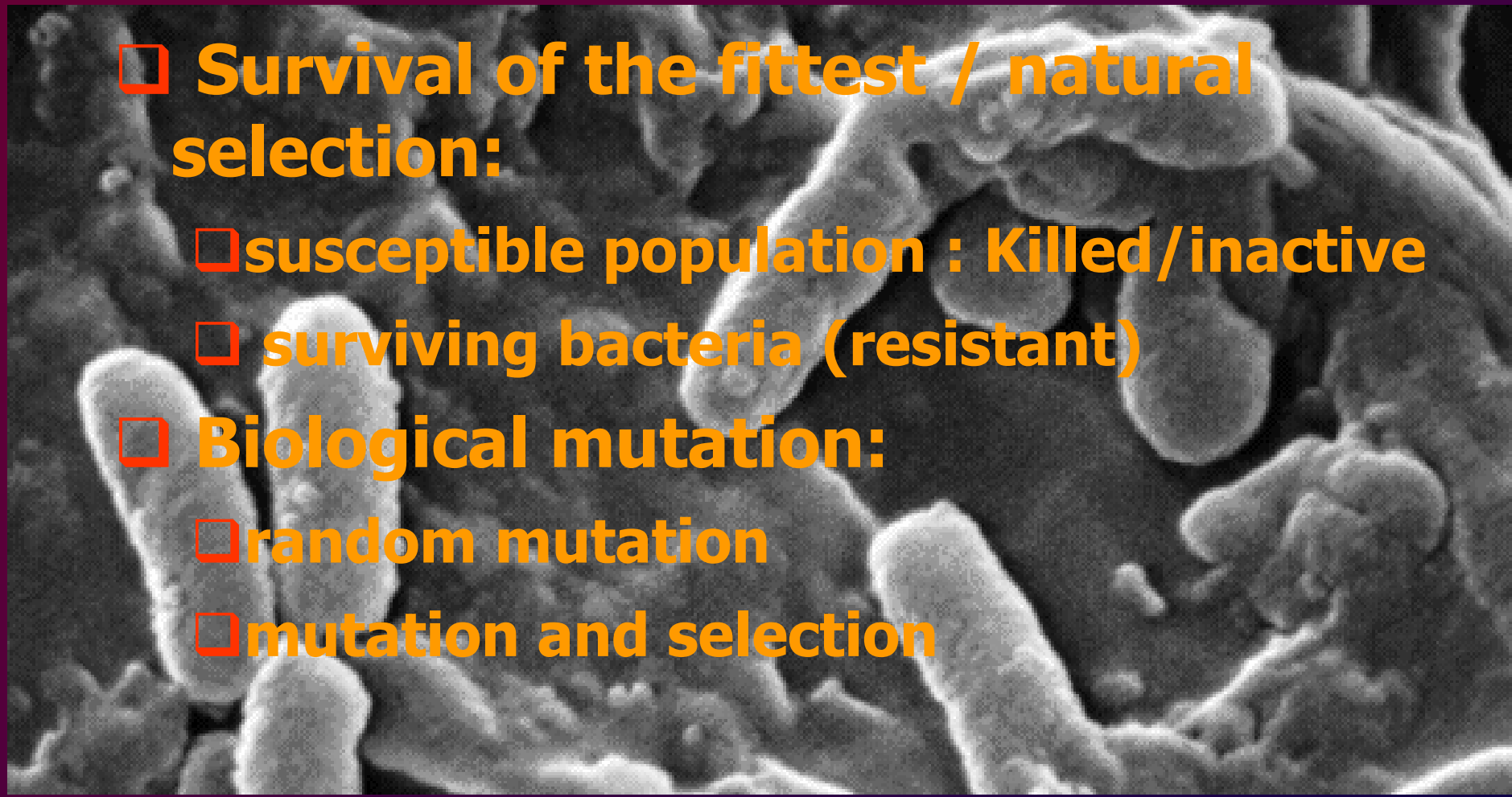
EMERGING INFECTIOUS DISEASES

BACTERIA DEFENSE SYSTEM



- Survival of the fittest / natural selection:
  - susceptible population : Killed/inactive
  - surviving bacteria (resistant)
- Biological mutation:
  - random mutation
  - mutation and selection

Emergence of Antibiotic Resistance FC Bacteria

Selection for Antibiotic Resistant Strains

Antibiotic Resistant Dominant Strains

Evolution of Drug Resistance

Antibiotic Resistance Among Pathogens Causing Hospital-Acquired Infections

IMPACT ON SEWAGE TREATMENT PLANTS

- Infected individuals using antibiotics inappropriately can produce antibiotic resistant bacteria in their systems and discharge them to the sewer system in urine and feces.
- Individuals may flush unused antibiotics down the sink or toilet, and pharmaceutical industry wastewater containing antibiotics may be discharged to the sewer system for disposal.
- This can result in antibiotic resistant bacteria being propagated within the sewage treatment plant.

IMPACT ON THE WATER ENVIRONMENT

- Research indicates that all types of bacteria entering a sewage treatment plant are reduced in number as they pass through the various treatment processes.
- However some bacteria do survive and leave the treatment plant in the effluent or biosolids.
- In rural areas where animal feedlot operations exist, antibiotic resistant bacteria may be present in animal feces which can enter local rivers and streams.

The Potential Role of Concentrated Animal Feeding Operations in Infectious Disease Epidemics and Antibiotic Resistance

Mary J. Gilchrist,[1] Christina Greko,[2] David B. Wallinga,[3] George W. Beran,[4] David G. Riley,[5] and Peter S. Thorne[5]
Environ Health Perspect. 2007 February; 115(2): 313–316.
Published online 2006 November 14. doi: 10.1289/ehp.8837.

CHALLENGES TO THE WASTEWATER TREATMENT INDUSTRY

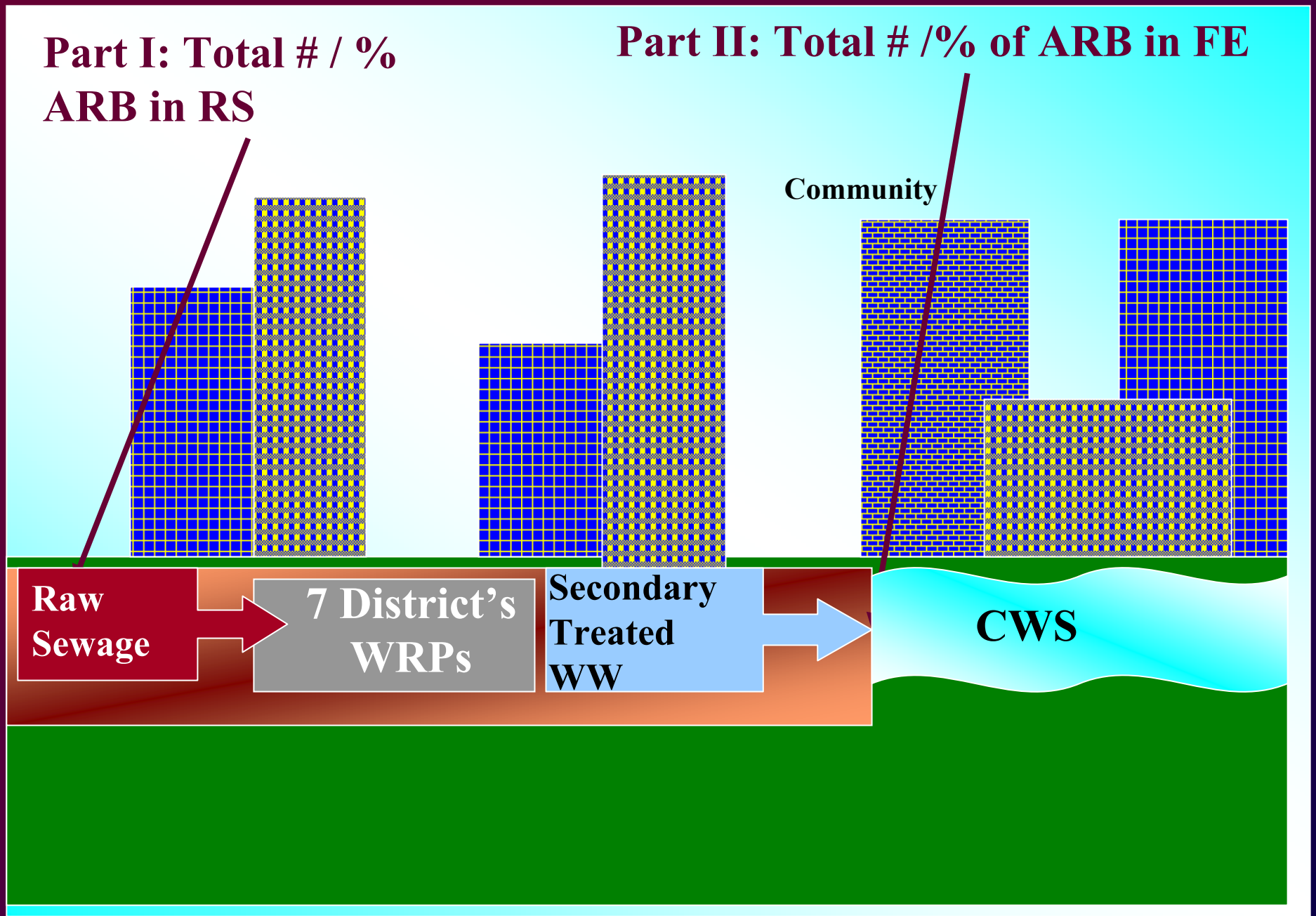
- Antibiotics enter the sewage treatment plant at very low concentrations. Treatment plants do not have the instruments to measure them.
- There is a hypothesis that antibiotic resistant bacteria could be multiplying during the sewage treatment process.
- Treatment plants do not have the sophisticated microbiological facilities needed to analyze for antibiotic resistant bacteria.

WHY SHOULD WE CARE?

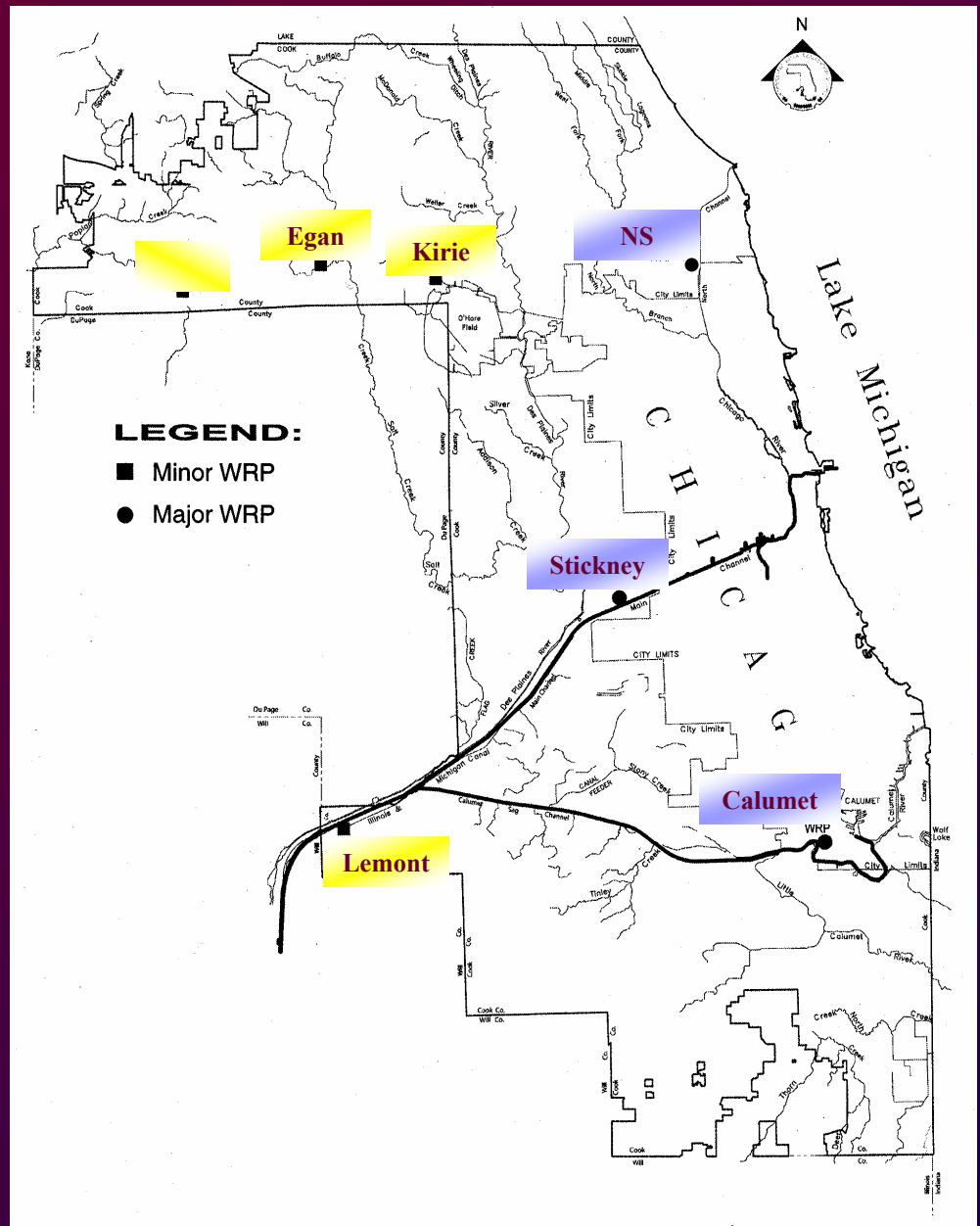
OBJECTIVES

- **To determine the total number and percentage of antibiotic resistant FC in RS and FE at each of the District's Seven WRPs.**
- **To analyze the data statistically to assess the effect of secondary sewage treatment at each WRP on the prevalence of antibiotic resistant FC in FE.**

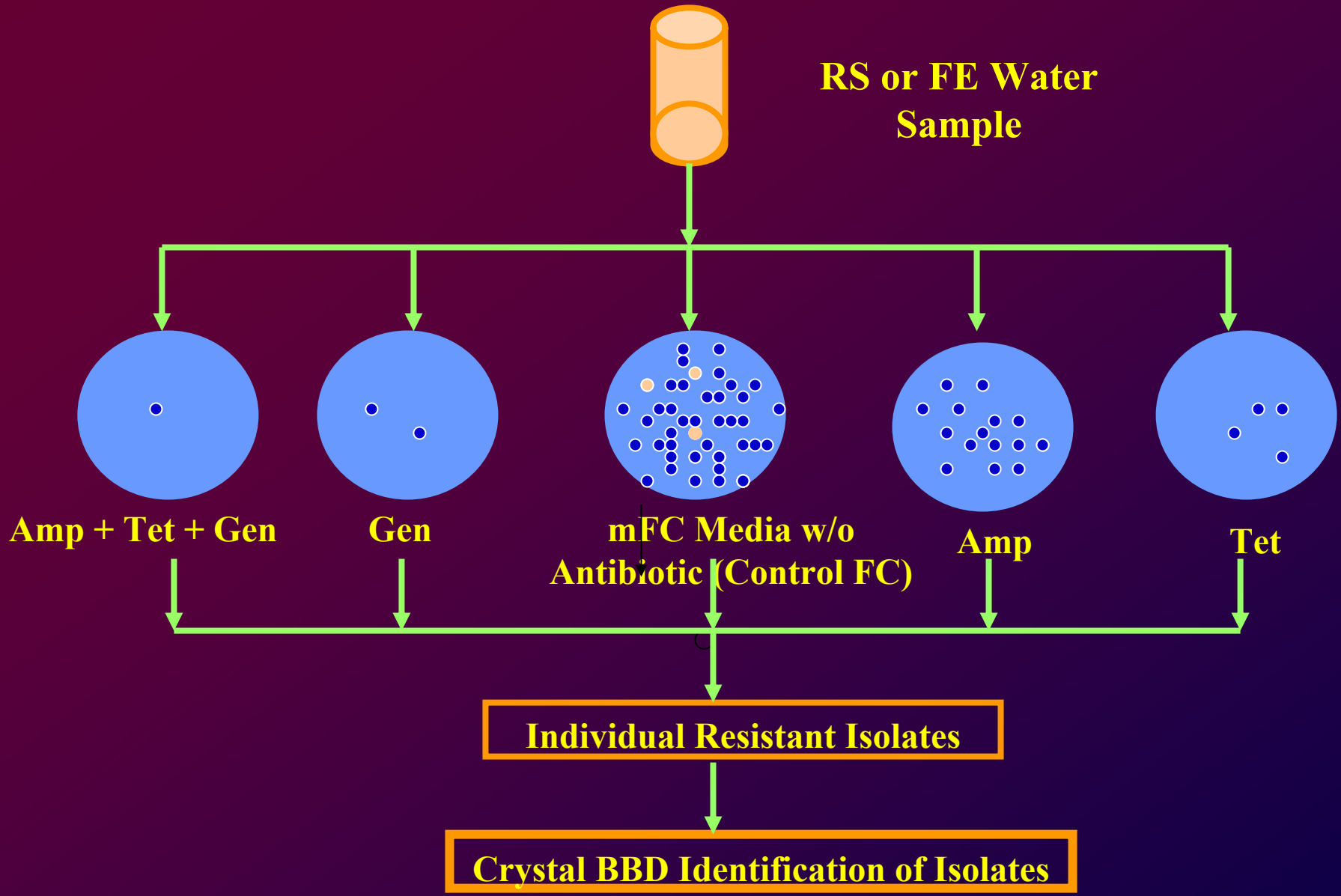
Schematic Representation of the ARB Project



DISTRICT'S SEVEN WRPs



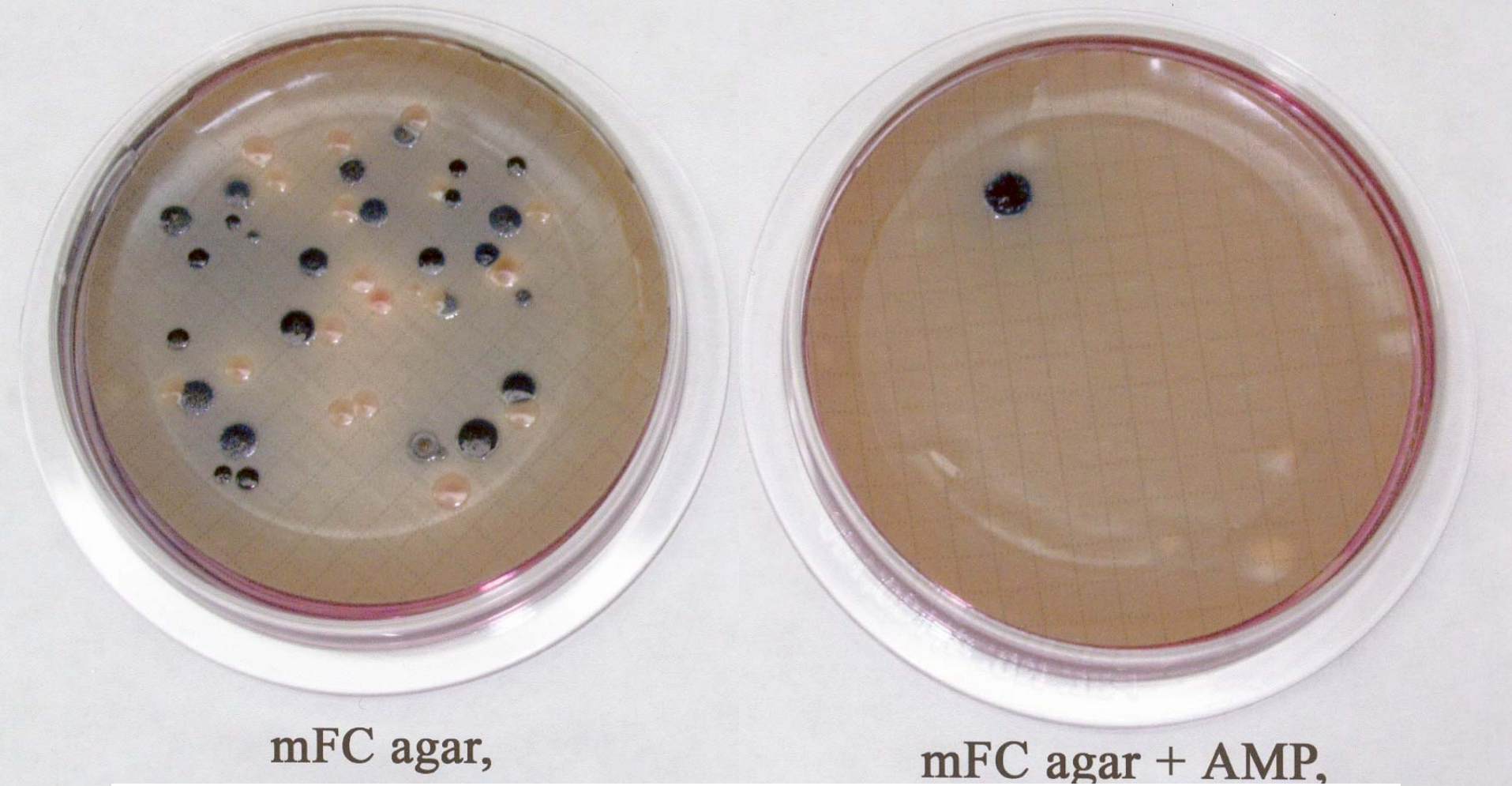
SCHEMATIC OF ARA PROCEDURE



FECAL COLIFORM

CONTROL

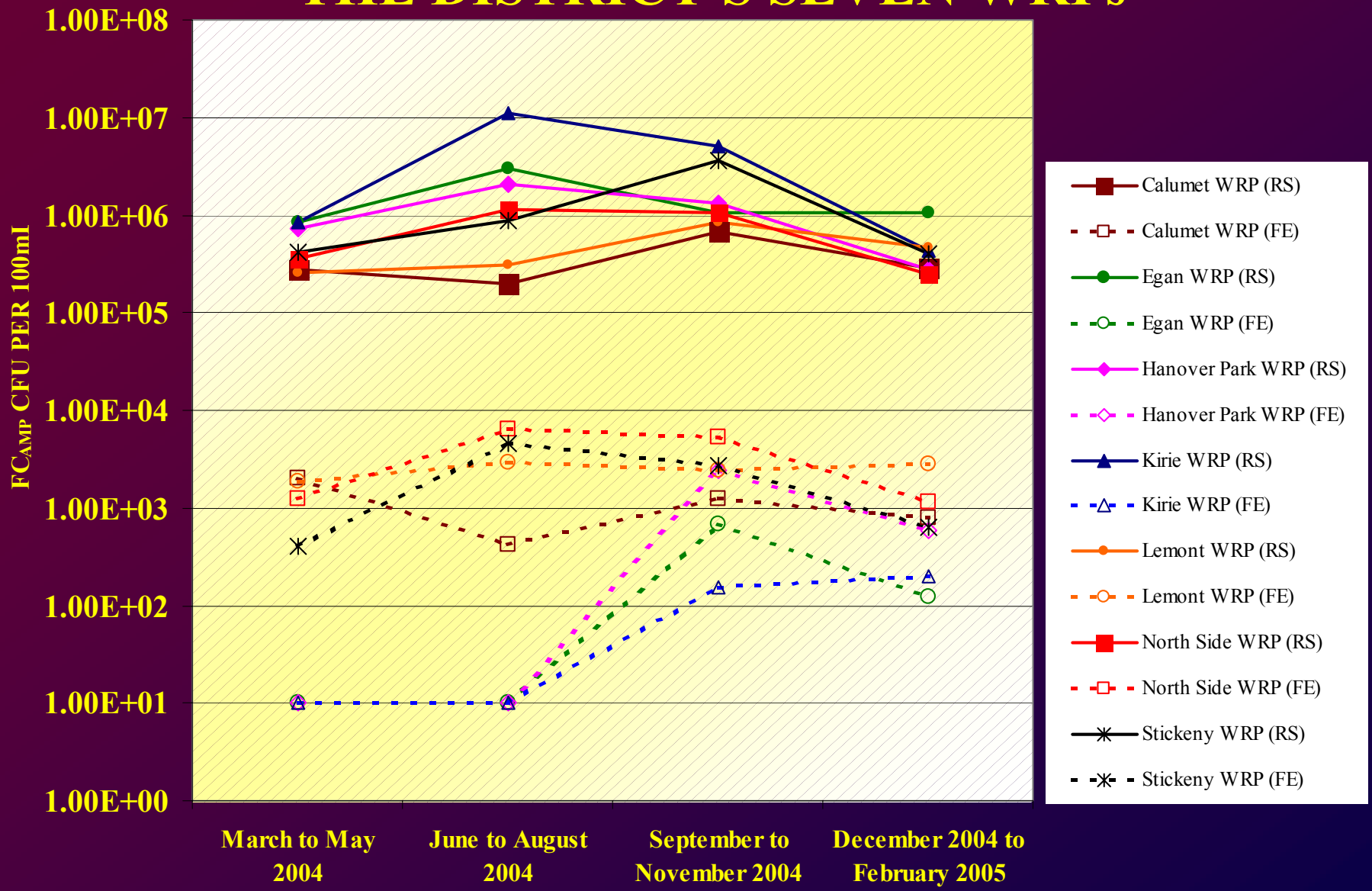
AMP-R



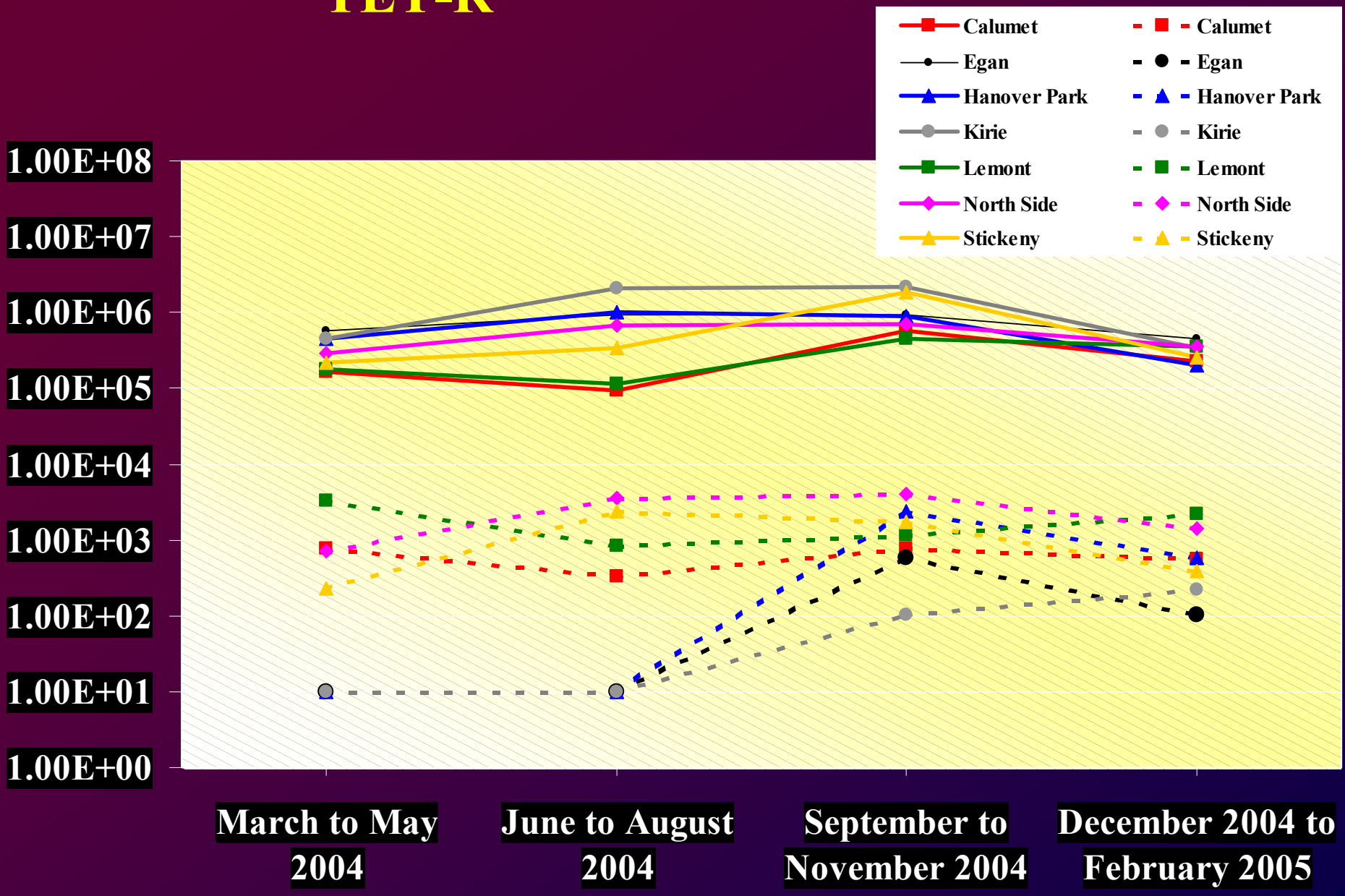
mFC agar,

mFC agar + AMP,

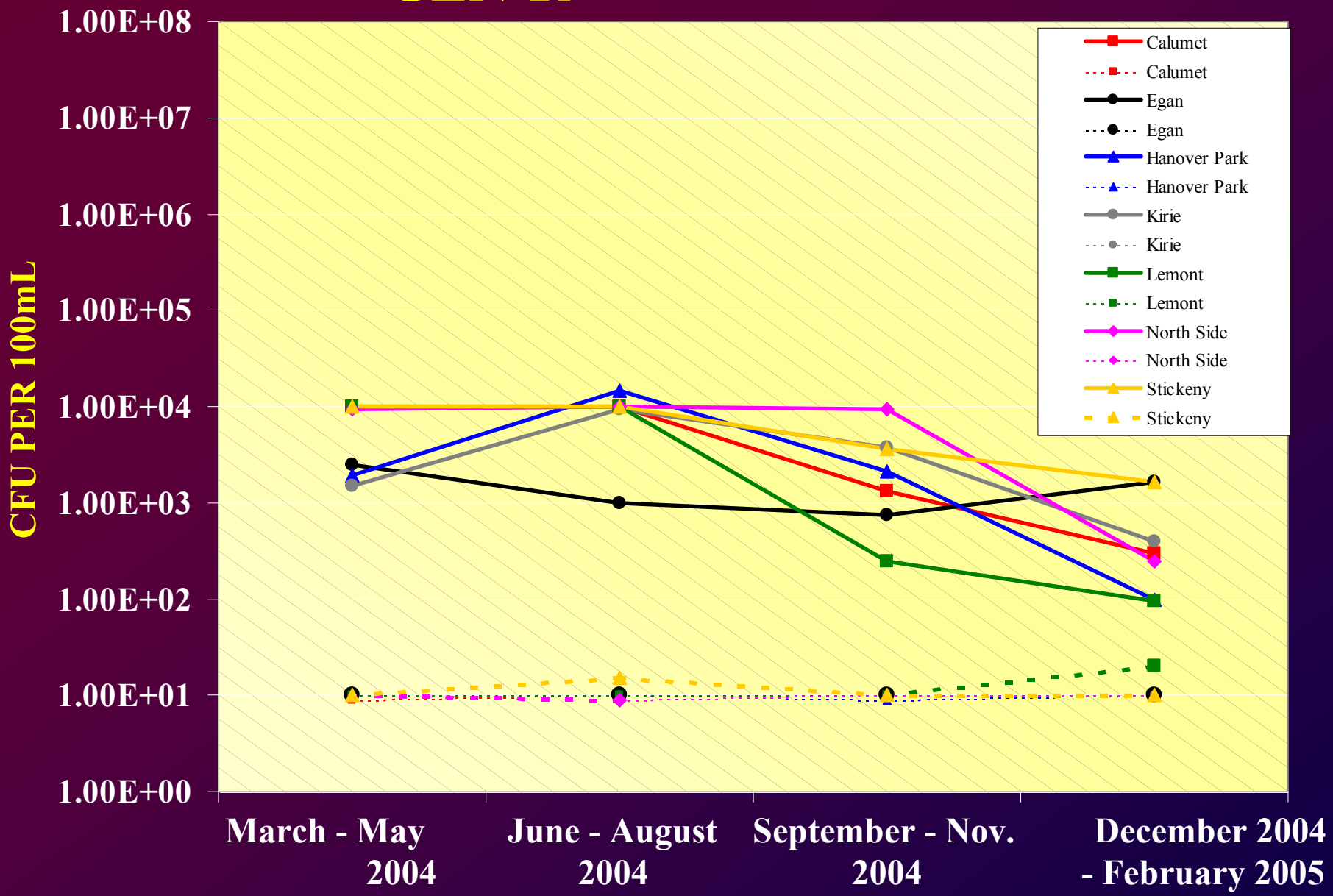
NUMBER OF FC_{AMP} PER 100 mL OF RAW SEWAGE (RS) AND FINAL EFFLUENT (FE) FROM THE DISTRICT'S SEVEN WRPs



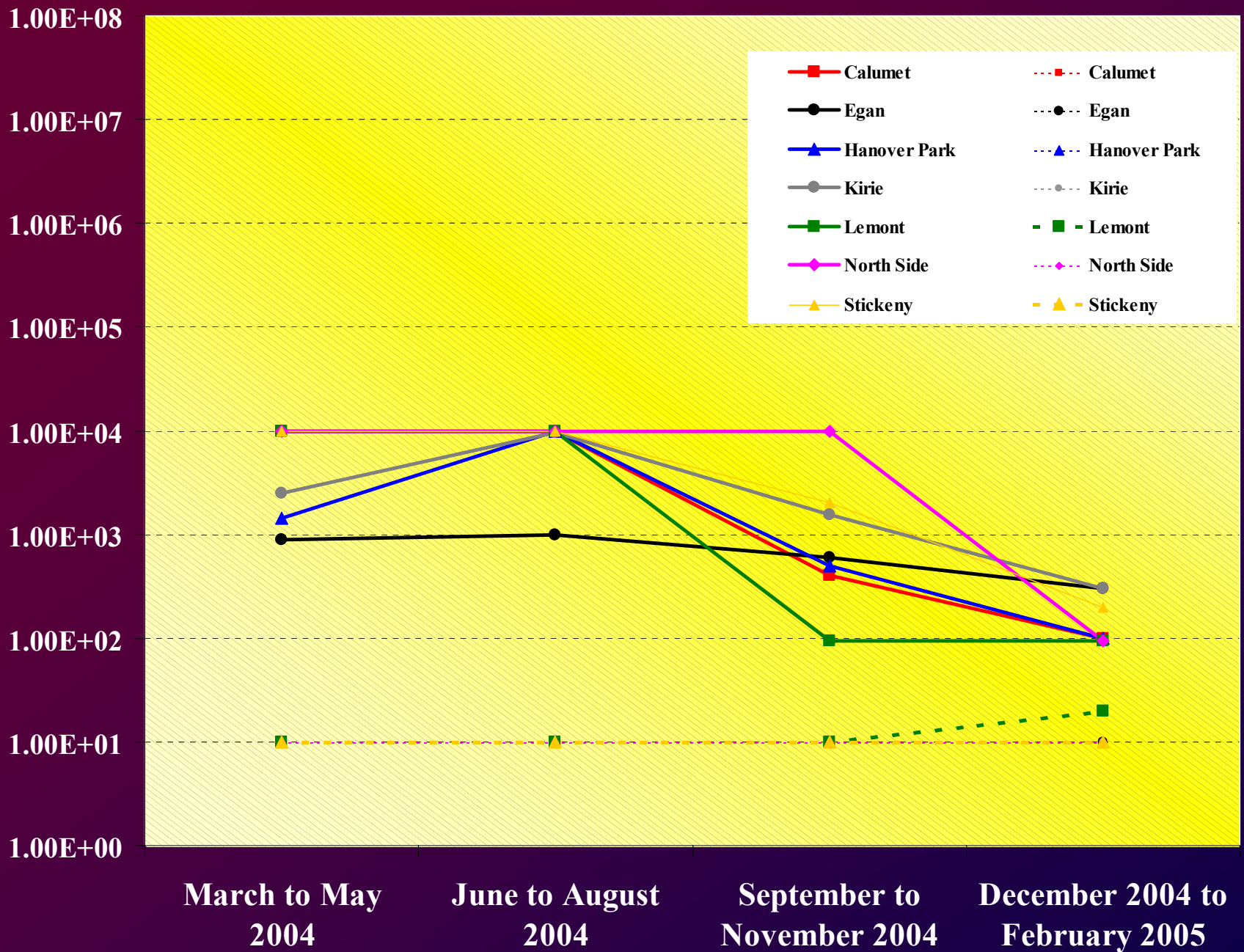
FC TET-R PER 100 mL



FC GEN-R PER 100 mL



FC AMP/TET/GEN-R PER 100 mL



WRP	Predicted Value	FE β_1	FE R^2	RS β_2	RS R^2	Sign. Prob. ($\beta_1 = \beta_2$) T-Test
Calumet	AmpR	0.7842	0.9966	0.8748	0.9998	0.001
Calumet	TetR	0.7250	0.9987	0.8611	0.9997	0.001
Calumet	GenR	0.2595	0.9972	0.4343	0.9931	0.001
Egan	AmpR	0.7497	0.9968	0.9188	0.9992	0.001
Egan	TetR	0.7302	0.9965	0.8777	0.9999	0.001
HP	AmpR	0.8058	0.9976	0.8984	0.9997	0.003
HP	TetR	0.8051	0.9980	0.8725	0.9997	0.006
HP	GenR	0.2545	0.9935	0.4435	0.9676	0.001
Kirie	AmpR	0.7335	0.9968	0.9123	0.9998	0.001
Kirie	TetR	0.7095	0.9871	0.8650	0.9992	0.006
Lemont	AmpR	0.8046	0.9992	0.8845	0.9997	0.001
Lemont	TetR	0.7627	0.9955	0.8534	0.9999	0.001
Lemont	GenR	0.2529	0.9881	0.3242	0.9834	0.007
Lemont	MR	0.2556	0.9900	0.3013	0.9997	0.001
NS	AmpR	0.8389	0.9987	0.8924	0.9999	0.001
NS	TetR	0.8060	0.9986	0.8886	0.9986	0.001
NS	GenR	0.2475	0.9857	0.4468	0.9766	0.002
NS	MR	0.2420	0.9927	0.4241	0.9627	0.004

IDENTITIES OF ARB

Source	FC_{AMP-R}	FC_{TET-R}	FC_{GEN-R}	$FC_{AMP/TET/GEN-R}$
RS	*E. coli*[1] (5)[2]	*E. coli* (4)	*E. coli* (6)	*E. coli* (9)
RS		*K. oxytoca*[3] (1)		
FE	*E. coli* (4)	*E. coli* (5)	*E. coli* (4)	*E. coli* (1)[4]
FE	*K. pneumoniae*[5] (1)	Unidentified (1)[6]		

[1]*Escherichia coli.*
[2]Number of isolates.
[3]*Klebsiella oxytoca*.
[4]Only one colony of $FC_{AMP/TET/GEN}$ was isolated from FE.
[5]*Klebsiella pneumoniae*.
[6]The biochemical profile of this organism is not in the Crystal™ ID System database.

CONCLUSION

- **The percentages of ARB observed in RS followed the trend:**
  $FC_{AMP\text{-}R}$ (11.6 to 46.8) > $FC_{TET\text{-}R}$ (5.8 to 35.7) > $FC_{GEN\text{-}R}$, (<0.01 to 0.29) and $FC_{AMP/TET/GEN\text{-}R}$ (<0.01 to 0.06).
  96% of the ARB isolates from RS (24 of 25 isolates) were identified as *E. coli*.

- **The percentages of ARB observed in FE followed the same trend observed in RS:**
  $FC_{AMP\text{-}R}$ (9.0 to 28.4) > $FC_{TET\text{-}R}$ (5.3 to 21.9) > $FC_{GEN\text{-}R}$ (0.03 to <1.05) and $FC_{AMP/TET/GEN\text{-}R}$ (0.03 to <1.05).
  $FC_{AMP/TET/GEN\text{-}R}$ was virtually eliminated by secondary sewage treatment.
  87 % of the ARB isolates (14 of 16 isolates) from FE were identified as *E. coli*.

CONCLUSION

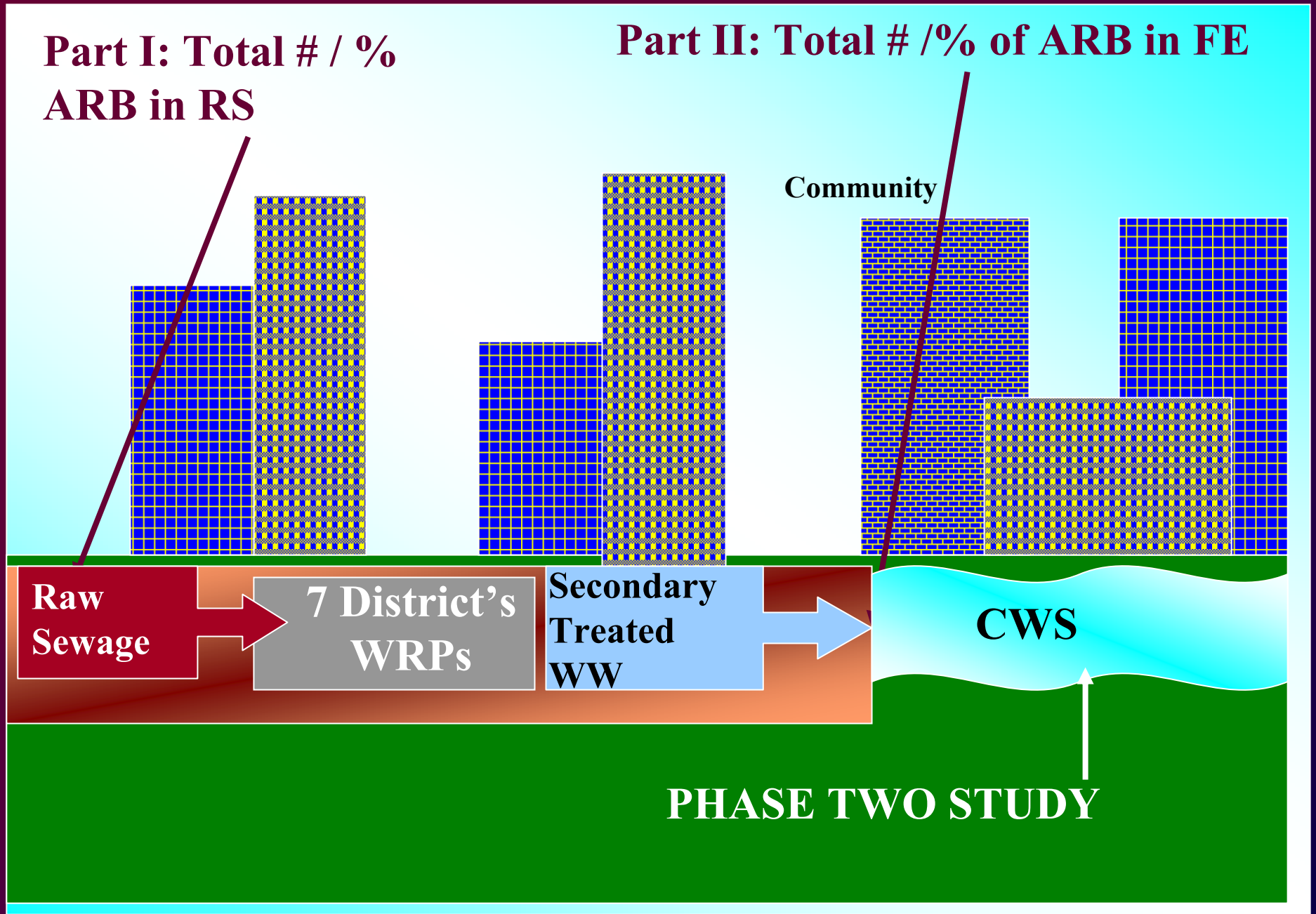
- **Regression Analysis Results to predict ARB concentrations in RS versus FE for equality showed that the percentages of all of these antibiotic resistant FC in the FE from all 7 District WRPs were lower than the percentages of these organisms in RS ($p = <0.01$).**

- **The secondary sewage treatment in the District reduces the numbers and percentages of $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, and $FC_{AMP/TET/GEN\text{-}R}$ in the FE and that the environments in the District's seven WRPs are not conducive to the propagation or survival of these antibiotic resistant organisms.**

PHASE II STUDY

- **The review of Antibiotic Resistant Bacteria study by Drs. Lue-Hing & Patterson.**
- **Study Expansion: to determine the impact of three WRPs FE on the CWS**

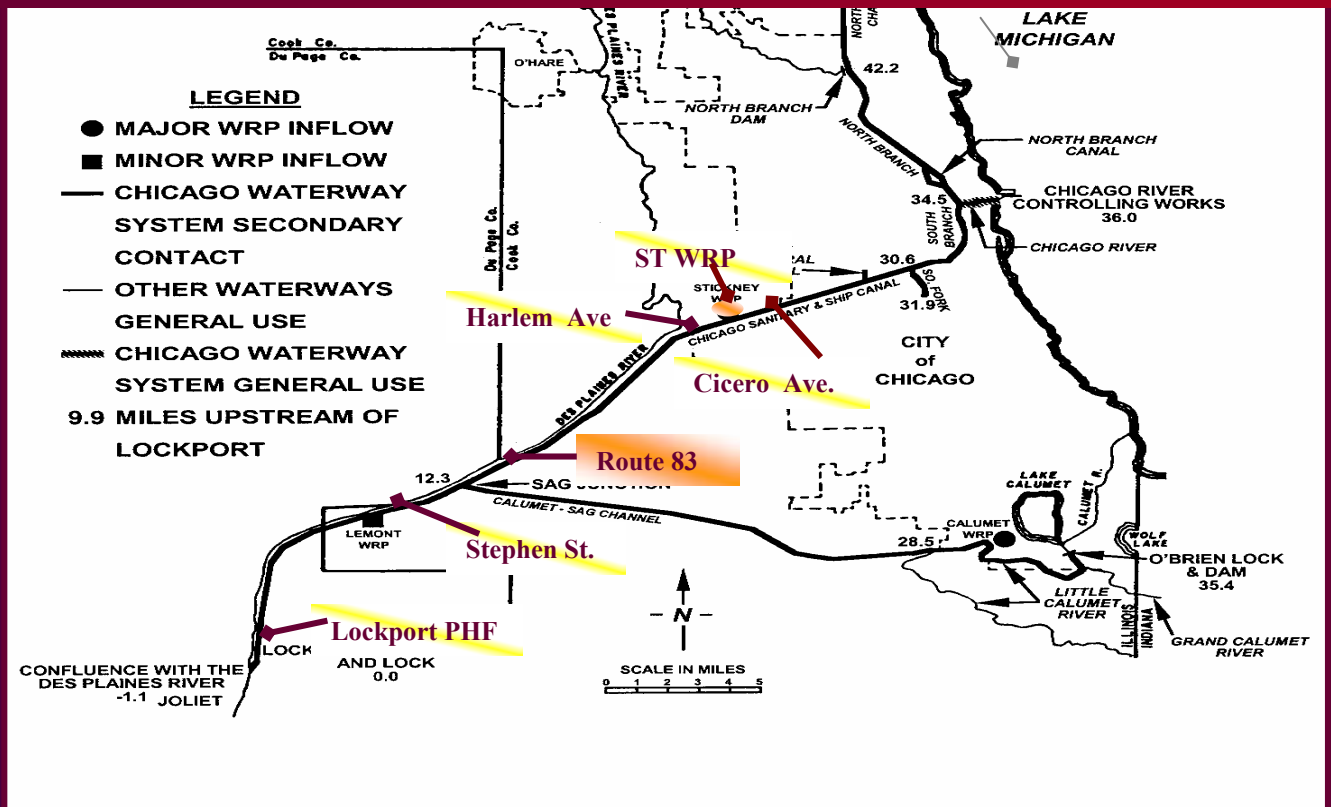
Schematic Representation of the ARB Project



PHASE II
STUDY OBJECTIVES

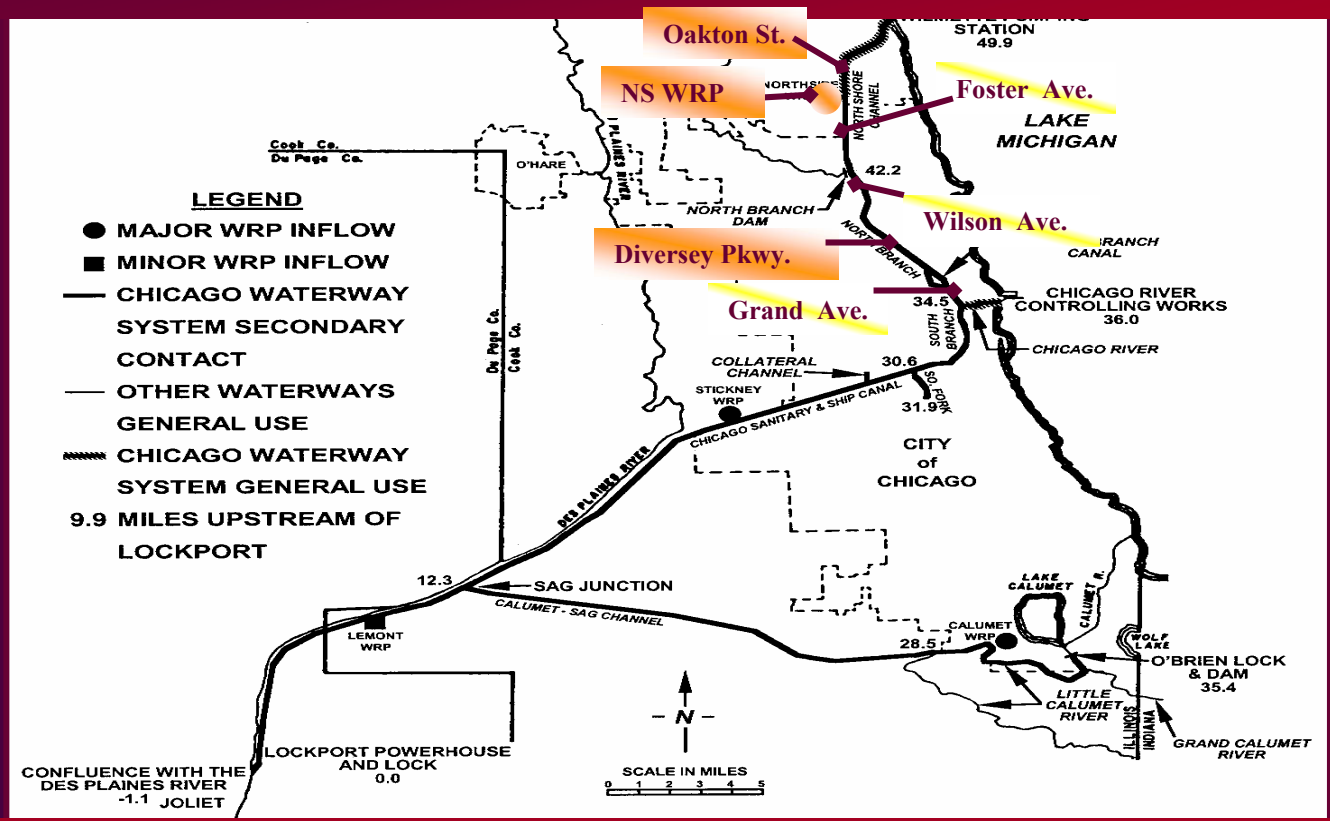
- Survey the Chicago Waterway System (CWS) for AMP-R, TET-R, GEN-R, and AMP/TET/GEN-R FC
- The number and spatial distribution of antibiotic resistant bacteria in the CWS
- Determine whether FC_{AR} represent a public heath hazard and what action, if any, should be taken

CSSC/ST-WRP MONITORING LOCATIONS ANTIBIOTIC RESISTANT FCB



December 2005-October 2006

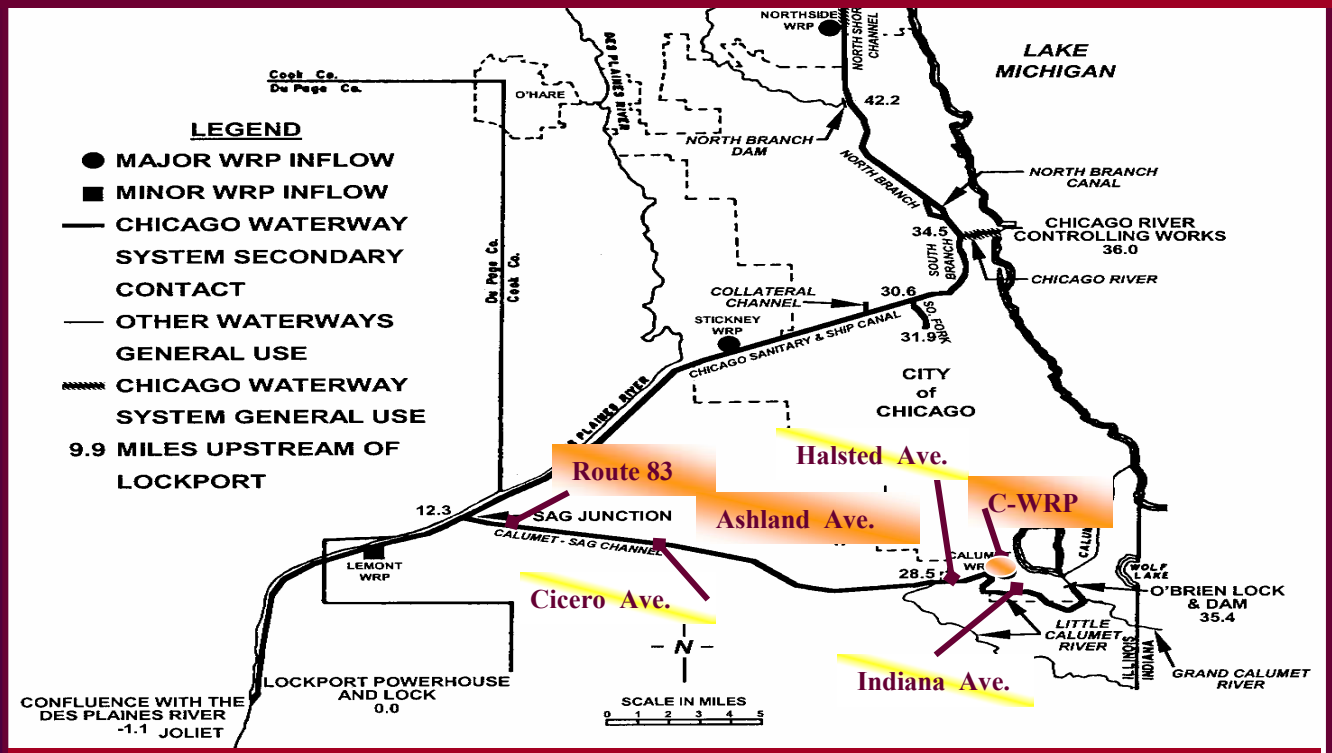
NSC/NBCR/NS-WRP MONITORING LOCATIONS ANTIBIOTIC RESISTANT FCB



December 2006-October 2007

5/6/02

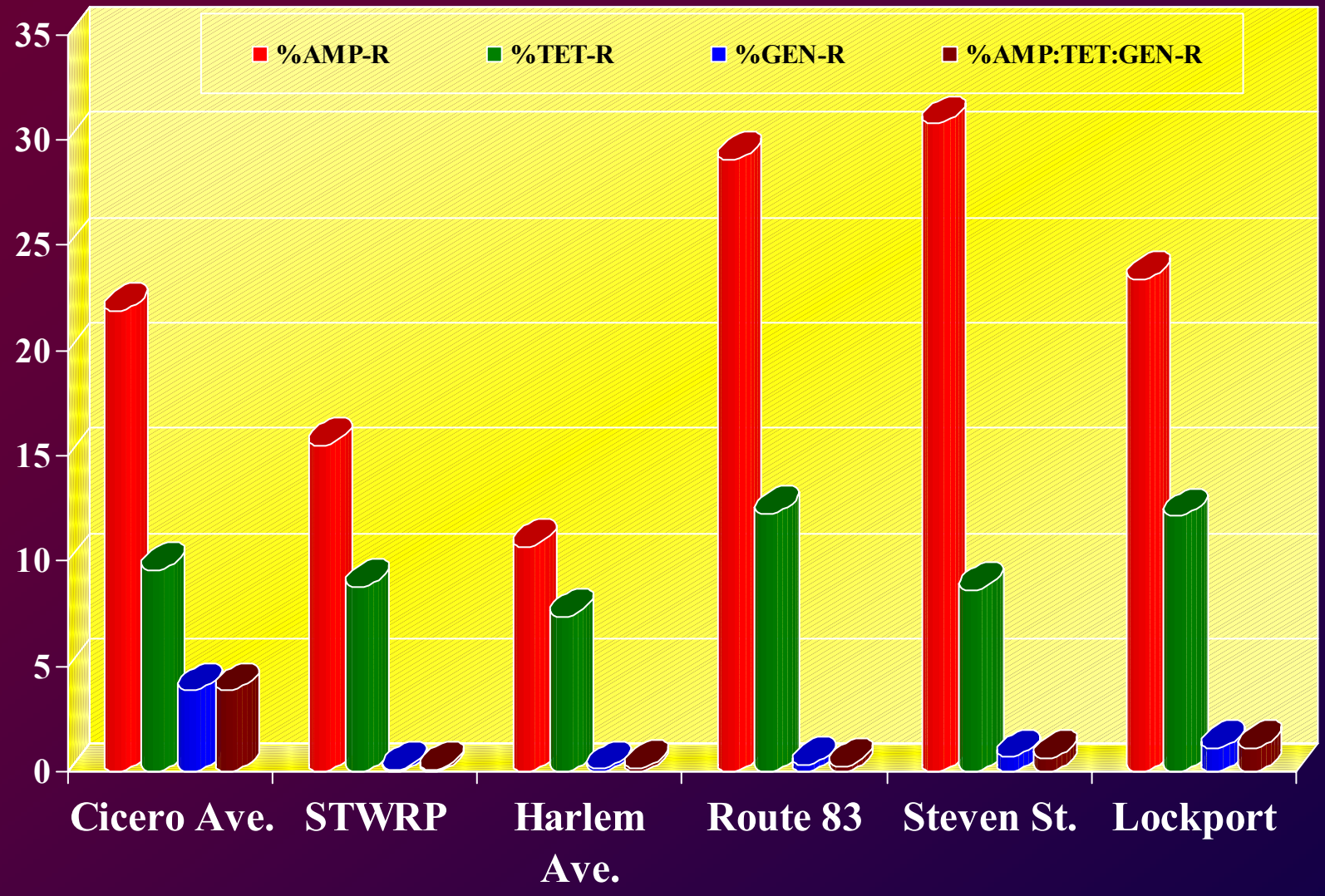
LCR/CSC/C-WRP MONITORING LOCATIONS ANTIBIOTIC RESISTANT FCB



December 2007-October 2008

5/6/02

PERCENTAGES OF FC RESISTANT BACTERIA IN STICKNEY WRP FINAL EFFLUENT AND THE CSSC LOCATIONS



Antibiotic Resistance FC Bacteria (ARB) CSSC Survey

- ARB Percentages in the STWRP final effluent were, in most cases, lower than the CSSC.
- The relative percentages of antibiotic resistant FC in CSSC : $FC_{ampR} > FC_{tetR} > FC_{genR} > FC_{amp/tet/genR}$.
- The identities of resistant FC species in the FE and CSSC were predominantly either *E. coli* or *Klebsiella spp*.

Antibiotic Resistance FC Bacteria (ARB) CSSC Survey

- **Based on this study result, it appears that the secondary treated wastewater without disinfection from the SWRP, do not affect the number, spatial distribution, and composition of ARB in the CSSC receiving waterways.**

CDC EMERGING INFECTIOUS DISEASES

Research

Antibiotic Resistance of Gram-Negative Bacteria in Rivers, United States

Ronald J. Ash,* Brena Mauck,* and Melissa Morgan*
*Washburn University, Topeka, Kansas, USA
Vol. 8, No. 7, July 2002

- The presence of antibiotic-resistant bacteria in freshwater samples from 16 U.S. rivers at 22 sites and measured the prevalence of organisms
- The range of % AMP Resistant gram negative bacteria (3.9 to 53) is larger than the range of % of FC $_{AMP-R}$ in FE (9-28.4) & in CSSC (10.7- 29.1).

WHAT SHOULD WE DO?

- More research needed. Study is in progress to survey the CAWs.
- Use antibiotics properly.
- Dispose of antibiotics properly.
- Study animal feedlot operations more carefully.

Campaign to Prevent Antimicrobial Resistance

Centers for Disease Control and Prevention
National Center for Infectious Diseases
Division of Healthcare Quality Promotion

Clinicians hold the solution!

- *Link to: Campaign to Prevent Antimicrobial Resistance Online*
- *Link to: Federal Action Plan to Combat Antimicrobial Resistance*

News & Public Affairs

Office of National Drug Control Policy

•Feb.20, 2007 CONTACT: Jennifer de Vallance, ONDCP
(202) 395–6648 / (202) 368–8422

•FEDERAL GOVERNMENT ISSUES NEW GUIDELINES FOR PROPER DISPOSAL OF PRESCRIPTION DRUGS:

•WHAT EVERY AMERICAN CAN DO TO PREVENT MISUSE OF PRESCRIPTION DRUGS

- Do not flush medications down the toilet instead, remove label and dissolve medications, mix with items (kitty litter, coffee grounds, etc) and seal in a bag
- Return unused, unneeded, or expired prescription drugs to pharmaceutical take-back locations that allow the public to bring unused drugs to a central location for safe disposal

ACKNOWLEDEMENTS

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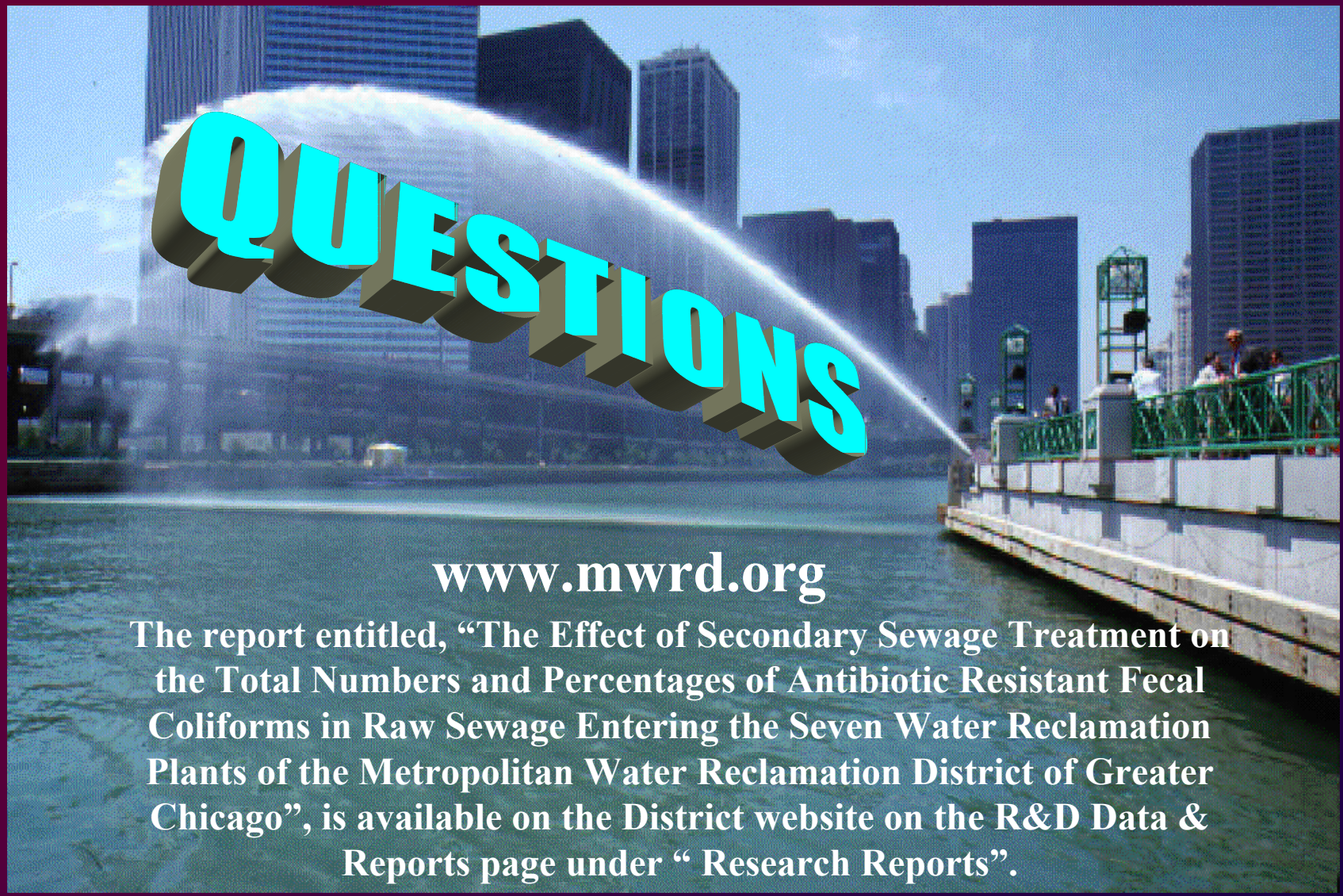

www.mwrd.org

The report entitled, "The Effect of Secondary Sewage Treatment on the Total Numbers and Percentages of Antibiotic Resistant Fecal Coliforms in Raw Sewage Entering the Seven Water Reclamation Plants of the Metropolitan Water Reclamation District of Greater Chicago", is available on the District website on the R&D Data & Reports page under " Research Reports".