Drug Resistance in Environments Associated with Aquaculture

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Aquaculture

- Important source of protein
- The number and type of aquaculture products will continue to increase
- Provides important source of jobs
- Provides important source of money for individuals, companies, countries

USA Aquaculture Production 2004

Catfish- 630 million lbs	**$ 439 million**
~140,000 acres	
Trout	$ 57 million
Salmon-394 million lbs	**$ 871 million**
Clams- 5.8 million lbs	$ 11 million
Oyster- 7.5 million lbs	$ 17 million
Mussels-1 million lbs	
Ornamental fish	$ 44 million
TOTAL	$ 1,439 million

From Aquaculture outlook report May 2005

Fish Farming

- 40% of world's fish consumption is farmed
- ~ 90% fish consumed in Japan & Norway is farm raised
- Marine fish systems-open system where waste is dumped directly into water or waste can be contained as done in Norway
- Land base systems- usually closed where seepage is into environment

Asia


Laboratory
Display and Quarantine House
Office
Breeding Greenhouse
Domitory
Breeding Ponds
Breeding Greenhouse #1
Spawning Greenhouse
Yamasaki Koi Farm

Gram-negative Aquaculture Associated Bacteria

- Pathogenic Bacterial species (7 genera):

  Aeromonas, *Edwardsiella*, *Flavobacterium*, *Pasteurella, Photobacterium, Vibrio, Yersinia*

- Associated bacteria normally not pathogenic (17 genera):

  Acinetobacter, *Alteromonas*, *Alcaligenes*, *Brevundimonas*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Morganella*, *Moraxella*, *Providencia, Pseudomonas*, *Ralstonia*, *Salmonella*, *Serratia*, *Sphingomonas, Stenotrophomonas, Vibrio*

Bacterial Movement

- Open marine based farm-fish food, fish waste into water- spread with tides
- Closed land based- water and solids distributed in environment during floods, typhoons, hurricanes, earthquakes, when ponds drained
- Move with fish eggs, fish, fish food (often dead fish) products, fish fertilizer, shipping material (ice)

Environment

- Fish farms increase bacterial population of surrounding environment
- Open marine fish farms –increases the bacterial population under the net 10,000-fold
- Land based-similar increase in bacteria at bottom of pond and circulating water

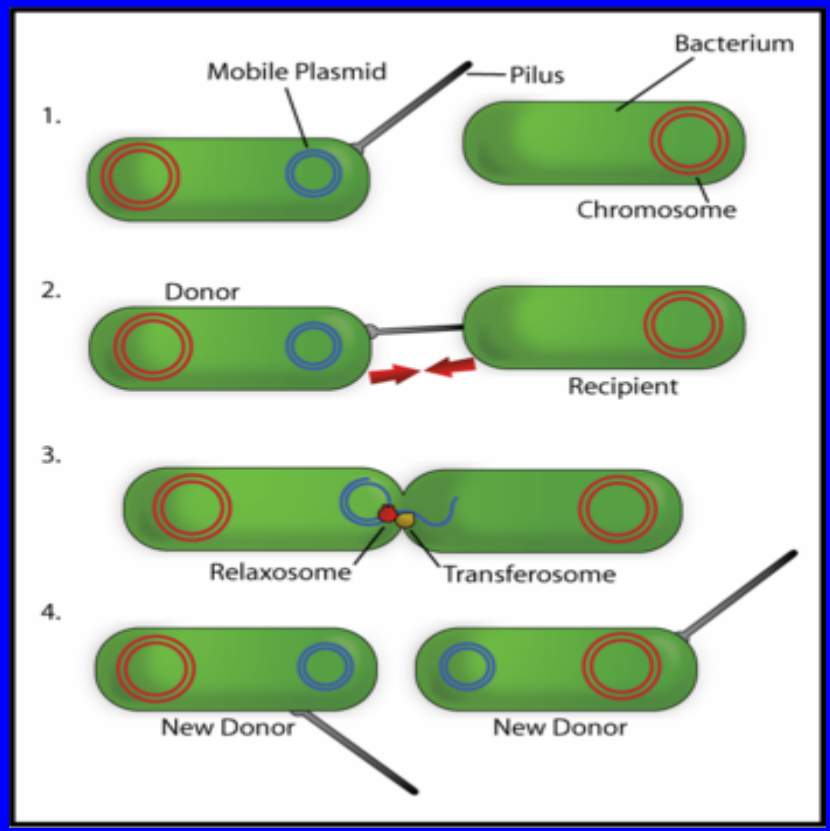
Antibiotics

- Widely available since 1950's
- Use for **treatment** of diseases in man, animal, fish and plants
- In No. America used to **prevent** disease in man, animal, fish and plants
- Used to treat non-infectious diseases
- Antibiotic resistant bacteria developed in response to antibiotic use

Antibiotic Resistant Bacteria

- Most resistance is due to the presence of new genes- usually associated with mobile elements
- Genes can move between bacteria that are related and not related
- Easy to spread through ecosystems
- EPA now considers antibiotic resistance genes contaminates which should be contained

Plasmid Antibiotic Gene Exchange [conjugation]



Disease due to Antibiotic Resistant Bacteria

- Reduction in therapeutic options
- Cost to treat higher
- Treatment is longer
- Increased mortality

Antibiotics and Aquaculture

- Tetracyclines have been commonly used in aquaculture (salt and fresh water) over last 50 years
- Salmon eat other fish- food is often fish which can be toxic so antibiotics mixed with the food especially in Asia
- Large numbers of genetically identical animals – increase problems with disease- thus often treated to prevent
- As a results Tc^r aquaculture associated bacteria are common

Catfish farm-land based

American Catfish Ponds

- Study done with USDA
- Bacteria from US catfish food were resistant to tetracycline
- Food labeled as antibiotic-free had varying levels of antibiotics

1993 Mol & Cell Probes, DePaola et al 7:345

American Catfish Ponds

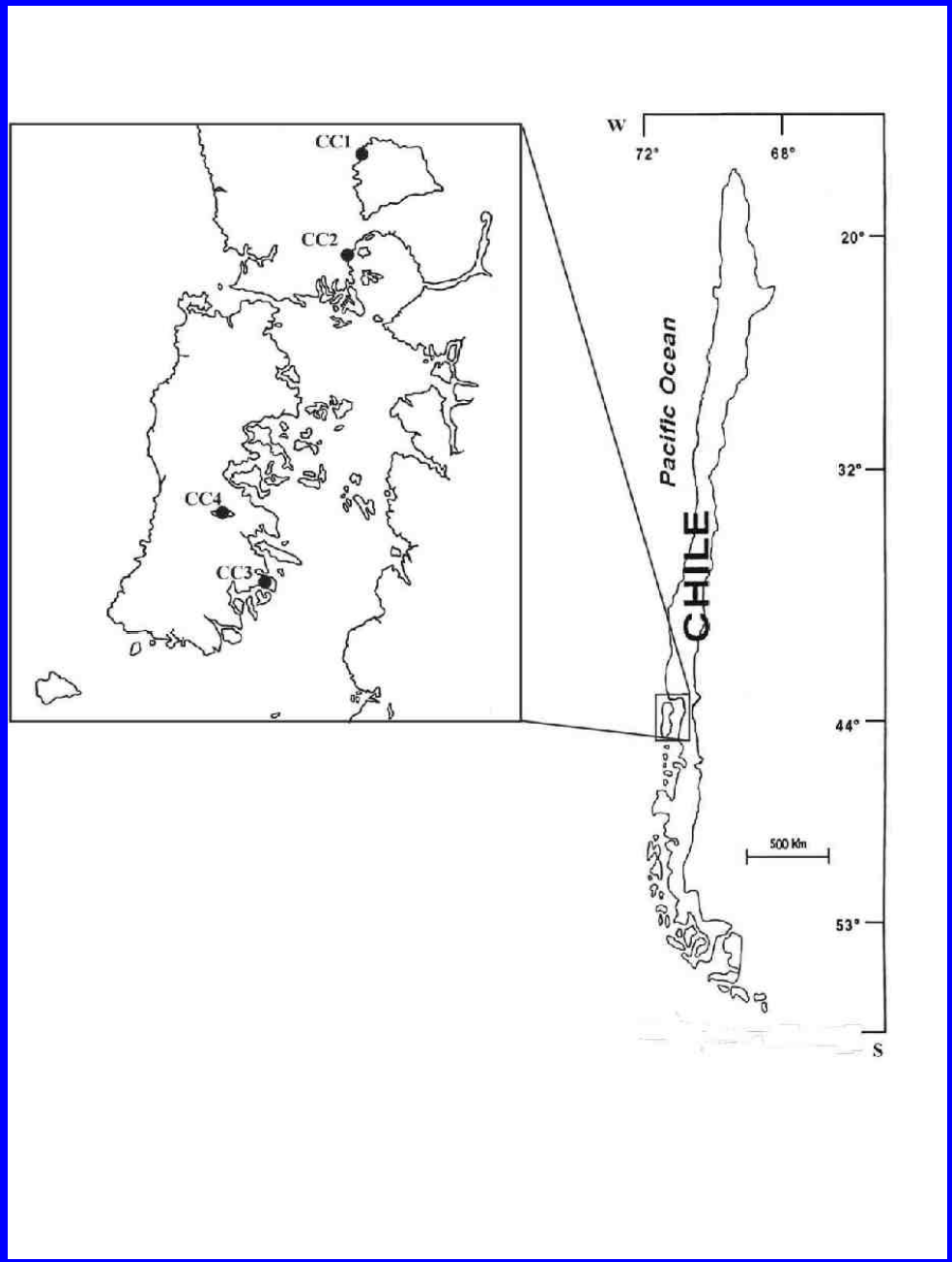
- Found tetracycline resistance (*tet*) genes which are common in bacteria causing human disease
- Found novel *tet* genes not previously found in clinical isolates
- Suggests that there is more diversity in resistance genes in aquaculture environment
- Data suggested that some *tet* genes were preferentially associated with water bacteria

1995 Mol & Cell Probes DePaola & Roberts 9:311

Chilean Salmon Farms

- Second largest salmon producer in the world- 679 million lbs (1.7 times 2004 US production) in year 2002
- Major exporter of salmon
- Intensive use of antimicrobials for prevention and control with tetracyclines most commonly used drug

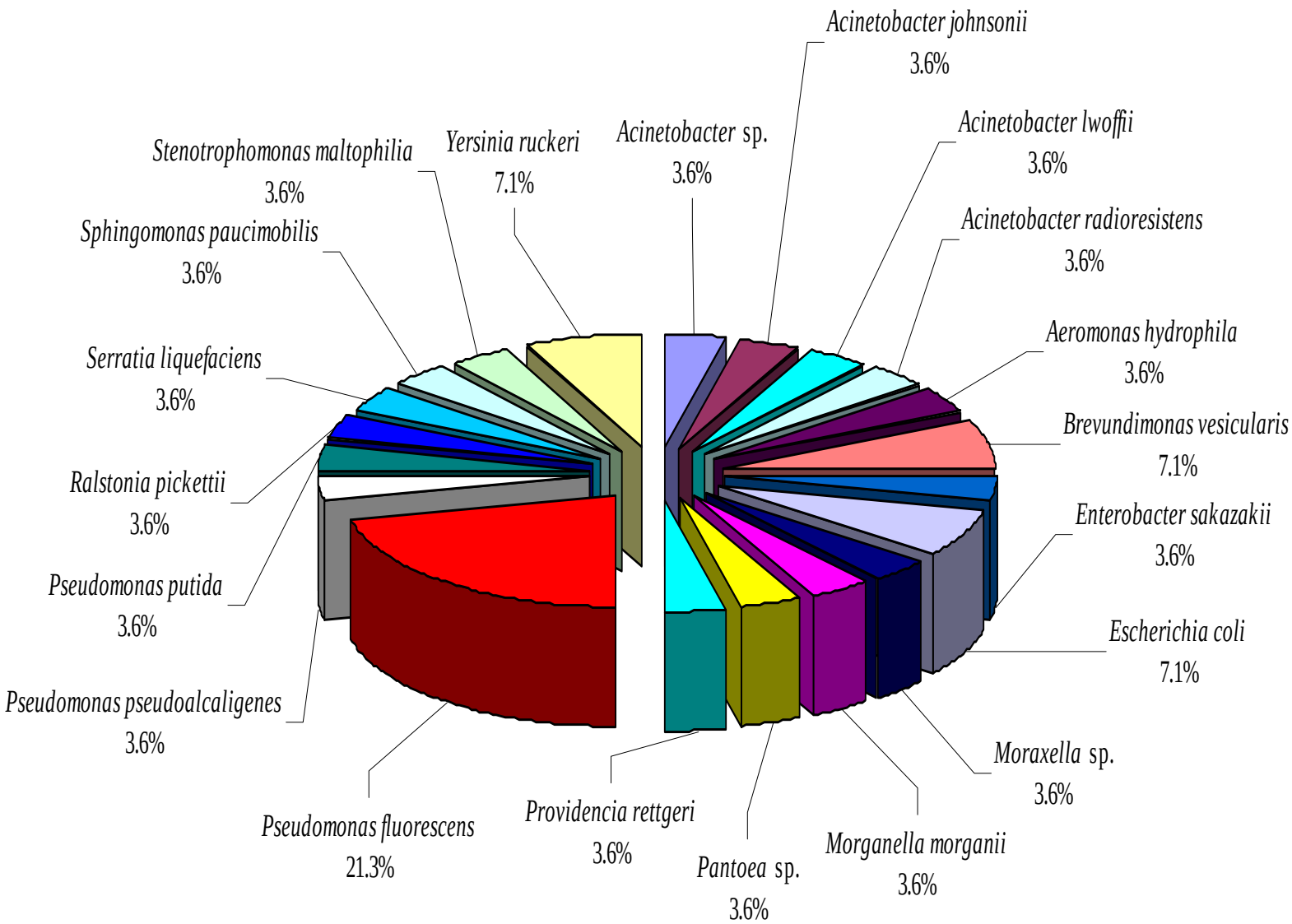
Site of Farms

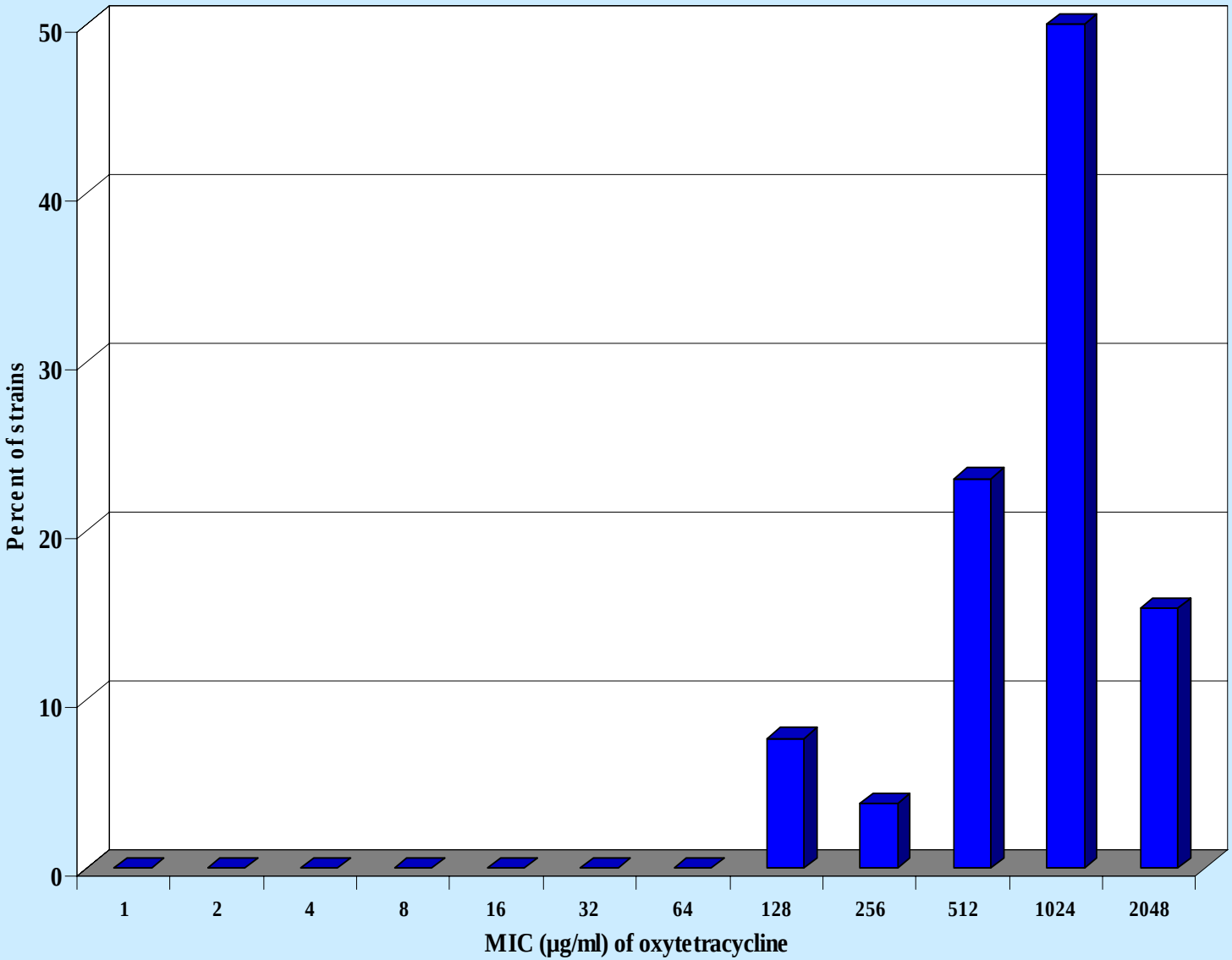


Chilean Salmon Farms

- Four fresh water salmon farms in Southern Chile
- No oxytetracycline exposure ($\geq$ 6 months)
- Farm # 1-3 land based, # 4 water based
- Cultures were from: farms water, water going in and out of the tanks, surface water non-medicated food and salmon fingerlings

Miranda, C.D. et al. 2003 AAC 47:883


Acinetobacter johnsonii
3.6%
Acinetobacter lwoffii
3.6%
Acinetobacter radioresistens
3.6%
Aeromonas hydrophila
3.6%
Brevundimonas vesicularis
7.1%
Enterobacter sakazakii
3.6%
Escherichia coli
7.1%
Moraxella sp.
3.6%
Morganella morganii
3.6%
Pantoea sp.
3.6%
Providencia rettgeri
3.6%
Pseudomonas fluorescens
21.3%
Pseudomonas pseudoalcaligenes
3.6%
Pseudomonas putida
3.6%
Ralstonia pickettii
3.6%
Serratia liquefaciens
3.6%
Sphingomonas paucimobilis
3.6%
Stenotrophomonas maltophilia
3.6%
Yersinia ruckeri
7.1%
Acinetobacter sp.
3.6%


50
40
30
20
10
0
Percent of strains
1
2
4
8
16
32
64
128
256
512
1024
2048
MIC (µg/ml) of oxytetracycline

Bacteria Cultured

- 103 Gram-negative tetracycline resistant environmental bacteria identified
- **74 (72%) of the isolates were resistant to 6-10 antimicrobials**
- Oxytetracycline MICs 128-2048 μg/ml
- Viable Tc^r bacteria common in the commercial food pellets used

Chilean Salmon Farms

- *tet* resistance genes found in 40-80% of the isolates from each farm
- Resistant bacteria found in fish food, fish and water samples
- 60% had known *tet* genes; 40% had unknown *tet* genes
- More diversity seen
- Same *tet* genes as previously found in bacteria from catfish ponds

Fish farm in British Columbia Canada

Salmon Production Eastern Canada

- 2004; > $250,000 Canadian & 35,000 MT produced
- Important employment to rural communities
- Government support

Aeromonas salmonicida

- In 1989 an atypical *A. salmonicida* isolated causes salmon and trout disease furunculosis
- Carried a unique plasmid
- 1992-2001 Eastern Canada had continuing problems with this disease in hatcheries and sea cage sites
- Examined the bacteria to determine if the problem was due to strain or multiple strains over 10 years

North American psychrophilic *Aeromonas salmonicida*

- Nine atypical *A. salmonicida* from 1992-2001 from farmed and wild fish Eastern Canada
- Atypical *A. salmonicida* grows between 4-15 ^{0}C
- Eight isolates 1992-1999 carried *tet*(A) on 58 kb plasmids- these were related strains
- One 2001 isolate carried *tet*(B) gene-new strain
- One dominate strain from 1992-1999 then different strain was identified in 2001

Casas et al., 2005. FEMS Micro Lett. 242:59-63.

North American psychrophilic *Aeromonas salmonicida*

- Six isolates could transfer their plasmids and *tet*(A) gene to *E. coli* at 15 ^{0}C
- Lowest temperature that conjugation has been documented to occur
- **Suggests direct transfer between the *A. salmonicida* and *E. coli* could occur in nature-this bacteria could act as a reservoir for antibiotic resistance genes**

Transfer from water bacteria

- *Chlamydia suis* obligate intracellular bacterium in pigs
- 10 kb region from *A. salmonicida* (aquaculture bacteria) plasmid pRAS3.2 with the *tet*(C) gene found in Tc^r *C. suis*, pig pathogen, from Midwestern US farm using tetracycline as food additive in the pig feed
- *A. salmonicida* grows at < 20 ^{0}C; *C. suis* does not
- Question: Unlikely direct transfer- what intermediate bacteria was involved in the transfer from *A. salmonicida* to *C. suis*? Both co-infect the same cell
- First documentation of horizontal gene transfer into an obligate intracellular bacterium

Dugan et al. 2004 AAC 48:3989

Tropical Fish

- 12 million Americans have ornamental fish tanks
- Australia- 2000 new multidrug resistant *S. paratyphi* [causes serious gastroenteritis] identified
- Same strain found in people and ornamental fish tanks
- Most fish imported from Asia- quarantined, dosed with high levels of antibiotics
- Hypothesized that instead of eliminating the pathogens process selected for the highly resistant *S. paratyphi*
- First time to verify that ornamental fish tanks are potential reservoir for antibiotic resistant pathogens

Levings et al., 2006. Emerging Infect. Dis 12:507;

Aquaculture Bacteria

- Aquaculture bacteria are a reservoir for human bacterial pathogens including:
  Salmonella typhimurium
  Yersinia enterocolitica & *Vibrio* spp.
- Aquaculture bacteria are a reservoir for antibiotic resistance genes and mobile elements for both human and other ecosystems
- Resistance genes once in one bacterium able to move through and between other bacterial populations and ecosystems

Aquaculture Bacteria

- Need to think of the world as a single connected system where changes at one location may lead to changes in distant locations in totally unrelated bacteria
- Aquaculture practices in the developing world does impact us locally- foreign raised food may contain antibiotic resistant bacteria, pathogenic bacteria and/or antibiotic residues

Conclusion

- Surveillance studies from aquaculture associated bacteria should be done from all countries that have fish farms
- Surveillance studies in fish farms from Europe, Japan, USA may not represent what is found in other areas
- *tet* genes in aquaculture associated bacteria are diverse and can differ from those found in man and animal associated bacteria
- Question: How do these genes move between bacteria and around the world?

Conclusion

- Aquaculture exposes many new bacteria to antibiotic residues, and resistance genes and their mobile elements
- We need to better define the risks that antibiotics and resistant bacteria pose to the aquaculture environment, the aquaculture stock, man & animals
- **Aquaculture bacteria are a reservoir for antibiotic resistant genes, the associated mobile elements, for man, animals, plants and the environment**
- **Evolution of acquired antibiotic resistance genes & associated elements is an on-going process which may differ in different ecosystems and over time**