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
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 result 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 (≥ 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
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\textsuperscript{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 °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

North American psychrophilic 
*Aeromonas salmonicida*

- Six isolates could transfer their plasmids and *tet*(A) gene to *E. coli* at 15°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 °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.