Food and Agriculture Organization of the United Nations

FMM/RAS/298: Strengthening capacities, policies and national action plans on prudent and responsible use of antimicrobials in fisheries Final Workshop in cooperation with AVA Singapore and INFOFISH

12-14 December, Concorde Hotel, Singapore

DIAGNOSTICS AND ANTIMICROBIALS ADMINISTRATION

Snježana Zrnčić,

zrncic@irb.hr

INTRODUCTION

THE AIM OF THE PRESENTATION:

- EMPHASIZE THE CONTRIBUTION OF DIAGNOSTICS TO THE RESPONSIBLE MANAGEMENT OF BACTERIAL DISEASES IN AQUACULTURE AND CONSEQUENTLY TO THE REDUCTION THE ANTIMICROBIALS USE (AMU) AND PREVENTION OF THE ANTIMICROBIAL RESISTENCE (AMR) DEVELOPMENT

SOLUTIONS AGAINST BACTERIAL DISEASES ?

1 - Short term solutions:

Fast actions for immediate disease control

◆ Treatment prescription: bath & medicated feed

◆ Supporting actions: Stress reduction, daily mortality removal, change nets, quarantine, movement restriction, eradication

2 – Long term solutions

◆Health Management Plans to avoid future outbreaks

◆ Introductions and movement control, biosecurity, health monitoring programs & vaccination

DIAGNOSIS AND TREATMENT

At first occurence of abnormal behaviour, farmer should inform fish health specialist

According to changes in environment, clinical appearance and results of necropsy –choice of media and analysis

Sensitivity testing of pure bacterial cultures to approved and indicated antimicrobials

Results of the testing using the standardized protocols will advice on the choice of microbials

Evaluation of the affected biomass will enable quantification of required medicated feed

Ordering of medicated feed from approved feed mil

Treatment of affected lot during 10 days at least

Table 2.1. Outline of steps for culture and identification.

Day	Activity	Method or technique
Day 1	1. Sample collection and preparation	2.1
	2. Inoculation of sample to primary isolation plate media (or broths where appropriate)	2.2, Table 2.2, Table 2.3
	3. Incubate at appropriate temperature and atmosphere	2.2, Table 2.2, Table 2.3
Day 2 (24 h)	1. Examine culture plates	2.3
	2. Select suspect colonies and subculture to BA or MSA-B to obtain pure growth (secondary plates)	Table 2.4 for cultural and microscopic appearance
	3. Re-incubate primary plates	2.2, Table 2.2 and 2.3
	4. Incubate all plates at appropriate temperature and atmosphere as before	
Day 3 (48 h)	1. Re-examine primary plates for slow-growing pathogens	2.3
	2. Check that subcultures on secondary plates are pure	
	3. Perform primary identification tests	2.4, Chapter 3, and media (Chapter 7)
	4. Inoculate appropriate biochemical identification set	2.5, 2.6
	5. Re-incubate primary plates as before	2.2, Table 2.2 and 2.3
Day 4 (72 h)	1. Re-examine primary plates for growth of slow-growing pathogens. Re-incubate if disease suggests a pathogen that requires more than 3 days for growth	2.3
	2. Examine biochemical identification set/s and record results at 24 h incubation	Table 3.1
Day 5 (96 h)	1. Examine biochemical identification set/s and record results for 48 h incubation. Add reagents for tests for indole, methyl red, nitrate, Voges-Proskaüer	Table 3.1 Chapter 3 and Tables 4.1 to 4.22 for biochemical results (Biochem set), and
	2. Interpret results from appropriate identification table	Tables 4.23 to 4.31 for results for API kits

BA, blood agar; MSA-B, marine salt agar.

In the case of slow growing and fastidious bacteria it may last even longer

IMPACT OF BACTERIAL DISEASES TO THE PROFITABILITY OF AQUACUTURE

DIRECT LOSSES

- Low survival rate
- Cost of diagnostic analysis
- Cost of treatment
- In the case of diseases re-occurence new treatment is needed

INDIRECT LOSSES

- Cost of feed already used for farming of sick aquatic animals
- Removal of dead animals
- Engagement of workers in application of medicated feed
- Retarded growth and poor FCR
- Risk of disease re-occurence of outbreak on the site (endemic)

FAST AND ACCURATE RECOGNITION AND DETECTION OF THE BACTERIAL PATHOGEN

- Main prerequisite for mitigation of losses caused by bacterial diseases outbreaks
- Very important factor of the health monitoring programmes
- Diagnostic skills should be continously improved regardless to the diagnostic capacity

Diagnostic capacity	Diagnostic method applied	Findings	Suspected pathogen
LEVEL I	CHANGES IN ENVIRONMENT	Increase or decrease of the temperature of the water, low oxygen saturation, poor water exchange, stress	Any bacterial pathogen, but suspicion on vibriosis (*Vibrio anguillarum* or *V. harveyi*, *V. salmonicida*) or even *R. salmoninarum*
		Long period of high temperature of the sea, heavy rainfalls and decreasing of salinity, deteriorated cultivation conditions	Pasteurellosis caused by *Photobacterium damselae* subsp. *piscicida,*
		Low water temperature, fluctuation in water temperature, feeding to satiation, stress	*Pseudomonas fluorescens, Pseudomonas anguilliseptica*
	GROSS SIGNS	Swimming apart from the shoal, on the surface/bottom, lethargy	*Nocardia* spp., *Mycobacterium* spp., *Clostridium botulini*, *Str. iniae*
		Erratic swimming, spiral movement	*Str. iniae/agalactiae, Lactoccocus garviae, Edw. ictluri, Cl. botulini*
		Dark pigmentation	*Flav. psychrophilum, Lactoccocus garviae, Y. ruckeri*
		Pale colouration of the skin, anemia	*Edwardsiella tarda, Nocardia* spp.
		Whitish layer on whole body of glass eels	*Ps. anguilliseptica*
		Hemorrhages on the skin and fin basis	*Aer. hydrophila, Lactoccocus garviae, Ps. fluorescens*
		Hemorrhages in the eyes	*L. garviae, Ps. anguilliseptica, Y. ruckeri, A. hydrophila*
		Hemorrhages in the mouth	*Ps. anguilliseptica, Y. ruckeri, A. hydrophila*
		Hemorrhages in the gills/opercular area	*F. branchiophilum & columnare, Ps. anguilliseptica*
		Hemorrhages on the skin and in the muscles	*Aer. hydrophila & salmonicida, Edw. ictaluri, Flav. columnare, Nocardia* spp., *Ps. anguilliseptica, Ps. fluorescens*
		Erosion on the head and tail and fins	*Y. ruckeri, Ps. fluorescens, Nocardia* spp., *Mycobacterium*
		Brownish necrotic areas in gills or fins	*Fla. columnare*
		Saddle like lesion on the back	*Flav. psychrophilum, Flav. columnare*
		Exophthalmia	*E. ictaluri, A. hydrorphila, botulism*
		Loss of mucus layer sometimes with enophthalmia	*Fla. columnare*
		Distended abdomen	*Aer. hydrophila & salmonicida, Edw. ictaluri & Edw. tarda, L. garviae, Ps. fluorescens, Renibacterium salmoninarum, botulism*
		Furuncles	*Aer. salmonicida salm.*
		Protruded anus	*E. tarda, Y. ruckeri, Aeromonas hydrophila*
		Everted stomach, flaccid paralysis	Botulism
	NECROPSY	Gas filled hollows in the muscle, deep ulcers	*Edwardsiella tarda*
		Presence of ascitic fluid in body cavity	*A. hydrophila & salmonicida, E. ictaluri & tarda, Y. ruckeri, Flavobacterium* spp., *L. garviae, Ps. fluorescens, R. salmoninarum,*
		Petechial haemorrhages in the muscle wall	#
		Hemorrhages in swim bladder	*Ps. fluorescens, Y. ruckeri*
		Granulomas in the internal organs	*Edw. tarda, Nocardia* spp., *Francisella noatunensis*
		Swollen kidney	*Edw. ictaluri, Ren. salmoninarum, etc.*

Diagnostic capacity	Diagnostic methods applied		Observations and results	Possible pathogen
LEVEL II	BACTERIOLOGY	Isolation, and view of bacterial growth on media and identification by biochemistry or slide agglutination, and antibiogram	MEDIA	
			TSA and BA	Most of freshwater bacteria in tropical, temperate or cold waters
			KDM2 or SKDM	*R.salmoninarum* with incubation lasting from 7 days to 19 weeks
			AOA or TYES	*Flavobacterium psychrophila*, F. *branhiophilum*, F. *Cytophaga*, Yellow colonies change colour to orange after addition of 10% KOH
			No brown pigment on solid agar, mostly no growth at 37°C	*atypical Aeromonas salmonicida*
			Brown pigment in solid agar, mostly no growth at 37°C	*Aeromonas salmonicida* subsp. *salmonicida*
			After obtaining pure culture: Gram staining and genus typing by biochemistry API strips (20E, 20NE,), API ZYM, API-Staph or any other miniaturized test may be used Agglutination of bacteria with specific antiserum against a bacterial species In case treatment is needed: susceptibility testing	
	HISTOLOGY	Tissues of fin fish should be preserved in the buffered formaldelhyde. When samples for histology are fixed it is useful to preserve some samples in ethanol or to freeze them to be available for molecular testing.	Histological observation is especially useful when culture methods for isolation of pathogenic bacteria failed. Findings in tissue could lead toward additional laboratory testing of the level III.	
LEVEL III	DNA based tests	PCR	All these methods are described in details in the chapter 2.2. Role of diagnostics, and the particular of each diseases is referenced in the specific paragraphs of chapter 3. on bacterial species	
		qPCR		
		16SrRNA sequencing		
		NGS		
	Spectrophoto metric testing	MALDI-TOF		

* exclude typical parasitological and viral clinical signs:

-parasitological: flashing against bottom or walls of basin, spiral swimming (whirling disease of young trout), white spots, thick slime;

-viral clinical signs: often popeye (rhabdoviruses, biRNA, etc.), or enophthalmus (carp/koi with herpes or pox virus), anemia, pale gills and internal organs (exception: infectious salmon anemia (ISA) of salmon: dark hemorrhagic liver) or hemorhagges in the internal organs (VHSV, IHNV)

FAST AND ACCURATE DIAGNOSTICS

Implementation in broodstock, hatchery, pregrowing and fattening units both for both short term solutions and long term solutions

Requirements:

- Good knowledge of the technology procedure (optimal ecological condition, normal appearance and behaviour of all stages of the farmed species during all steps of the cultivation – from broodstock to market size fish)
- Knowledge of the ecological and environmental favourable conditions for disease outbreak occurence
- Knowledge of the clinical appearance of the particular bacterial disease, post mortem signs and histopathological changes in affected tissue as primary methods for diagnostics − often lack specificity and it is difficult to detect the pathogen in the animals without clinical signs of the diseases (early recognition of any changes and setting up the suspicion)

- effective control and treatment of bacterial diseases requires rapid, reliable and highly sensitive diagnostic methods
- Plating and cultivating of pathogen bacteria is also a widely used method, but it is time consuming and there are some very hardly cultivable, fastidious bacterial pathogens
- a possibility to implement immunological, protein-based and molecular methods solves all mentioned limitations
- The most important is fast commencement of diagnostic procedures (notifying the suspicion to the diagnostic lab or starting the diagnostic procedure in the own facility if there is any)

HEALTH MANAGEMENT PLANS:
1. REDUCING THE BACTERIAL PATHOGEN PRESSURE (GAP)

- **Good management practices (separation of generation, stocking density, proper feeding, feed quality, reducing stress, water quality control etc...)**
- **Hygiene practices**
  - **Biosecurity measures: introduction of certified stocks, awareness of the diseases history on the farm, control of aquaculture animals movement between and within farm, movement of people and vehicles, control of birds, predators etc., removing of dead fish**
  - **Cleaning and disinfection procedures of equipment, containars, nets, boats, etc.**

2. HEALTH MONITORING PROGRAMMES
BROODSTOCK CONTROL

Example:

Although biosecurity measures have been implemented we should be aware of vertical transmission of different bacterial diseases like:

-*Streptococcus iniae*, *Fransicella noatunensis* subsp *orientalis* through fertilized eggs in tilapia

-bacterial kidney disease caused by *R. salmoninarum* in salmonid fish via eggs

-*Flavobacterium psychrophilum* in salmonids,

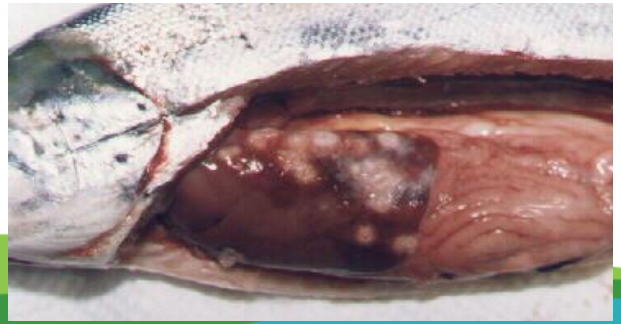
-photobacteriosis caused by *Photobacterium damselae* in marine fish

-piscirickettsiosis (salmonid rickettsial syndrome – SRS), caused by *Piscirickettsia salmonis* in salmonis fish in Chile

Streptococcus iniae

Fransicella noatunensis subsp *orientalis*

Flavobacterium psychropilum



Piscirickettsia salmonis

- Monitoring in hatcheries – sometimes biosecurity measures could fail and bacterial infection in the hatchery could appear – testing of fry (the most susceptible life stage)
- Transport from hatchery to ongrowing units – stress and the latent infections could occur after the transportation
- Knowledge of predisposing factors/periods for endemic diseases occurence requires disease monitoring procedures with diagnostic testing
- In absence of specific immunoprophylaxis (vaccination against endemic bacterial pathogens) losses could be mitigated only by prudent and responsible use of antimicrobials
- Key of successful treatment – continuous monitoring and early diagnosis

ROLE OF RAPID DIAGNOSTIC IN AMR PREVENTION

◆Use antimicrobials to better treat infections

◆Slow the rise of drug resistance by reducing the unnecessary use of antimicrobials, in particular antibiotic

◆Ultimately change our approach to treat bacterial infections through targeted and precise therapy

ADMINISTRATION OF ANTIMICROBIALS

Use of antimicrobials can be divided into:

- **Therapeutic** antimicrobial use is the treatment of established infections.

- **Metaphylaxis** is a term used for group-medication procedures, aimed to treat sick animals while medicating others in the group to prevent disease.

- **Prophylaxis** means the preventative use of antimicrobials in either individuals or groups to avoid development of infections.

- **Growth promotion** use is when an antimicrobial agent is used as feed supplement in food animals to promote growth and enhance feed efficiency.

ADMINISTRATION METHODS

- **Medicated feed** – preferred method of antimicrobials administration, more often commercially prepared as sinking or floating pellets
- **Baths and dips, flush** – not effective as some other treatment methods, particularly for systemic infections, poor absorption of the antimicrobials used
- **Topical** – usually only necessary for more valuable individual fish, such as ornamental varieties or broodstock – treatment of ulcers or injuries
- **Injection** – more effective than using medicated feed – practical use for valuable individuals – requires anaesthesia – i/p and i/m
- **Administration through bioenrichment** of live feed organisms most often Artemia or rotifers either directly or indirectly for fish larvae and shrimp postlarvae

EFFICACY OF BACTERIAL DISEASES TREATMENT USING MEDICATED FEED

- Medicated feed - Incorporation of antimicrobial substance into the feed via powdered premix (active ingredient + carrier)
- Always under veterinary prescription

Prerequisite for successful treatment:

- Rapid diagnosis (Vibriosis in sea bass;treatment starts 1.st day- Mt=1,5%; treatment starts after 1 week, Mt=16%)
- Antimicrobial selection and dosis
- Manufacturing process
- Administration

ANTIMICROBIAL SELECTION, DOSAGE AND WITHDRAWAL TIMES

Antimicrobial agent	Dosage	Indication	Withdrawal period
Oxytetracycline	60-80mg/kg body weight daily in feed	Vibriosis, aeromoniasis, edwardsielosis, flavobacteriosis*, tenacibaculosis, francisellosis, streptococcosis, lactococcosis	400-600 °days
Trimethoprim/ Sulphafurazol	50 mg /kg body weight daily in feed	Vibriosis, aeromoniasis, edwardsielosis, pseudomoniosis, tenacibaculosis	350°days
Quinolones (oxolinic, nalidixic acid, flumequine)	12-50 mg/kg body weight daily in feed	Vibriosis, aeromoniasis, edwardsielosis, pseudomoniosis, flavobacteriosis*, photobacteriosis	80 °days
Florfenicol	10–30 mg/kg body weight daily in feed	aeromoniasis, edwardsielosis, yersiniosis, flavobacteriosis	150 °days
Erythromycin	100 mg/kg body weight daily in feed	BKD, mycobacteriosis, streptococcosis, lactococcosis	700 °days
Fluoroquinolones	25-40 mg/kg body weight daily in feed	Tenacibaculosis, francisellosis, lactococcosis	500 °days
Amoxycillin	40-80 mg/kg body weight daily in feed	Furunculosis, streptococcosis, lactococcosis	500°days

CHOICE OF ANTIMICROBIALS

Historical records (continuous monitoring needed followed by sensitivity testing)

Final premix dosE adjustment:

- concentration of active ingredient – to avoid sub-dosing
- biomass to treat – to avoid sub-dosing
- daily feed ratio – to avoid sub-dosing

DURATION OF TREATMENT – AT LEAST THREE DAYS AFTER CESSATION OF THE SYMPTOMS OF THE DISEASE BUT NOT LESS THAN 7 DAYS

POSSIBLE MISTAKE IN USE OF ANTIMICROBIALS

- Starting too late
- Inadequate medicine / dosage selection
- Wrong duration of treatment
- Use of antibiotics as prophylactics, “just in case”
- Use of antibiotics in viral infections
- Repeatedly use of same medicines

USE OF ANTIMICROBIAL ON THE FARM

- Starvation before medicated feed administration
- Reduction of daily feeding ratio and medicated feed should be the first meal or it should be adapted to the age and number of daily meals
- Administration:
  - ✓ Preferred manually
  - ✓ Small air-cannons in big cultivation units/off-shore cages with high biomass per unit

BETTER PREVENT THAN CURE

THANK YOU FOR YOUR ATTENTION