‘Resistant’ or ‘Susceptible’ May Not Always be Your Answer

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AST Methods

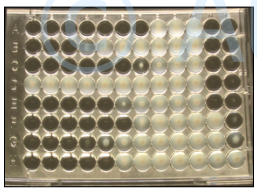
Agar diffusion

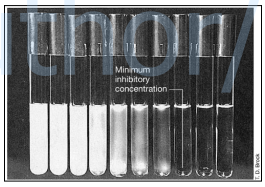
Disk diffusion (Kirby-Bauer)

Broth dilution

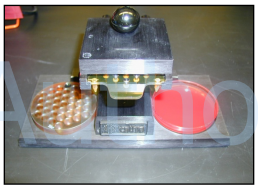
Microdilution

Macrodilution



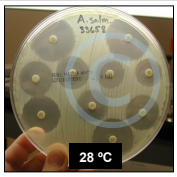


Agar dilution

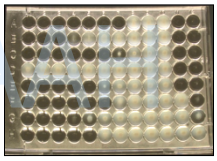


Agar diffusion

E-test



Use Standardized Methods Whenever Possible

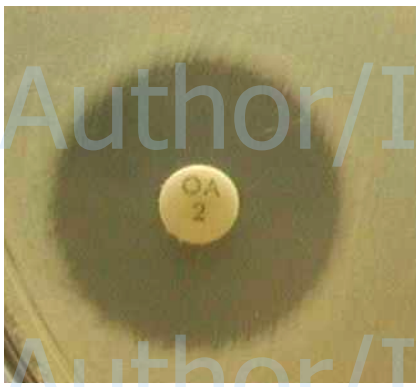


AST data are most reliable and reproducible if quality control procedures are used.

- *Quality control testing should be performed each test day for MIC tests performed less than once a week.*

Quality Control

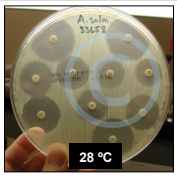
QC ranges are used to monitor performance



Zone = 34 mm

QC range 25-32 mm

Must retest !



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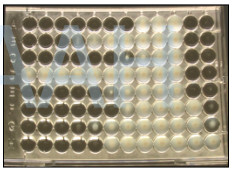
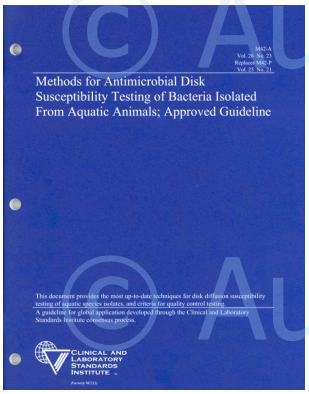


M42-A Guideline

Disk diffusion testing at 22 °C and 28 °C

M49-A Guideline

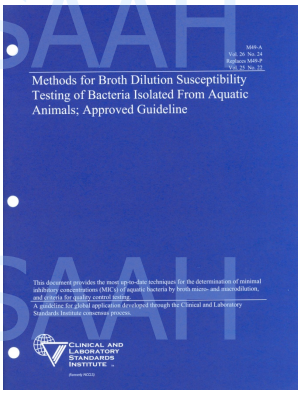
MIC testing at 22 °C and 28 °C

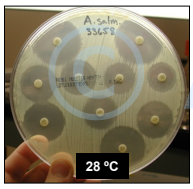


CLINICAL AND LABORATORY STANDARDS INSTITUTE ™

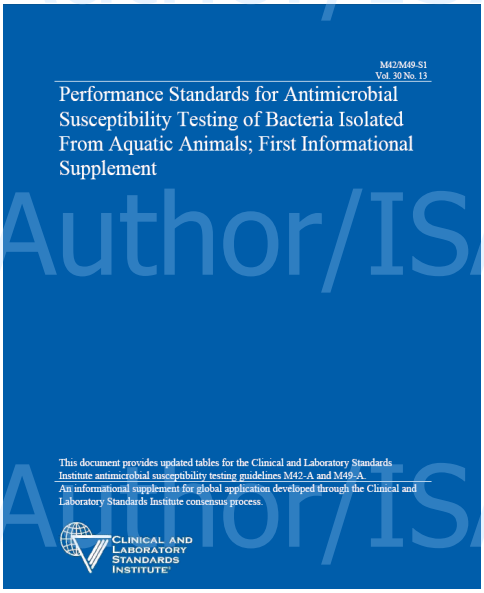
Global consensus documents

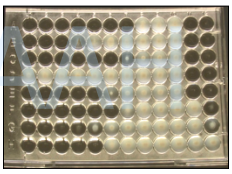
Provide methods and QC parameters, but no criteria for data interpretation





New - M42/M49-S1





Antibiotic Susceptibility Testing

Results can be used to…

Guide the clinical selection of an effective therapy (S, I, R)

clinical breakpoints **~ for veterinarians**

Monitor changes in susceptibility (wild-type cutoffs)

epidemiologic cutoff values **~ for epidemiologists**

Reason for this Supplement:

Clinical Breakpoints Currently Used in Fish Medicine

bacterial species-independent! – genera grow very different *in vitro*

Table 4
Frequency distribution of breakpoints (mm) currently in use in responding laboratories for the nine most commonly tested antimicrobial agents

Zone (mm)	AMX 25 µg		ENR 5 µg		ERY 15 µg		FLO 30 µg		FLU 30 µg		OXA 2 µg		OTC 30 µg		SFO 25 µg		SFT 25 µg	
	S	R**	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
6–7																1		
8–9																		3
10–11				1				1		2	1	6		4		3		7
12–13		3		2		3		5		3	2		1	3			1	4
14–15								1		1	2	1		10				3
16–17	1		1	5		2	1	3	1	2	1		2	1	3		9	1
18–19	1		2	1	3		5	1	3		1	1	12	2			6	
20–21			1				4	2	2	3			2	1			1	
22–23	1		5		1		1						1		1			2
24–25							2	1			1	1					1	3
26–27									2		1							
28–29					1								1				3	1
30–31			1				1		2				1					
32–33							1		1				1				1	
34–35														1			2	
36–37																		
38–39													1					
40–41																		
42–43																		
44–45									1	1								

Abbreviations: AMX, amoxycillin; ENR Enrofloxacin; ERY, erythromycin, FLO florfenicol; FLU, flumequine; OTC, oxytetracycline; OXA oxolinic acid; SFO, ormetoprim/sulfadimethoxine; SFT, trimethoprim/sulfmethoxazole; S* indicates breakpoints used to determine sensitivity. R** indicates breakpoints used to determine resistance.

This survey revealed many labs may be advising antibiotic treatment of fish or fish populations infected with bacteria that are in fact resistant to that antibiotic.

survey from P. Smith, Aquaculture. 2006

M42/M49-S1

Includes the 1st Clinical Breakpoints* for any aquaculture pathogen

(*Aeromonas salmonicida*)

Antimicrobial Agent	Disk Content	Zone Diameter Breakpoint (mm)			MIC Breakpoint (µg/mL)			Comments
		S	I	R	S	I	R	
TETRACYCLINES								
Oxytetracycline	30 µg	≥ 28	22-27	≤ 21	≤ 1	2-4	≥ 8	Class representative for tetracyclines Established based on zone diameter and MIC distributions of 323 *A. salmonicida* isolates (Miller and Reimschuessel, 2006; Smith et al., 2007) and clinical correlations from 2 studies (Coyne et al., 2004)
QUINOLONES								
Oxolinic acid	2 µg	≥ 30	25-29	≤ 24	≤ 0.12	0.25-0.5	≥ 1	Established based on zone diameter and MIC distributions of 323 *A. salmonicida* isolates ((Miller and Reimschuessel, 2006; Smith et al., 2007)) and clinical correlations from 4 studies (O'Grady et al., 1987; O'Grady and Smith, 1992; Smith and O'Grady, 2006; Hastings and McKay, 1987).

Example 1

Clinical situation

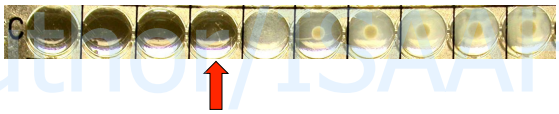
- ***Aeromonas salmonicida*** isolate from the spleen of an Atlantic salmon
- Drug of interest for **treatment** purposes – **oxytetracycline**
- Testing methods – disk diffusion and broth microdilution

How do we interpret this data?

Aeromonas salmonicida

Zone = 20 mm

MIC = 8 µg/mL



Susceptible?
-an infection may be appropriately treated with the dosage regimen

Intermediate?
-an infection may be appropriately treated in specific body sites or when a high dose of drug can be used

Resistant?
-pathogens are not usually inhibited by achievable concentrations, and clinical efficacy has not been reliable in treatment studies

M42/M49-S1

Includes the 1st Clinical Breakpoints* for any aquaculture pathogen
(*Aeromonas salmonicida*)

Antimicrobial Agent	Disk Content	Zone Diameter Breakpoint (mm)			MIC Breakpoint (µg/mL)			Comments
		S	I	R	S	I	R	
TETRACYCLINES								
Oxytetracycline	30 µg	≥ 28	22-27	≤ 21	≤ 1	2-4	≥ 8	
QUINOLONES								
Oxolinic acid	2 µg	≥ 30	25-29	≤ 24	≤ 0.12	0.25-0.5	≥ 1	

Interpreted as…

Aeromonas salmonicida

Zone = 20 mm

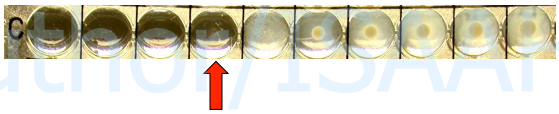
R

Resistant?

-pathogens are not usually inhibited by achievable concentrations, and clinical efficacy has not been reliable in treatment studies

R

MIC = 8 µg/mL



Oxytet Breakpoints Currently Used in Fish Medicine

Zone (mm)	OTC 30 µg S	R
6–7		
8–9		
10–11		4
12–13	1	3
14–15		10
16–17	2	1
18–19	12	2
20–21	2	1
22–23	1	
24–25		
26–27		
28–29	1	
30–31	1	
32–33	1	
34–35		1
36–37		
38–39	1	
40–41		
42–43		
44–45		

Many labs may be advising treatment with oxytetracycline to fish populations infected with bacteria that are in fact resistant.

CLSI clinical breakpoints for *A. salmonicida*

survey from P. Smith, Aquaculture. 2006

Example 2

Surveillance/Monitoring situation

- ***Aeromonas salmonicida*** isolate from the spleen of an Atlantic salmon
- Drug of interest for ~~treatment~~ classification purposes – **florfenicol**
- Testing methods – disk diffusion and broth microdilution

How do we interpret this data?

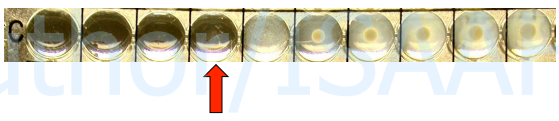
Aeromonas salmonicida

Zone = 24 mm

Wild-type?
- implies isolate is susceptible to the antimicrobial (no resistance mechanisms)

Non Wild-type?
- implies the isolate possesses acquired and/or mutational resistance mechanisms

MIC = 8 µg/mL



M42/M49-S1

Includes the 1st Epidemiologic Cutoff Values* for any aquaculture pathogen
(*Aeromonas salmonicida*)

Antimicrobial Agent	Disk Content	Zone Diameter Cutoff (mm)		MIC Cutoff (µg/mL)		Comments
		WT	NWT	WT	NWT	
AMINOGLYCOSIDES						
Gentamicin	10 µg	≥ 18	≤ 17	-	-	Established based on a zone diameter distribution of 106 *A. salmonicida* isolates (Smith et al., 2007)
MACROLIDES						
Erythromycin	15 µg	≥ 14	≤ 13	-	-	Established based on a zone diameter distribution of 106 *A. salmonicida* isolates (Smith et al., 2007)
PHENICOLS						
Florfenicol	30 µg	≥ 27	≤ 26	≤ 4	≥ 8	Established based on zone diameter and MIC distributions of 323 *A. salmonicida* isolates (Miller and Reimschuessel, 2006; Smith et al., 2007)
FOLATE PATHWAY INHIBITORS						
Ormetoprim-sulfadimethoxine	1.25/23.75 µg	≥ 20	≤ 19	≤ 0.5/9.5	≥ 1/19	Established based on zone diameter and MIC distributions of 217 *A. salmonicida* isolates (Miller and Reimschuessel, 2006)
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥ 20	≤ 19	-	-	Established based on zone diameter distributions of 106 *A. salmonicida* isolates (Douglas et al., 2007)

Interpreted as…

Aeromonas salmonicida

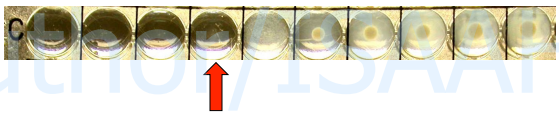
Zone = 24 mm

NWT

Non Wild-type?
- implies the isolate possesses acquired and/or mutational resistance mechanisms

NWT

MIC = 8 µg/mL



Florfenicol Breakpoints Currently Used in Fish Medicine

Zone (mm)	FLO 30 µg S	FLO 30 µg R
6–7		
8–9		
10–11		1
12–13		5
14–15		1
16–17	1	3
18–19	5	1
20–21	4	2
22–23	1	
24–25	2	1
26–27		
28–29		
30–31	1	
32–33	1	
34–35		
36–37		
38–39		
40–41		
42–43		
44–45		

Most labs are potentially misclassifying the susceptibility of *A. salmonicida* isolates to florfenicol.

CLSI epidemiologic cutoff for *A. salmonicida*

P. Smith, Aquaculture. 2006

Interpreting Antibiotic Susceptibility Test Data

Use clinical breakpoints (S, I, R) when available, to guide the clinical selection of an effective therapy

~ **for veterinarians**

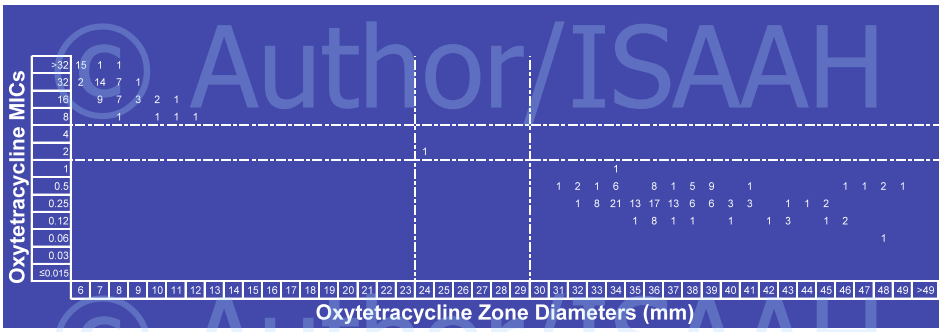
Use epidemiologic cutoff values (NWT, WT), if you need to monitor for changes in susceptibility

~ **for epidemiologists**

So What Data are Still Needed?

1. **Susceptibility <u>frequency</u> data distributions for various fish pathogen : drug combinations**

   To set Epidemiologic Cutoff Values



So What Data are Still Needed?

2. **Clinical reports of treatment successes and failures related to MICs and/or zone diameters for the causative pathogen.**

   Data needed for all fish pathogens!!!

So What Data are Still Needed?

3. **Pharmacokinetics data in serum/plasma during and after the dosing interval.**

   Provides vital data on achievable drug concentrations using a given dose under specific conditions.

- CLSI
  - Subcommittee for Veterinary AST

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Thank you!

Questions?

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