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

Review of AMR in aquaculture work in China

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PRESENTATION OUTLINE

- part 1: AMR in cold-water fish
- part 2: AMR of aeromonads
- part 3: Integron-mediated resistance mechanism of Aeromonas
- part 4: AMR of vibrios
- part 5: annual changes of AMR

- This review just includes some representative investigations done in the mainland of China, but the results were published on Chinese journals for most of them over the past 5 years.
- Various species of Aeromonas and Vibrio are the predominant fish pathogenic bacteria for freshwater aquaculture and mariculture, respectively. So they have been the focus of AMR study.
- Too many fish species and other aquatic animals are being cultured in China. This greatly increased the complexity of AMR and the difficulty of controlling it.
- Currently florfenicol, quinolone, neomycin, sulfonamides/trimethoprim and tetracycline are the major species of antimicrobials used in aquaculture of China.

Part 1

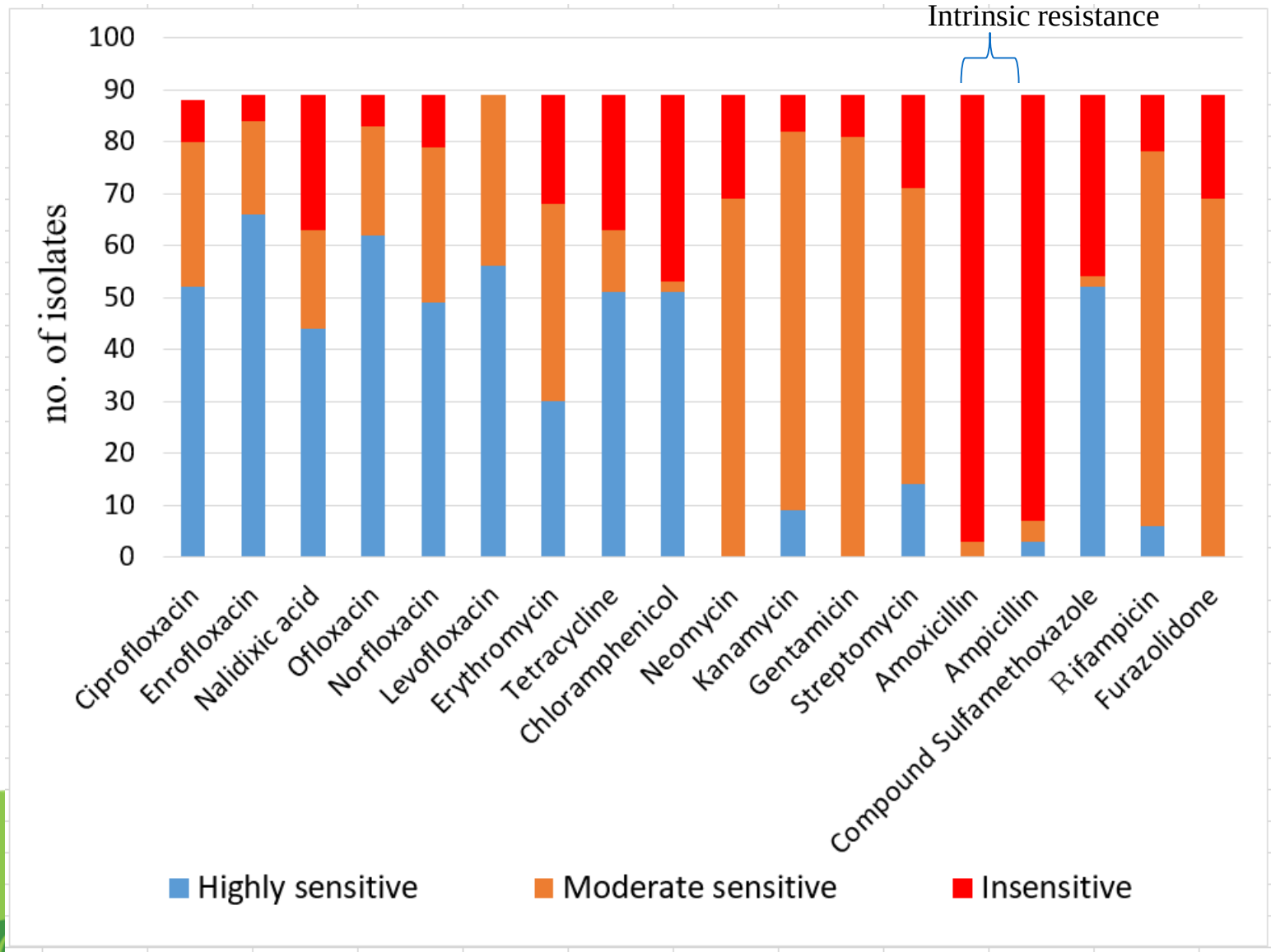
AMR of fish pathogenic bacteria isolated from cold-water fish cultured in three north areas of China

——Lian, et al. Acta Agriculturae Universitatis Jiangxiensis. 2015,37(2):339-345

The host fish and the species of 89 bacterial isolates involved in this study

Fish species	Species and No. of the isolates	Fish species	Species and No. of the isolates
silver salmon	*Aeromonas salmonicida* (12)	Rainbow trout	*Aeromonas sobria* (9)
silver salmon	*Acinetobacter* sp. (1)	Rainbow trout	*Flavobacterium* spp. (2)
Atlantic salmon	*Aeromonas salmonicida* (14)	Rainbow trout	*Yersinia ruckeri* (2)
Atlantic salmon	*Acinetobacter* spp. (6)	Siberian sturgeon	*Acinetobacter* spp. (3)
Atlantic salmon	*Aeromonas hydrophila* (2)	Siberian sturgeon	*Aeromonas veronii* (2)
Atlantic salmon	*Aeromonas sobria* (6)	Siberian sturgeon	*Aeromonas hydrophila* (2)
Atlantic salmon	*Flavobacterium* spp. (2)	Siberian sturgeon	*Flavobacterium* sp. (1)
Rainbow trout	*Acinetobacter* spp. (6)	Siberian sturgeon	*Strptococcus iniae* (3)
Rainbow trout	*Aeromonas veronii* (6)	Siberian sturgeon	*Streptococcus dysgalactiae* (10)

The sensitivity to 89 bacterial isolates against various antimicrobials



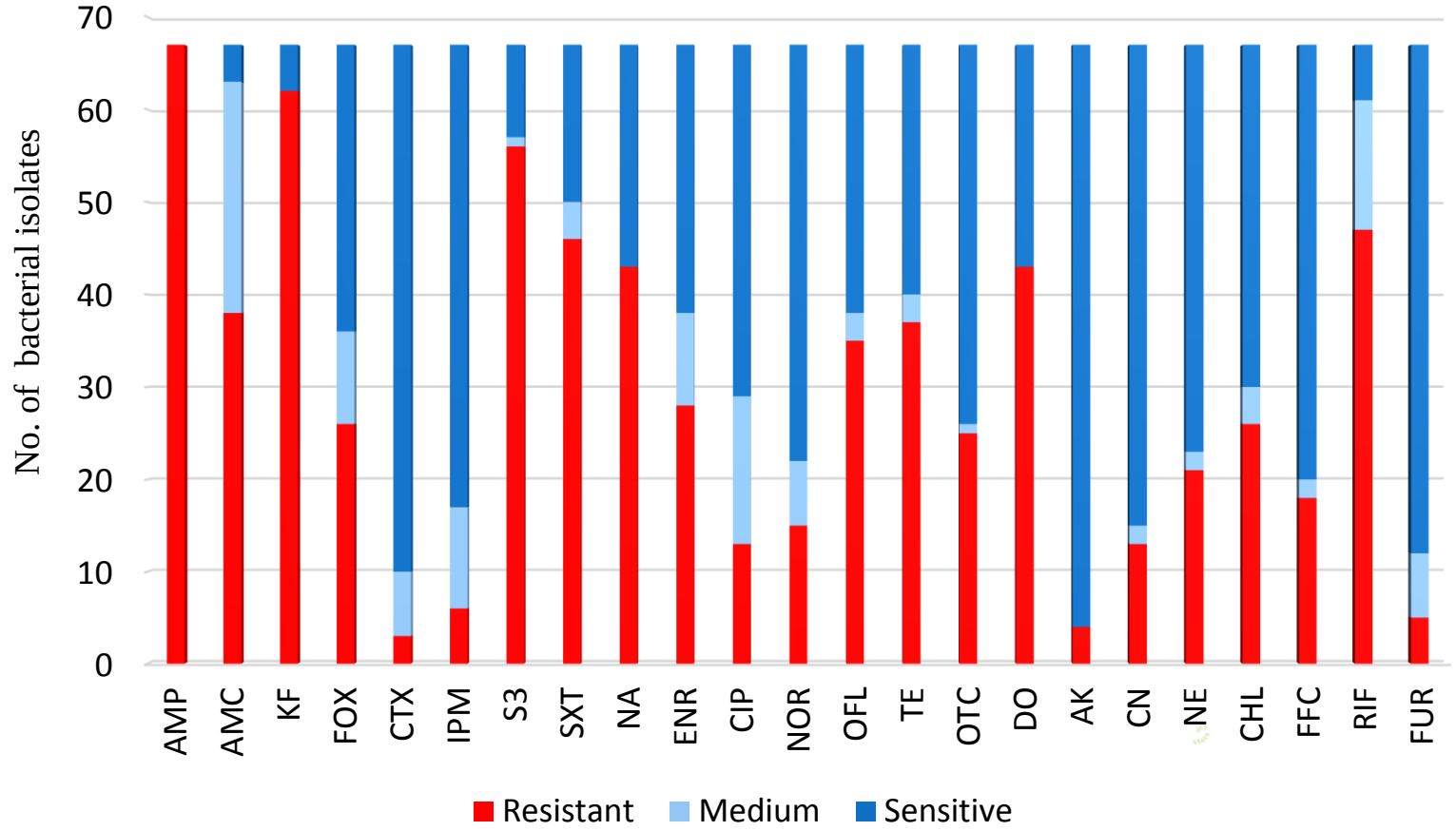
It seems that in cold water fishes, AMR is not too serious

Part 2

AMR situation of *Aeromonas* species isolated in south China

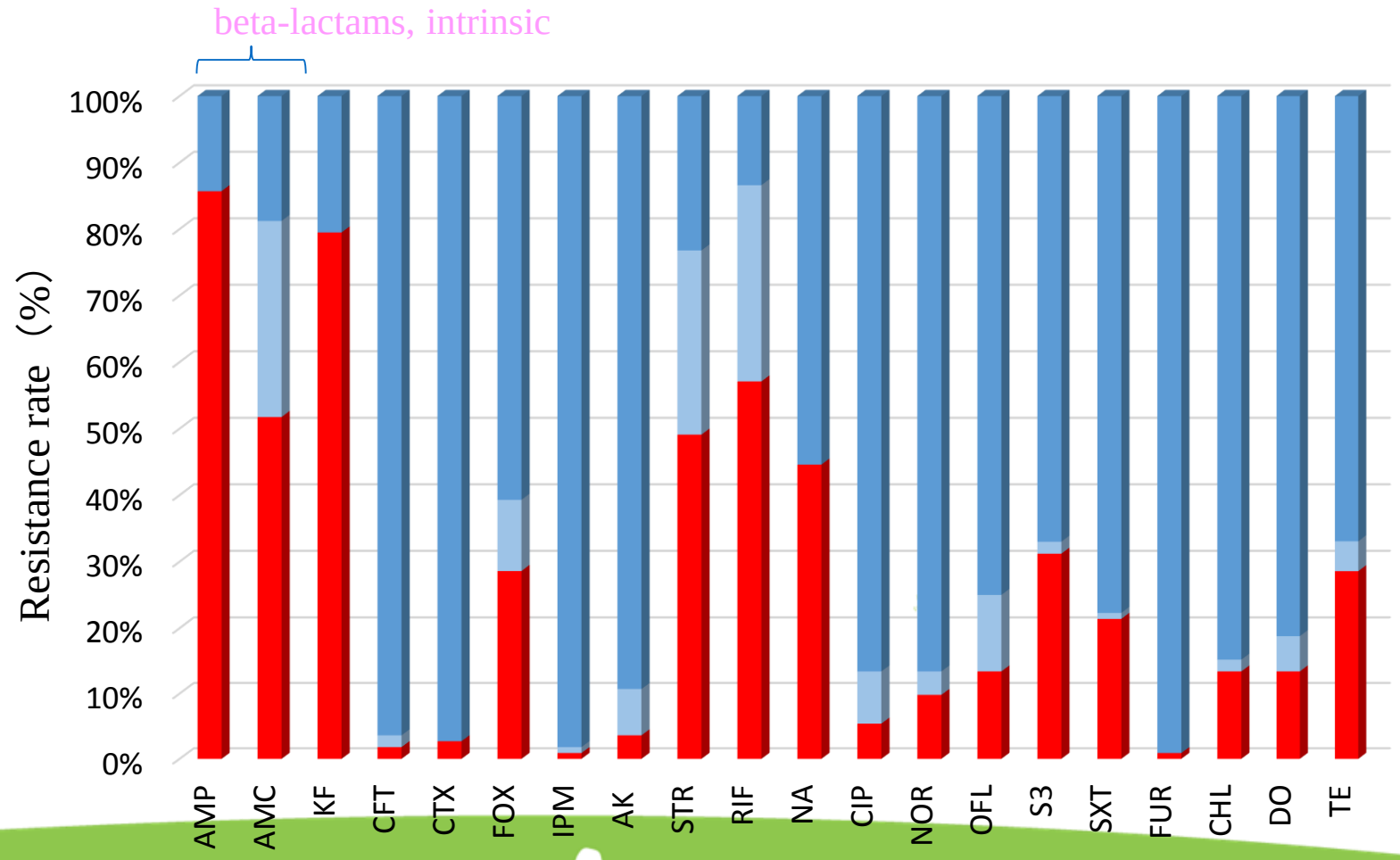
——Tan AP, et al. Journal of fisheries of china. 2014,38(7):1018-1025
——Wu YL, et al. Journal of Shanghai ocean university, 2013,22(2) :219-224
——Tan AP, et al. Master's Thesis of Shanghai ocean university, 2016.

2.1 Susceptibility of 67 *Aeromonas* isolated from turtles to 23 antimicrobial agents



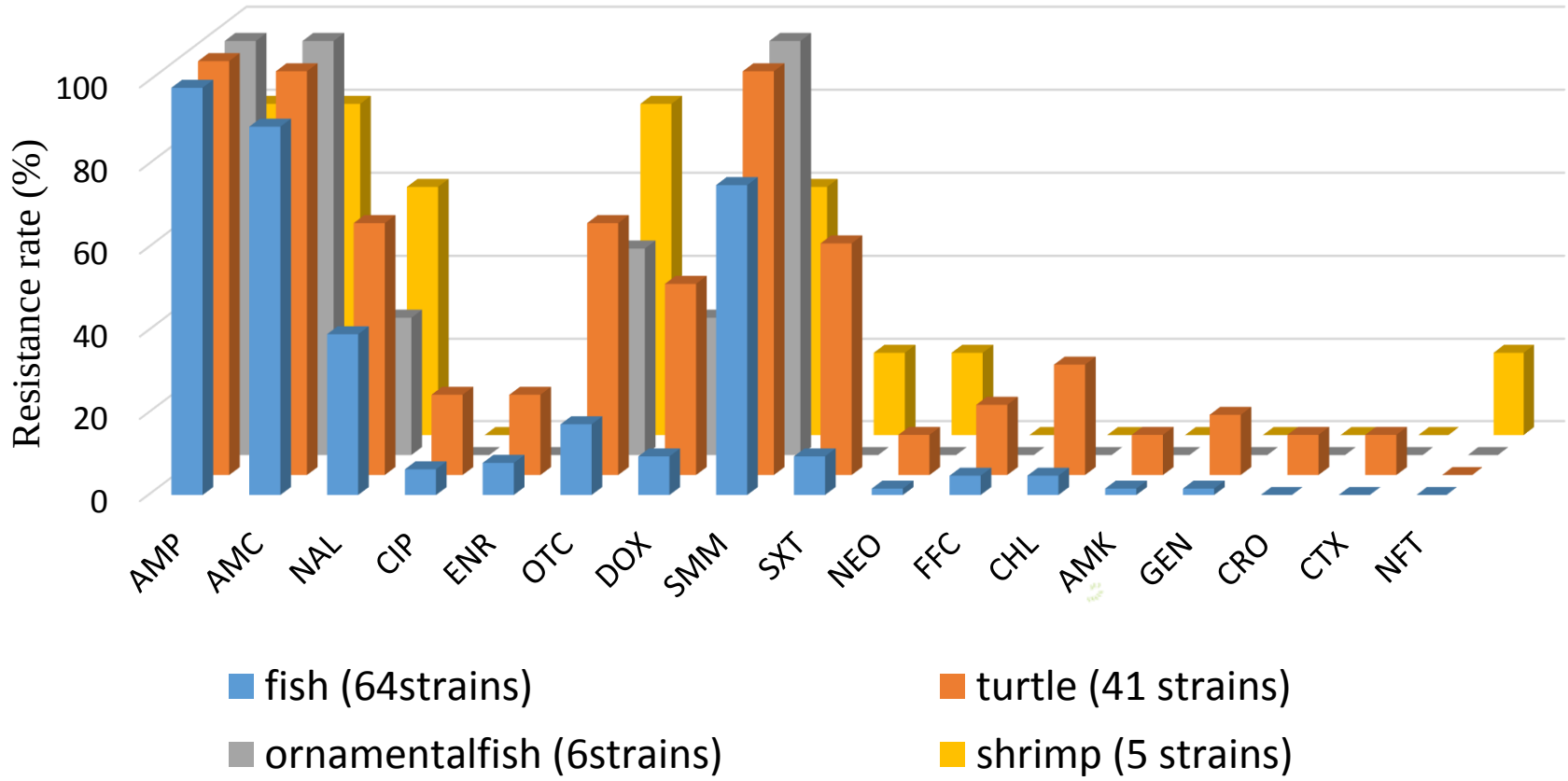
The abbreviations： Ampicillin (AMP);Amoxicillin/clavulanic acid (AMC); cefalothin (KF); cefoxitin (FOX); cefotaxime (CTX); Cefatriaxone(CFT);imipenem (IPM); Sulfanilamide (S3);Sulfamethoxazole/trimethoprim (SXT); nalidixic acid (NA); enrofloxacin (ENR);ciprofloxacin (CIP); Norfloxacin(NOR); ofloxacin (OFL); tetracycline (TE); doxycycline (DO); oxytetracycline (OTC); Florfenicol(FFC);chloramphenicol (CHL); Furazolidone (FUR); Amikacin (AK); Streptomycin(STR); rifampicin (RIF); gentamicin (CN); neomycin (NE) ; Thiamphenicol (THI); Nitrofurantoin(NFT); Oxolinic Acid(OXA) ; spectinomycin （SPM); novobiocin (NOB); tobramycin (TBM), Josamycin (JS); Erythromycin(ERM

Susceptibility of 112 strains of *Aeromonas* isolated from various aquatic animals against 20 antimicrobials



W YL

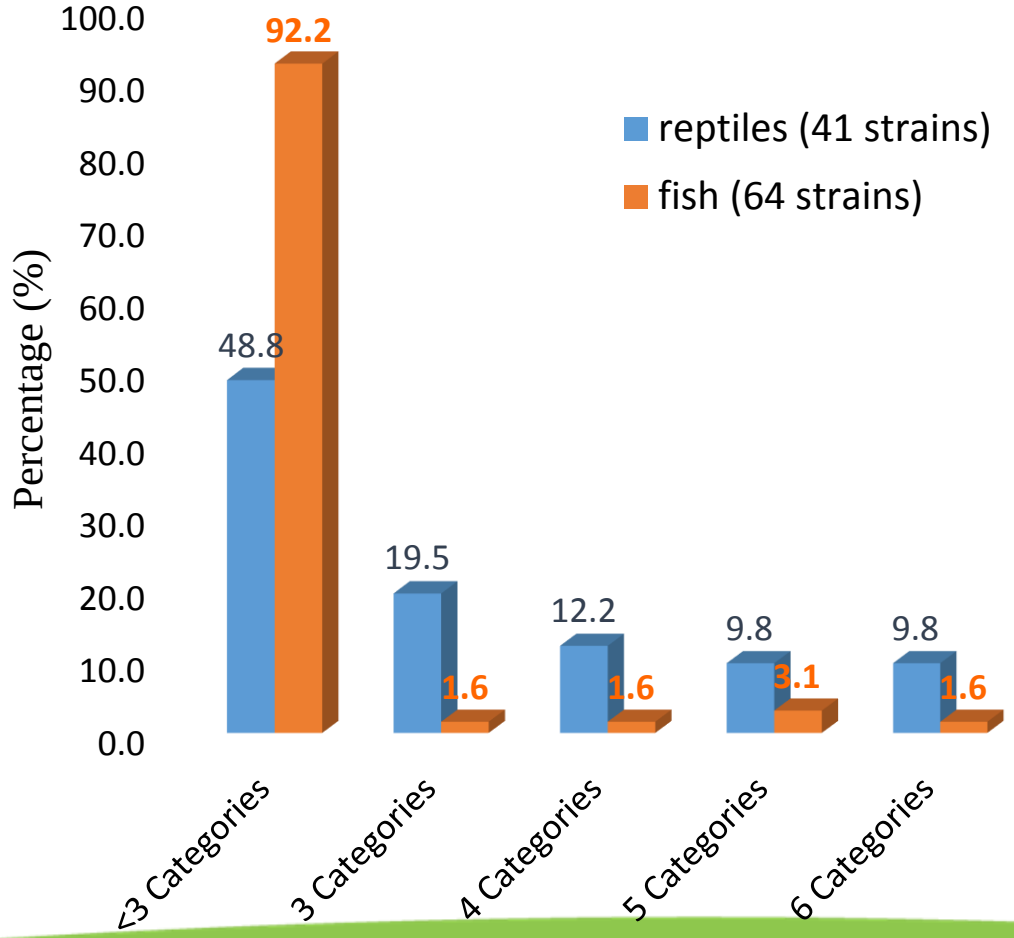
Resistance rates of *Aeromonas* strains isolated from different aquatic animals against 20 antimicrobial agents



Proportion of drug-resistant bacteria originating from fish were less than those from reptiles

Wu YL

Comparison of percentage of multidrug resistant (MDR) strains between fish origin and reptile origin



Multiple drug resistance (MDR) is AMR to at least **3** kinds of antimicrobial drugs

Tan AP

Categories include ①β-lactan; ②sulfonamides; ③quinolones; ④tetracyclines; ⑤aminoglycosides; ⑥amphenicols; ⑦rifampicin; ⑧nitrofurans

MIC_{50} and MIC_{90} of 22 kinds of antimicrobials against 116 strains of *Aeromonas*

Antimicrobials	MIC_{50} （mg/L）	MIC_{90} （mg/L）
NAL	2	>128
ENR	0.12	4
CIP	0.06	2
SMM	>512	>512
SM_2/TMP	≤2.5/0.5	>20/4
SDT/TMP	≤2.5/0.5	>20/4
SMZ/TMP	≤9.5/0.5	>4/76
OTC	≤0.5	>16
DO	1	>16
NE	≤1	4
GEN	1	4
AMK	2	8
STR	4	64
AMP	>256	>256
AMC	32/16	32/16
CTX	≤0.03	1
CFT	0.12	1
THI	≤2	>64
FFC	≤2	≤2
CHL	≤2	32
NFT	≤4	16
OXA	0.12	>4

Correlation between ARG type and MIC of NA and CIP against 116 strains of fish pathogenic *Aeromonas*

ARG type		No. of strains	Nalidixic acid (NA) (mg/L)			Ciprofloxacin (CIP) (mg/L)		
PMQR	QRDR		MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}
+	+	11	>128	>128	>128	0.5~>8	>8	>8
-	+	50	≤0.5~>128	>128	>128	≤0.004~>8	0.25	2
+	-	7	≤0.5~4	2	2	≤0.004~0.5	0.06	0.25
-	-	48	≤0.5~8	≤0.5	≤0.5	≤0.004~0.06	≤0.004	0.08
aac(6')-Ib-cr	+	9	>128	>128	>128	0.5~>8	>8	>8
aac(6')-Ib-cr , qnr S2	+	1	>128	>128	>128	0.5~>8	>8	>8
qnr S2	+	1	>128	>128	>128	0.5~>8	>8	>8
qnr S2	-	7	≤0.5~4	2	4	≤0.004~0.5	0.06	0.25

The mutation of QRDR on chromosome and gene *aac(6')-Ib-cr* on plasmid contribute the most to the resistance of *Aeromonas* to quinolones, and gene *qnr S2* alone on plasmid has weak

Deng YT

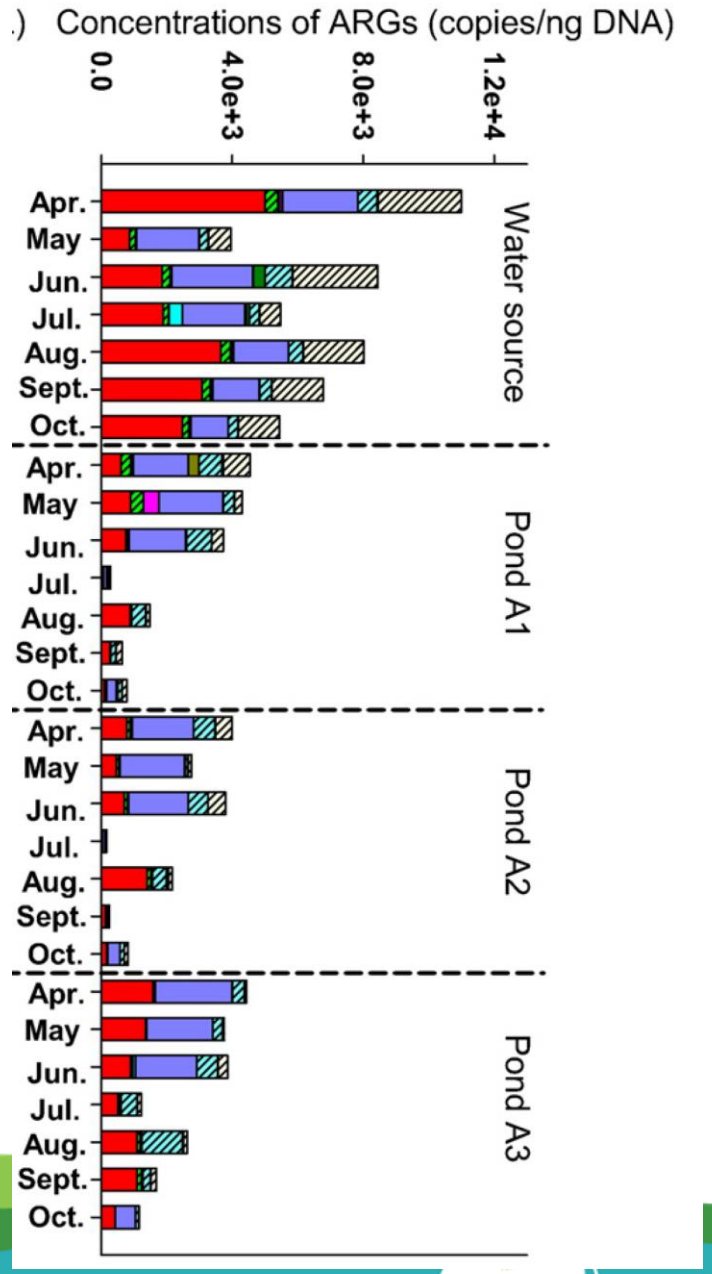
Mutation of QRDR and quinolones resistance phenotypes in the PMQR positive strains

Strains	Isolation year	PMQR	QRDR mutation		quinolones resistance profiles
			gyrA	*parC*	
28A	2003	*aac(6')-Ib-cr*	Ile83	Ile87	NA
17A	2005	*qnrS2*			
44A	2007	*aac(6')-Ib-cr*	Ile83	Ile87	NA \ENR\OFL\CIP\NOR
1203	2012	*qnrS1*			NA\ENR\OFL
1204	2012	*qnrS1*	Ile83		NA\ENR\OFL
1205	2012	*aac(6')-Ib-cr*	Ile83	Ile87	NA\ENR\OFL
12044	2012	*qnrS1*	Ile83		NA\ENR\OFL
12045	2012	*qnrS1*	Ile83		NA\ENR\OFL\CIP\NOR
12087	2012	*qnrS2*			
kidney	2013	*qnrS1*	Ile83	Ile87	NA\ENR\OFL
12002	2013	*qnrS1*			NA\ENR\OFL
13036	2013	*aac(6')-Ib-cr, qnrS2*	Ile83	Ile87	NA\ENR\OFL\CIP\NOR
liver	2013	*aac(6')-Ib-cr, qnrS2*	Ile83	Ile87	NA\ENR\OFL\CIP\NOR

Mutation of *GyrA* not *parC* on QRDR is the major contributor to the quinolone resistance in *Aeromonas*

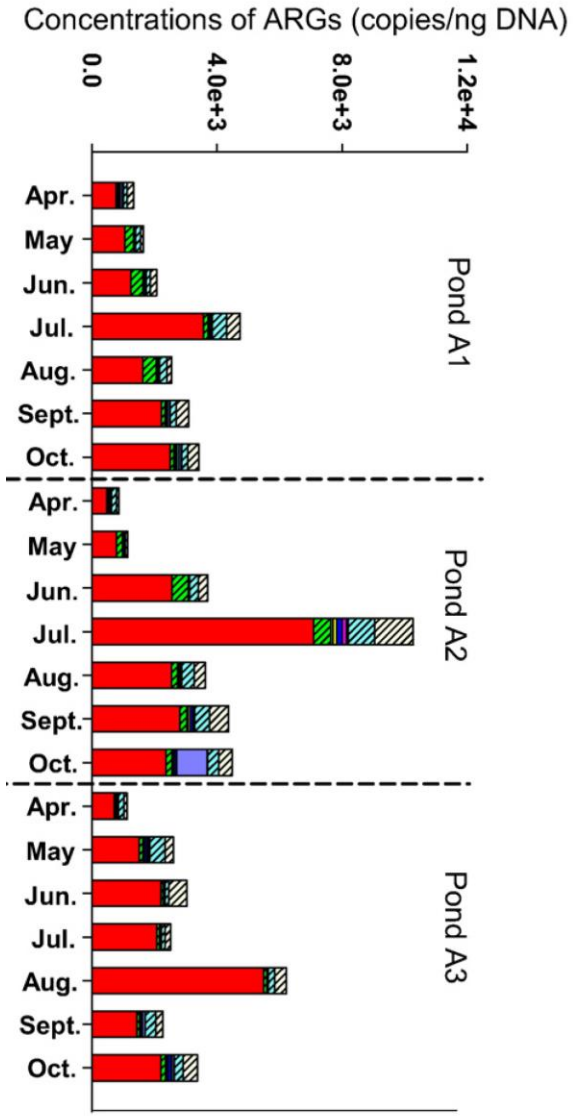
Tan AP

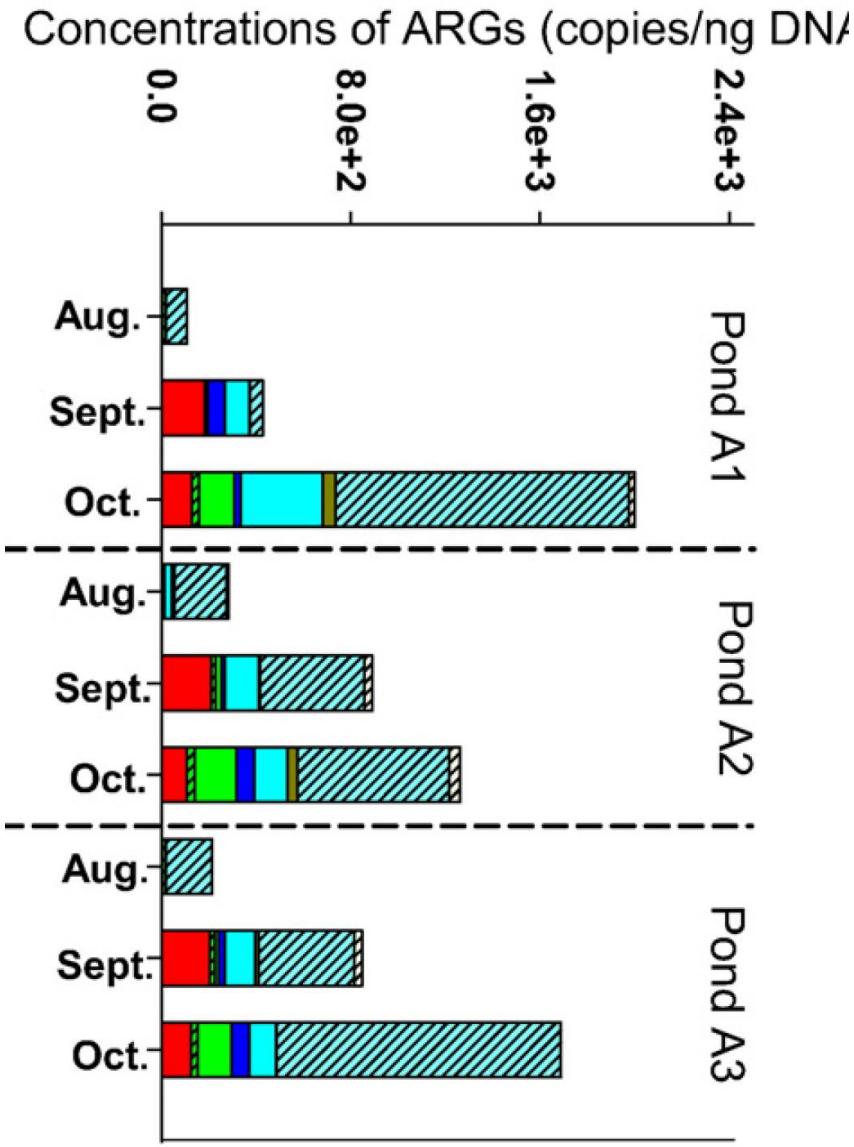
2.2 Occurrence and temporal variation of antibiotic resistance genes (ARGs) in shrimp aquaculture: ARGs dissemination from farming source to reared organisms



—Su et al. Science of the Total Environment 607–608 (2017) 357–366

Contents of target ARGs in water samples





Contents of target ARGs in sediment samples

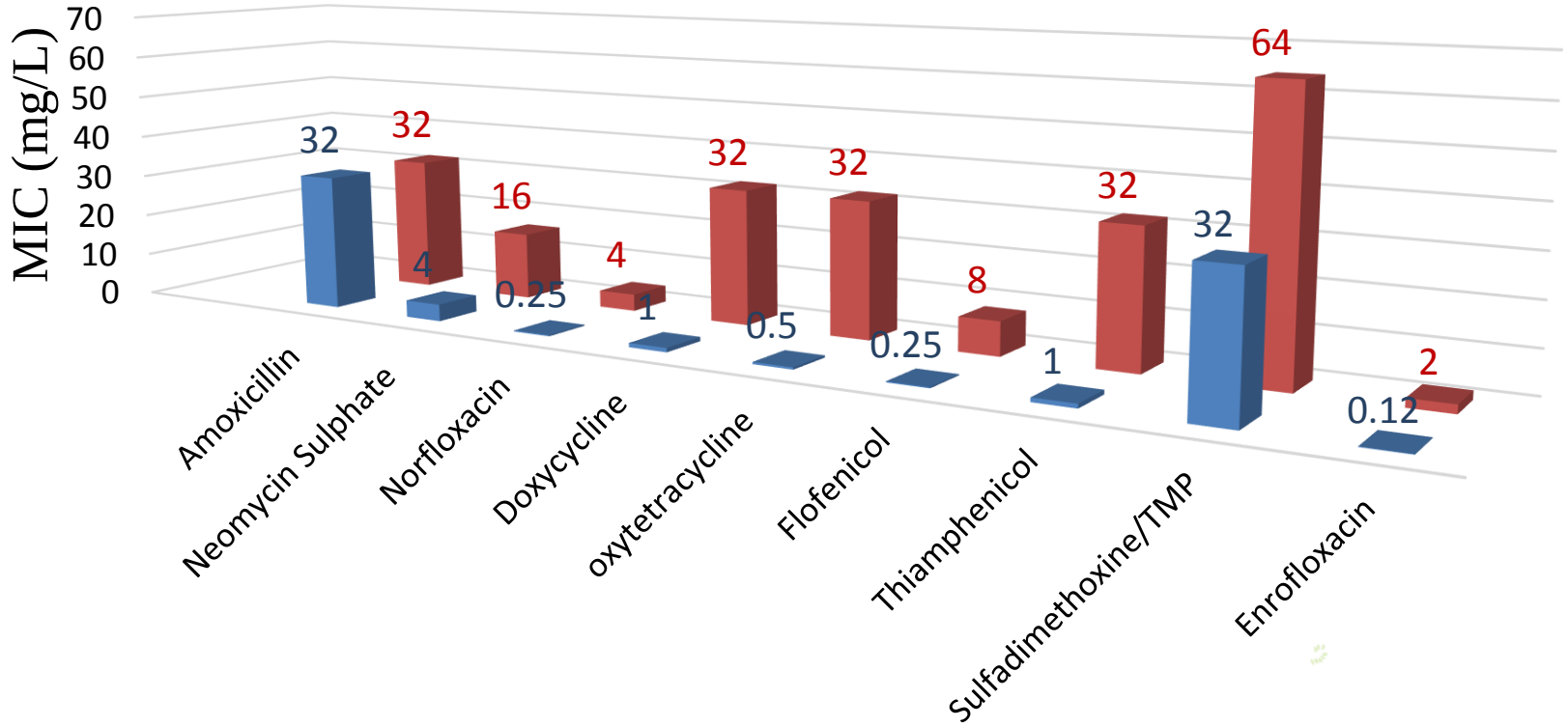
Contents of target ARGs in shrimp intestinal tract samples

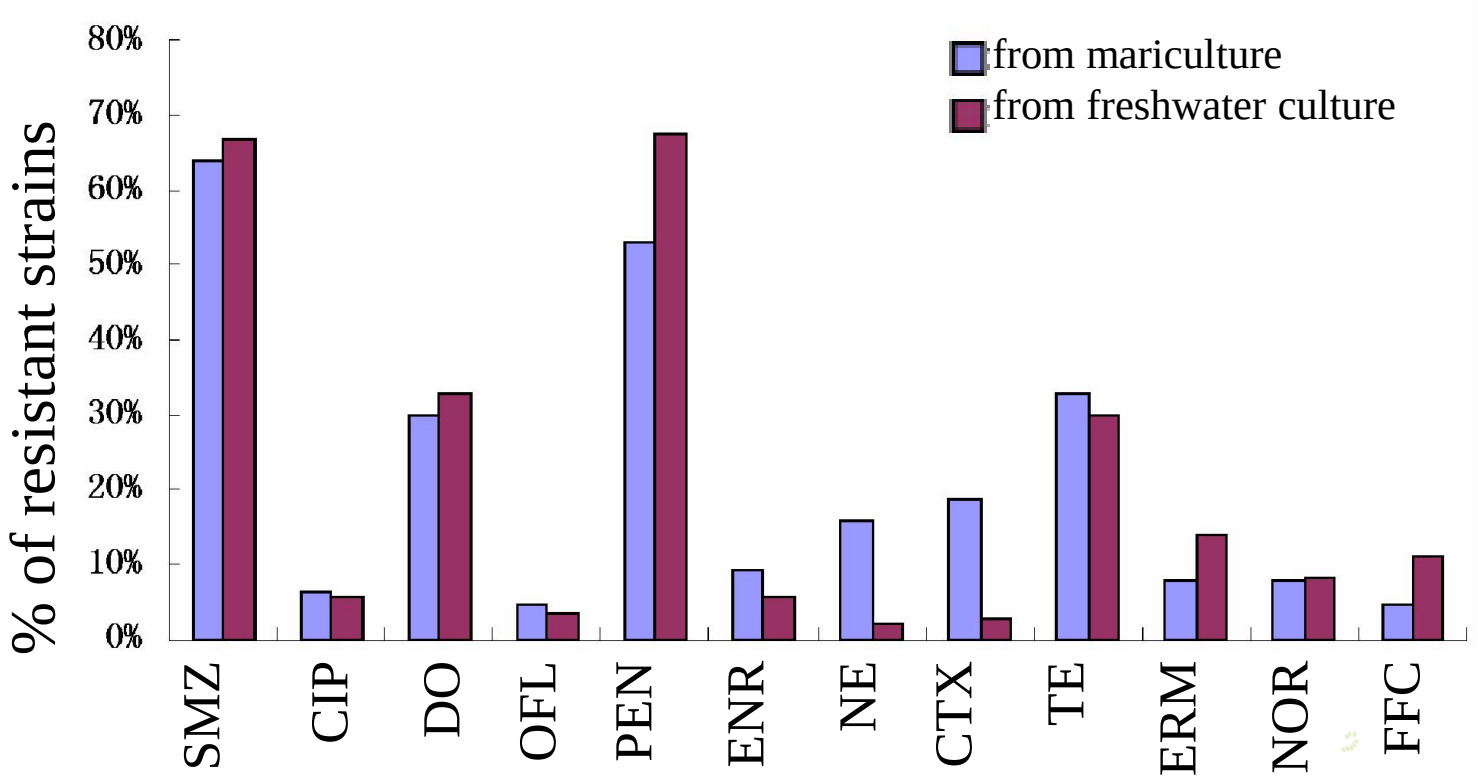
- Water source was an important medium disseminating ARGs to the aquaculture environments and reared organisms.
- The results showed that ***sul1*, *qnrD*, *cmlA*, and *floR*** were the predominant ARGs in the aquaculture environment and were different from those in the intestinal tract of shrimp.
- The total abundances of ARGs in intestinal tract of adult shrimps were 4.48~19.0 times higher than those in juvenile shrimps.

2.3 Investigations in the eastern coastal areas of Jiangsu province with diverse bacteria and diverse fish host

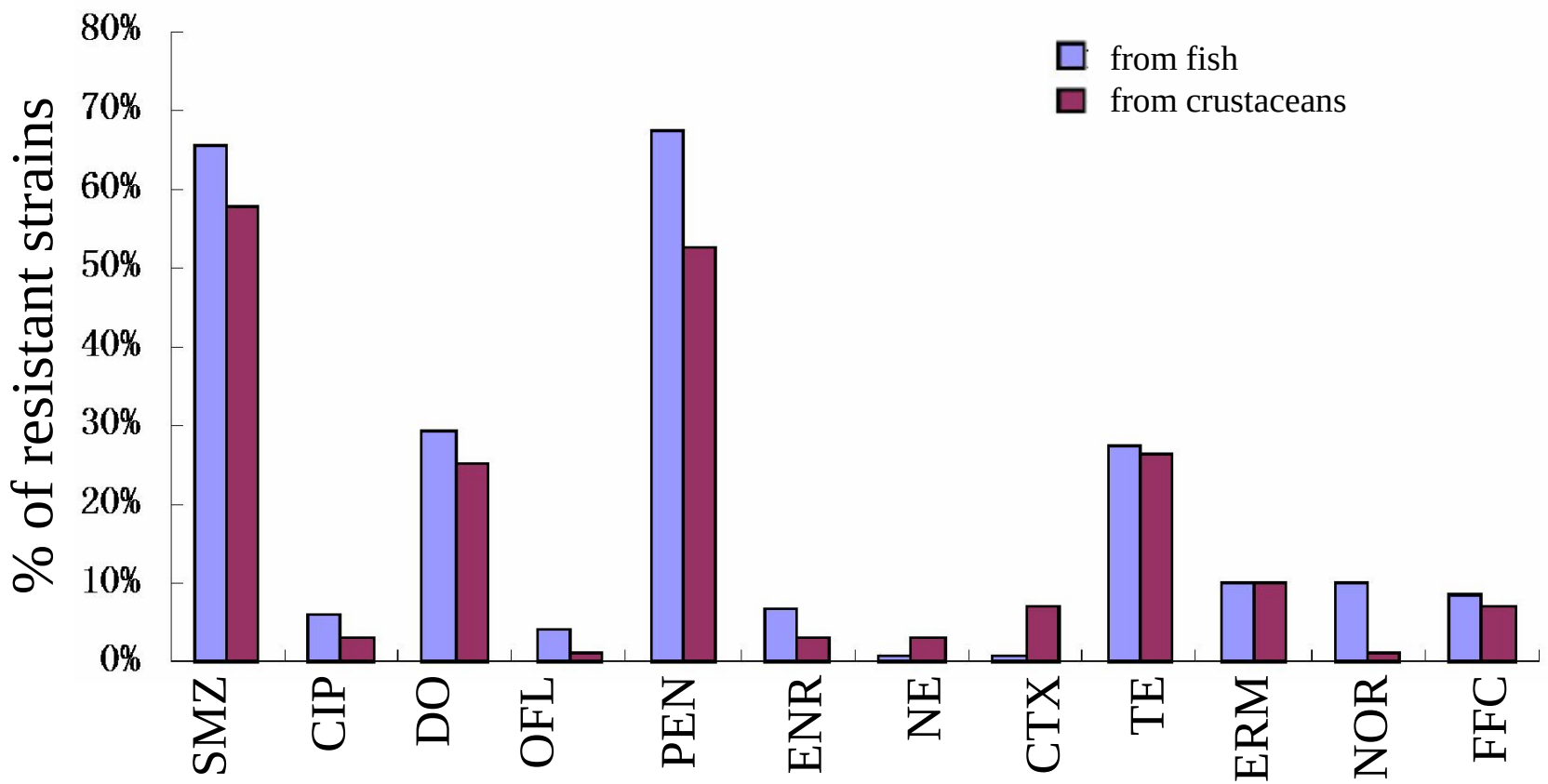
-Qiao & Wan. Master's thesis of Shanghai marine university, 2015

MIC_{50} and MIC_{90} of miscellaneous fish pathogenic bacteria isolated in Jiangsu province



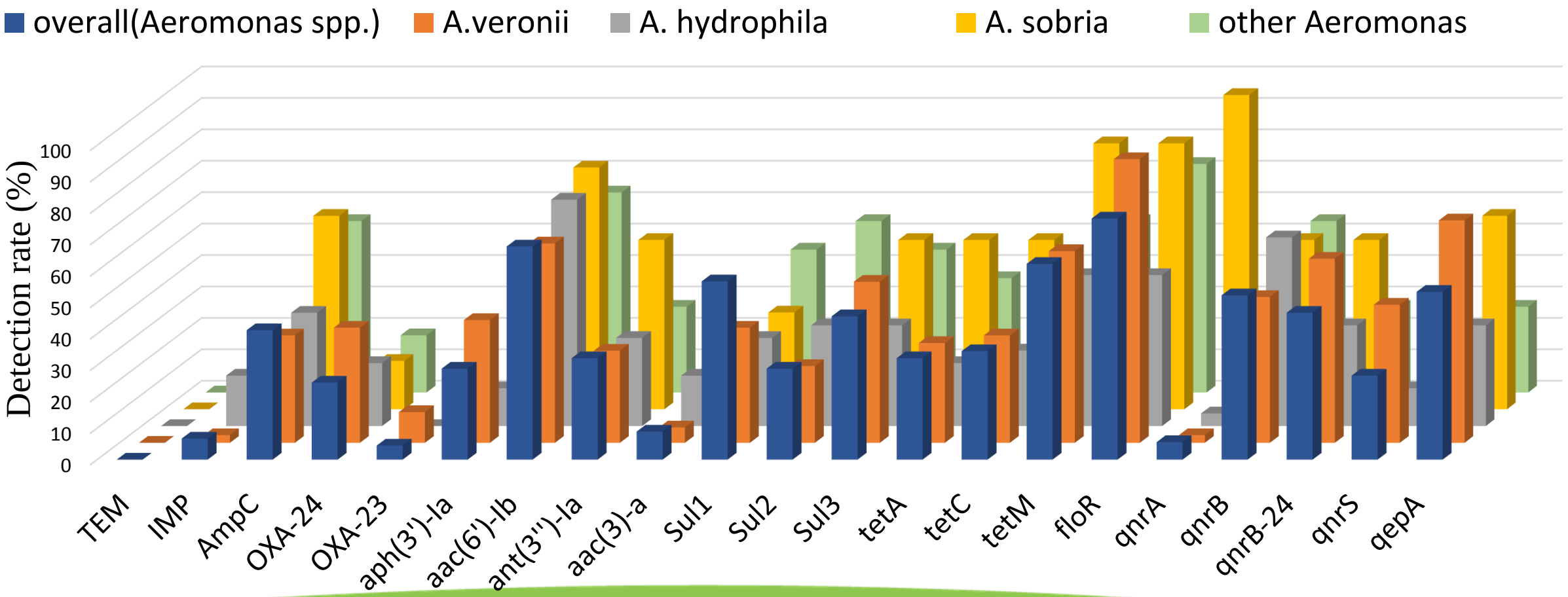


Comparison of resistance rate (%) of bacterial strains isolated from mariculture and freshwater aquaculture in Jiangsu province



Comparison of resistance rate (%) of bacterial strains isolated from fish and crustaceans cultured in Jiangsu province

Detection rate of various kinds of ARGs from 90 strains of *Aeromonas* spp. collected from coastal areas in Jiangsu province



Part 3
Integron-mediated Resistance Mechanism in *Aeromonas*

——Deng et al. Frontier in Microbiology, 2016:doi.org/10.3389/fmicb.2016.00935
——Li, et al. Journal of Fishery Sciences of China, 2013, 20(5):1015-1022

The integron-gene cassette system is one of the main spreading mechanisms of AMR. Integron can express the foreign R genes with the help of its strong promoter by capturing, integrating and recombining. A single integron can integrate several different R gene cassettes, by this way bacteria can be able to obtain multiple drug resistance.

Molecular characterization of integron-gene cassettes of 22 strains of class 1 integron-positive *Aeromonas* isolates from aquatic animals.

Classes	No.	Variable region（bp）	Gene cassette array
A	1	＞2000	*aac(6')-II*+*bla*OXA-21+*cat3*
B	12	2000	*drfA12+orfF+aadA2*
C	2	2000/ 900/750	*drfA12+orfF+aadA2/ catB3/ arr-3*
D	1	2000/ 1700/ 1500	ND
E	1	2000/ 800	*drfA12+orfF+aadA2/ drfA17*
F	2	1200/ 900	*drfA12+orfF/ catB3*
G	2	800	*dfrA17*
H	1	130	NA

***drfA*，*aadA* and *orfF* are the most common gene cassettes**

——Deng et al, Frontiers in Microbiology, 2016

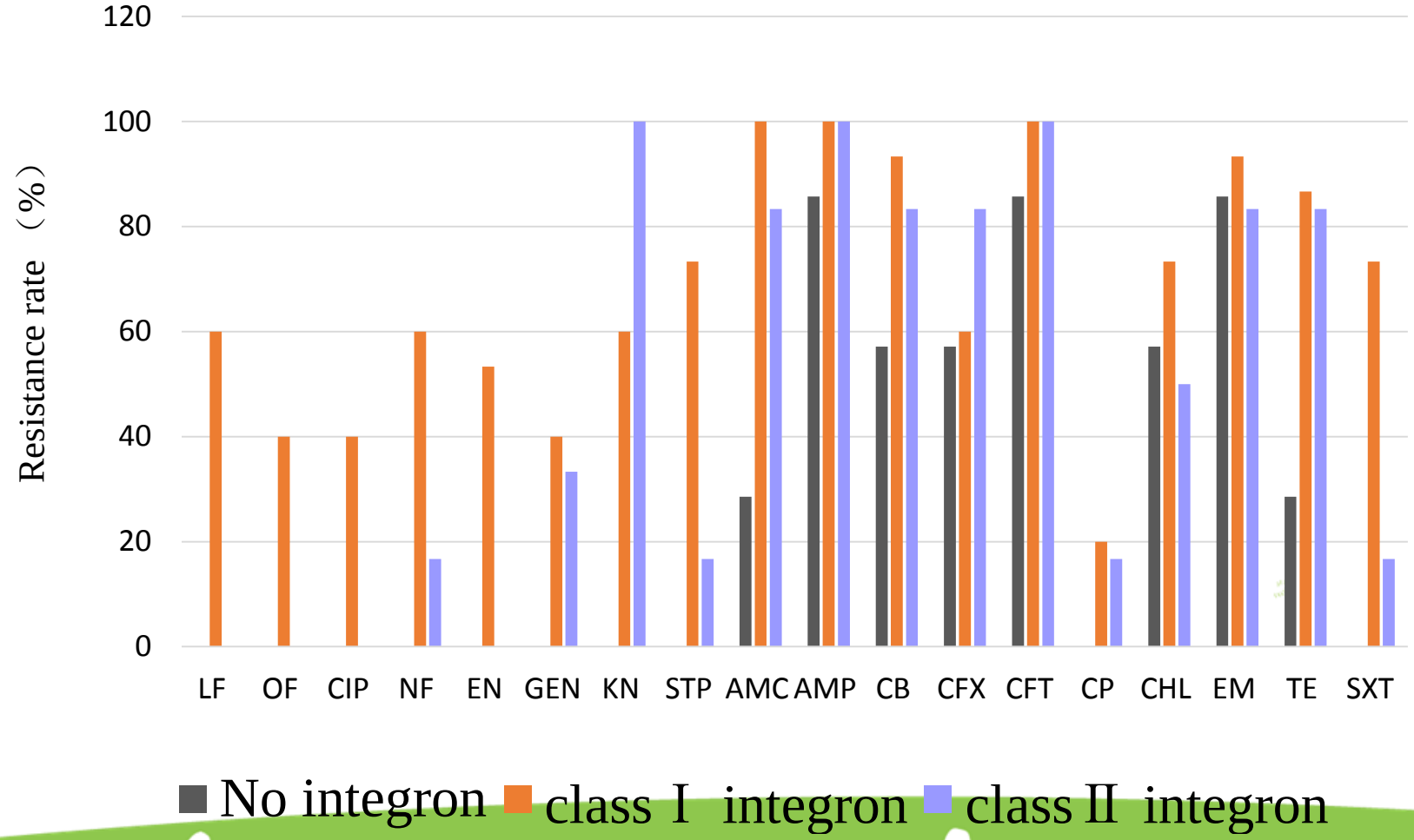
Distribution of various type of gene cassette array in variable region of 15 class I integron positive *Aeromonas* strains

gene cassette array	No. of strains
aadA2+dfrA12	4
aadA2+dfrA27	1
aadA2+aadA4a	1
aadA2	2
aadB	1
dfrA1+orfC	2
catB3+aadA2	2
blaOXA21+catB3	1
aadB+catB3	1

- *aadA*、*dfrA*、*catB* gene cassettes are the most common gene cassette, and mediate the resistance to aminoglycoside, sulfonamides and chloramphenicol, respectively
- Class I integron locates on both chromosome and plasmid, Class II was found only locating on plasmid

——Li SW, et al. Journal of Fishery Sciences of China, 20(5):1015-1022

Correlation between integron-gene cassette system and multi-drug resistance (MDR) in fish pathogenic *Aeromonas hydrophila*



- Class I integron: 15 strains
- Class II integron: 6 strains
- No integron:7 strains
- Class III integron was not detected

Correlation between drug resistance phenotype and integron carrying in *Aeromonas*

Strains	Antibiotics resistant profile	Class1 Integron	Class 2 Integron
AH1	PEN/AMP/ CRO/CEZ/ KAN/STR/SPE/AMI/TET/DOX/ERY/CLI/VAN/ FUR/RIF/TMP/CPFX	+	+
AH2	PEN/AMP/ CRO/CEZ/ KAN/STR/SPE/AMI/TET/DOX/ERY/CLI/FUR/ RIF/TMP/NOR/CPFX	+	+
AH3	PEN / CRO /CEZ/STR/KAN/SPE /TET/DOX/ERY/CAP/ CLI / FUR /RIF/ TMP/ LEVO /NOR/CPFX	+	+
AH4	PEN/AMP/CEF/ CRO /CEZ/STR/KAN/SPE /AMI /TET/DOX/ERY/CAP/ CLI / VAN/ FUR /RIF/ TMP /NOR	+	+
AH5	PEN/AMP/ CRO / STR/ SPE / TET/DOX/ERY/CAP/ CLI / FUR /RIF/ TMP/ LEVO /NOR/CPFX	+	+
AH6	PEN/AMP/ CRO / STR/ TET/DOX/ERY/ CLI / VAN/ FUR /RIF/ TMP/ CPFX	+	+
AH7	PEN/AMP/CEF/ CRO /CEZ/STR/KAN/SPE /AMI /TET/DOX/ERY/CAP/ CLI / VAN/ FUR /RIF/ TMP/ NOR/CPFX	–	+
AH8	PEN/AMP/CEF/ CRO /SPE /AMI /TET/DOX/ERY// CLI / FUR /RIF/ TMP	–	+
AH9	PEN/AMP/CEF/ CRO /SPE /AMI /TET/DOX/ERY/ CLI/VAN / FUR /RIF/ TMP	–	+
AH10	PEN/AMP/CEF/ CRO /SPE /AMI /TET/DOX/ERY/ CLI/VAN / FUR /RIF/ TMP	–	+
AH11	PEN/AMP/CEF/ CEZ/STR/KAN/SPE / TET/DOX/ERY/CAP/ CLI / VAN/ FUR /RIF/ TMP/ NOR/CPFX	–	+
AH12	PEN/AMP/CEF/ CEZ/STR/KAN/SPE / TET/DOX/ERY/CAP/ RIF/ TMP/ LEVO /NOR/CPFX	–	+
AH13	PEN/AMP/CEF/ CEZ/STR/KAN/SPE /AMI /TET/DOX/ERY/CAP/ VAN/ RIF/ TMP/ LEVO /NOR/CPFX	–	+
AH14	PEN/AMP/CEF/ CEZ/STR/KAN/SPE / TET/DOX/ERY/CAP/ VAN/RIF/ TMP/ LEVO /NOR/CPFX	–	+
AH15	PEN/AMP/CEF/ CRO /CEZ/STR/KAN/SPE /AMI /TET/DOX/ERY/CAP/ CLI / VAN/ FUR /RIF/ TMP/ LEVO /NOR/CPFX	–	+
AH16	PEN/AMP/CEF/ CRO /CEZ/STR/KAN/SPE /AMI /TET/DOX/ERY/ FUR	–	–
AH17	PEN/AMP/CEF/ CRO /CEZ/STR/KAN/SPE /AMI /TET/DOX/ERY/ CLI / VAN/RIF/ TMP/ LEVO /NOR/CPFX	–	+
AH18	PEN/AMP /CEZ/ CLI / NOR/	–	–

The resistance gene profiles carried by these bacteria were basically consistent with the corresponding resistantce phenotype, but there were some exceptions

-Xiao GH.2012. Master's thesis of Jiangxi Agriculture University.

Comparison on the AMR of 50 integron-positive and 267 integron negative isolates

	Integron-positive strains			Integron-negative strains			
Antimicrobials	Resistance rate	MIC_{50}	MIC_{90}	Resistance rate	MIC_{50}	MIC_{90}	P values
SMM	98%	>512	>512	55.43%	512	>512	0.000**
SXT	96%	>76/4	>76/4	3.74%	≤9.5/0.5	≤9.5/0.5	0.000**
SM2/TMP	-	>20/4	>20/4	-	≤2.5/0.5	5/1	-
SD/TMP	-	>20/4	>20/4	-	≤2.5/0.5	5/1	-
DO	18%	4	16	2.24%	≤0.5	4	0.000**
TE	16%	4	16	0.37%	≤0.5	4	0.000**
NA	92%	>128	>128	46.82%	1	>128	0.000**
CIP	26%	0.5	4	0.37%	0.015	0.25	0.000**
NOR	20%	2	16	0.37%	≤0.03	1	0.000**
ENR	6%	0.25	2	0.00%	0.015	0.25	0.001**
NE	8%	1	4	0.00%	≤1	2	0.000**
CN	2%	1	4	0.00%	0.5	1	0.147
AK	0%	1	4	0.00%	1	2	-
STR	-	16	128	-	4	8	-
AMP	96%	>128	>128	97.00%	>128	>128	0.987
CTX	22%	0.06	64	0.00%	0.06	0.25	0.000**
CHL	44%	16	>64	1.50%	≤2	≤2	0.000**
FFC	62%	32	>32	4.49%	≤1	2	0.000**
THI	-	>32	>32	-	≤2	≤2	-
FUR	0%	≤4	8	0.00%	≤4	≤4	-

—Feng YY. Master's thesis of Shanghai marine university, 2016

Correlation between Gene cassette array and MDR profile in *Aeromonas*

Gene cassette type	Gene cassette array	MDR isolate/total isolate	Resistance pattern
A	*dfrA17*	3/7	FFC/SXT/(ENR)/(CIP)/(CTX)
B	*dfrA12-orfF-aadA2*	0/6	FFC/(C)/SXT/(DO)
C	*dfrB4-catB3-aadA1*	2/6	(FFC)/(C)/SXT/(DO)/(CIP)(NOR)/(ENR)
D	*catB8*	0/5	SXT
E	*aac(6')-Ib-cr-arr-3*	5/5	FFC/C/SXT/DO/TE/CIP/NOR/CTX
F	*aac6-* Ⅱ*-blaOXA-21-catB3*	0/3	(FFC)/(C)/SXT
G	*aar2-aacA4-drfA1-orfC*	0/1	SXT/CIP
H	*aac(6')-Ib-cr*	1/1	FFC/C/SXT/CIP/NOR/CTX
I	*dfrA15*	0/1	SXT
J	*dfrB4-catB3-blaOXA-10-aadA1*	0/1	SXT
K	Empty integron	7/14	C/C/SXT/(DO)/(TE)/(CIP)/(NOR)/(N)/(CN)/(CTX)
	Integron-positive strains	**18/50**	
	Integron-negative strains	**2/267**	

Note: Resistance to antimicrobial agents appearing in parentheses was not present in all isolates.

- Among 317 *Aeromonas* isolates, 50 (15.77%) isolates were positive for *inti1*.
- Integron-positive isolates have higher resistance rate than integron-negative strains, and most were multidrug resistant.
- There were 16 different gene cassettes encoding resistance to aminoglycosides (*aadA1*,*aadA2*,*aac6- II*,*aacA4*),trimethoprim (*drfA1*,*dfrA12*,*dfrA15*,*dfrA17*,*dfrB4*), β-lactams(*blaoxA-10*,*blaoxA-21*), chloramphenicl (*catB3*,*catB8*),rifampicin (*arr-2*,*arr-3*) and plasmid-mediated quinolone resistance (PMQR) gene *aac(6')-Ib-cr*.
- The resistance phenotypes were corresponding to the gene profiles in the gene cassettes in all of the integron-positive isolates, suggesting that there were a close relationship between class 1 integron and multi-drug resistance in *Aeromonas* isolates.

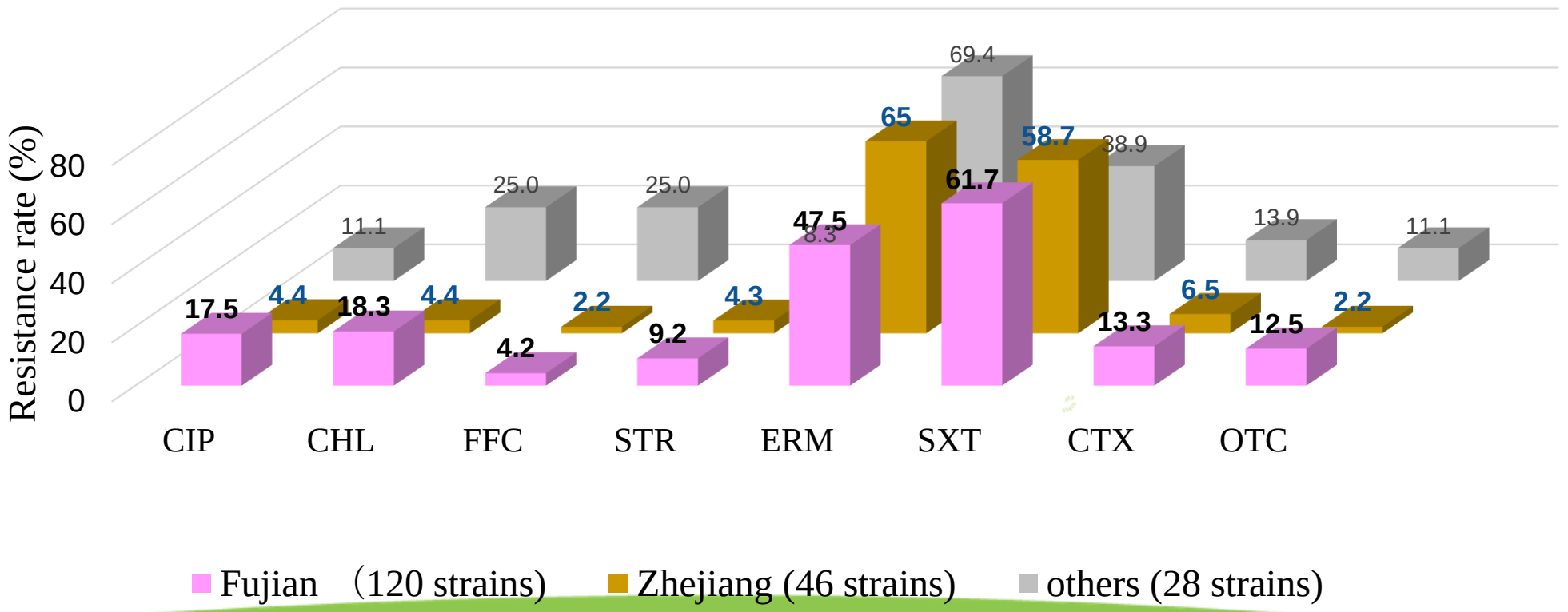
—Feng YY. Master's thesis of Shanghai marine university, 2016

Part 4

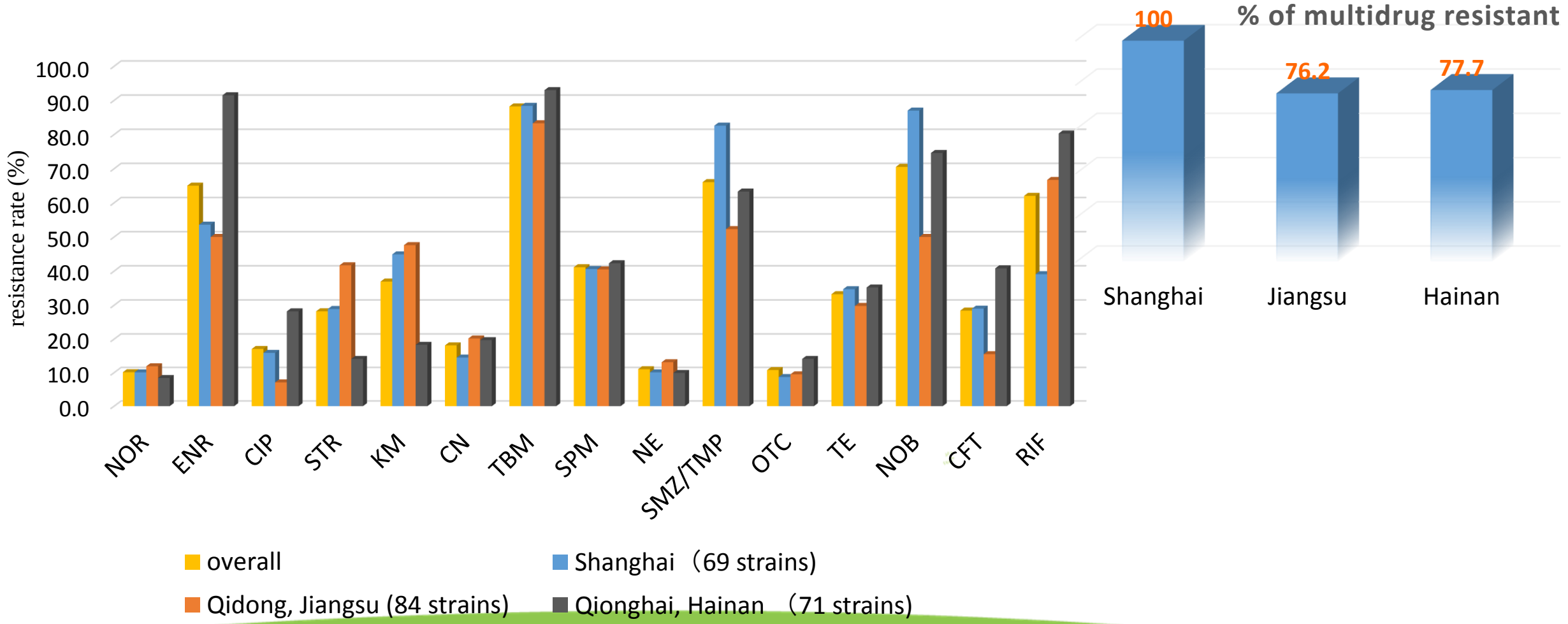
AMR of *Vibrio* strains isolated from marine fishes

—Xie et al. Marine Fisheries, 2011,33(4):442-446
—Jiang et al. Journal of Food Safety and Quality,2015,6:3474-3479
—Hu MH. Master's thesis of Shanghai marine university, 2015
—Jiang et al. South China Fisheries Science,2016,12(6):99-106
—Zhen et al. China Fisheries,2017.

4.1 Regional AMR comparison of vibrios from miscellaneous sources to 8 kinds of antimicrobials



4.2 Comparison of AMR of *Vibrio* isolates from different regions of China



Xie LY, 2012

Detection of class I integron in vibrios isolated from three different regions

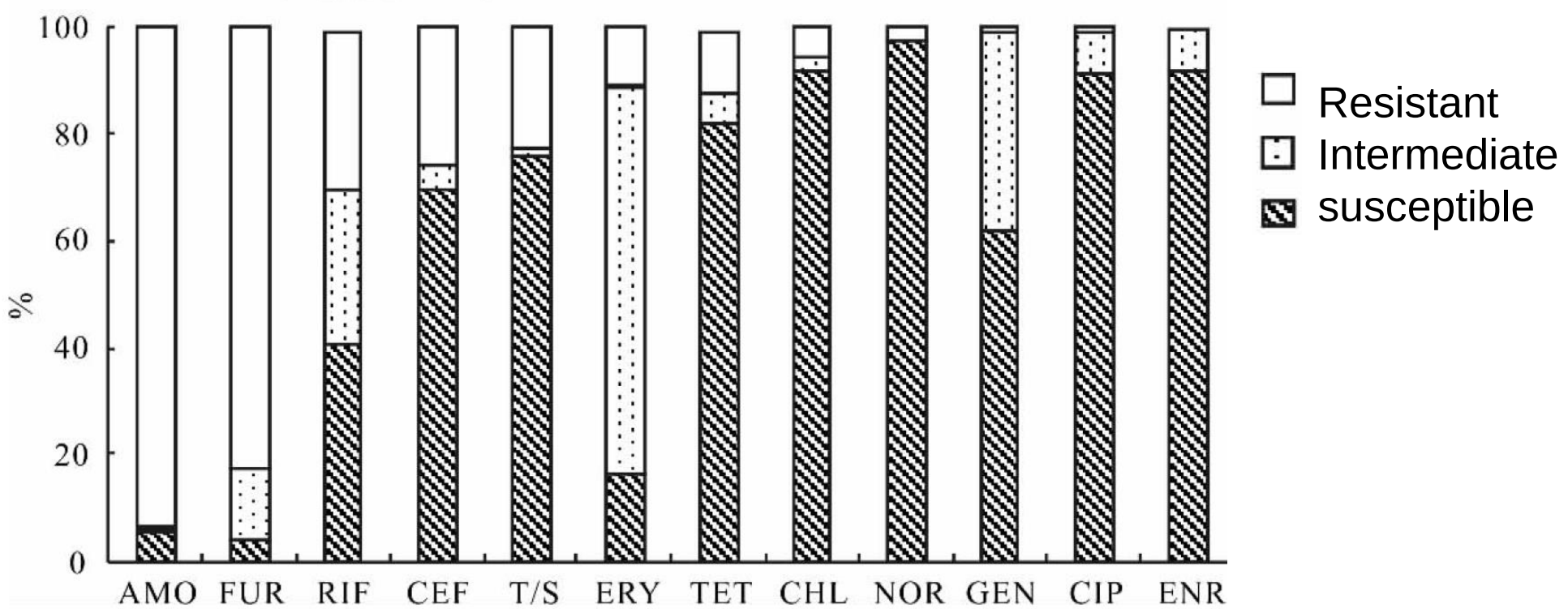
Gene detected	Shanghai (69 strains)		Jiangsu (64 strains)		Hainan (55 strains)		Overall (188 strains)	
	Genome	plasmid	Genome	plasmid	Genome	plasmid	Genome	plasmid
IntI1	48	32	8	10	28	14	84 (44.7%)	56 (29.8%)
Variable region	22	15	6	9	16	10	44	34
3'-S	25	22	7	9	16	12	48	43

- The gene cassette array : *dfrA12+orf+* aadA2 was the only type of cassette array identified from both chromosome and plasmid indicating the transfer of R genes between the two locations.
- No gene cassette resistant to quinolones was detected even if resistance against quinolones was very common suggesting its resistance is mediated by QRDR.

Xie, 2012

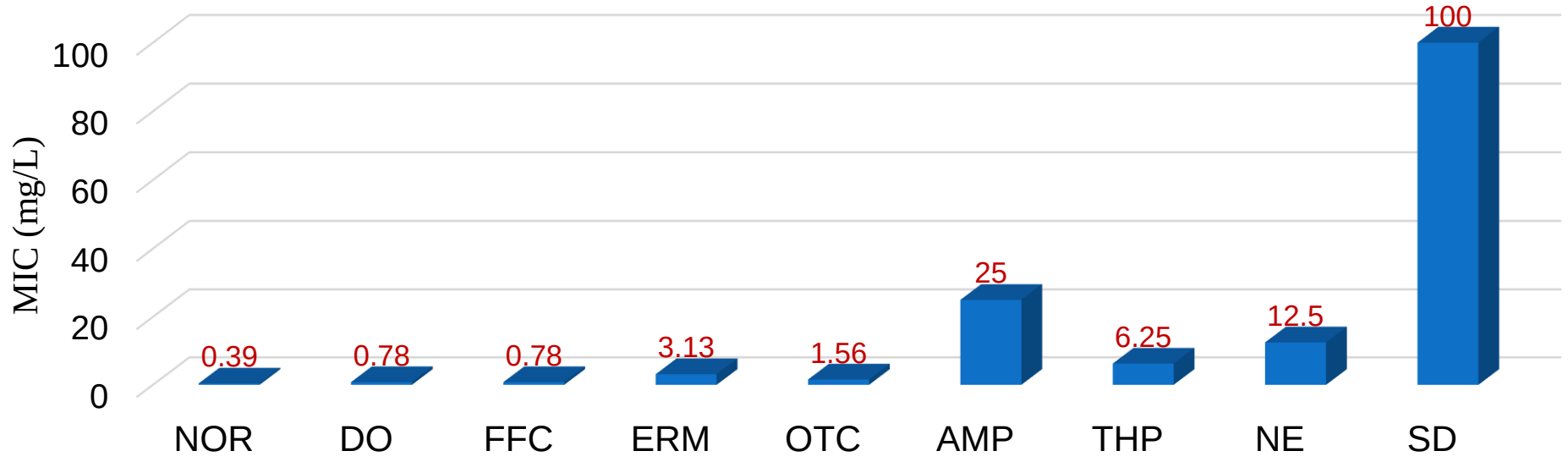
- In this study, the class I integron positive strains were all multidrug-resistant.
- It suggests that there is a close relationship between the multidrug resistance of bacteria and class I integron.

4.3 AMR of *Vibrio harveyi* (90 strains) isolated from maricultured fish in the South China during 2012 - 2014

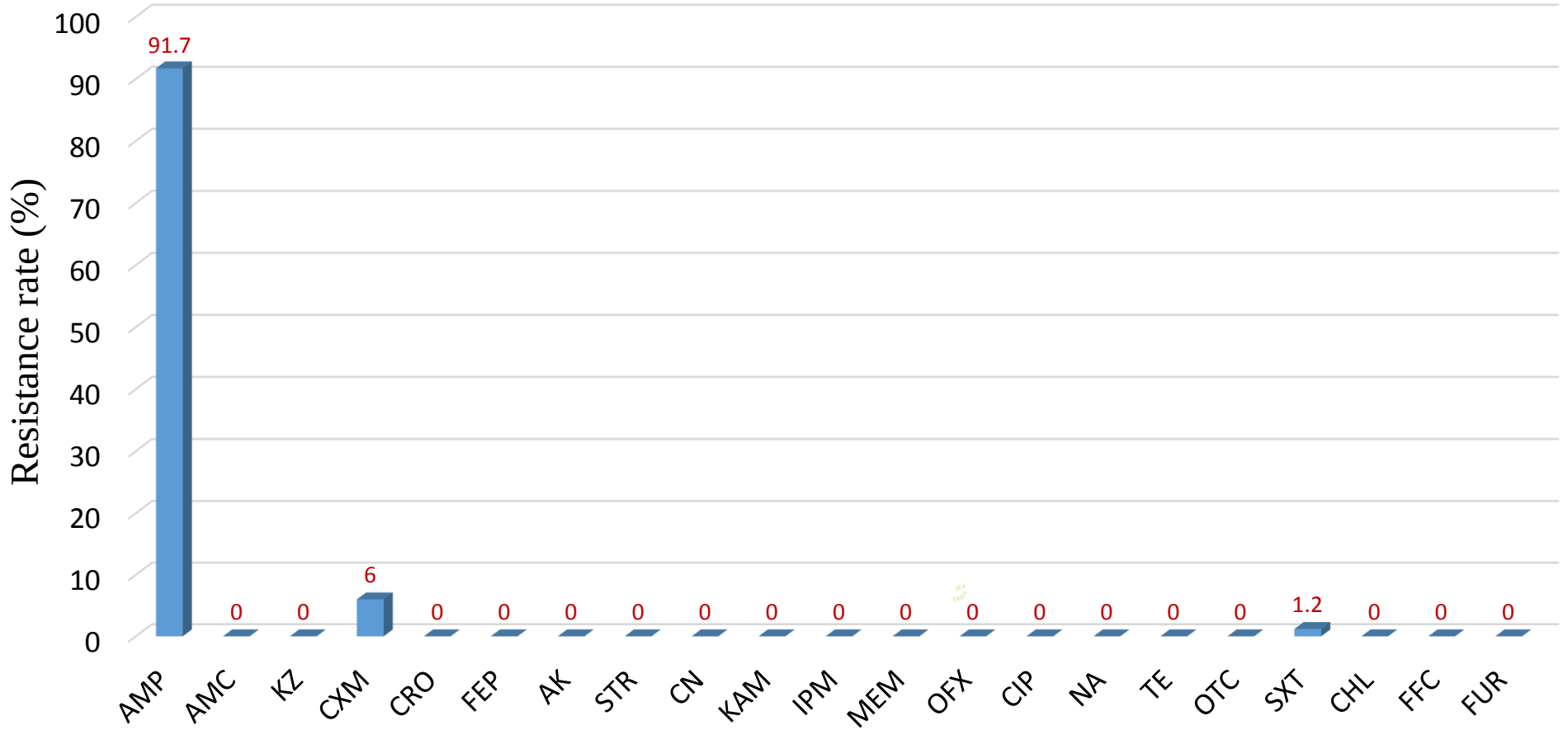


Jiang et al, 2016

4.4 MIC_{50} of 104 strains *Vibrio* spp. isolated from cultured turbot in Liaoning province in 2016



4.5 AMR of *V. parahaemolyticus* (84 strains) isolated from maricultured shellfish



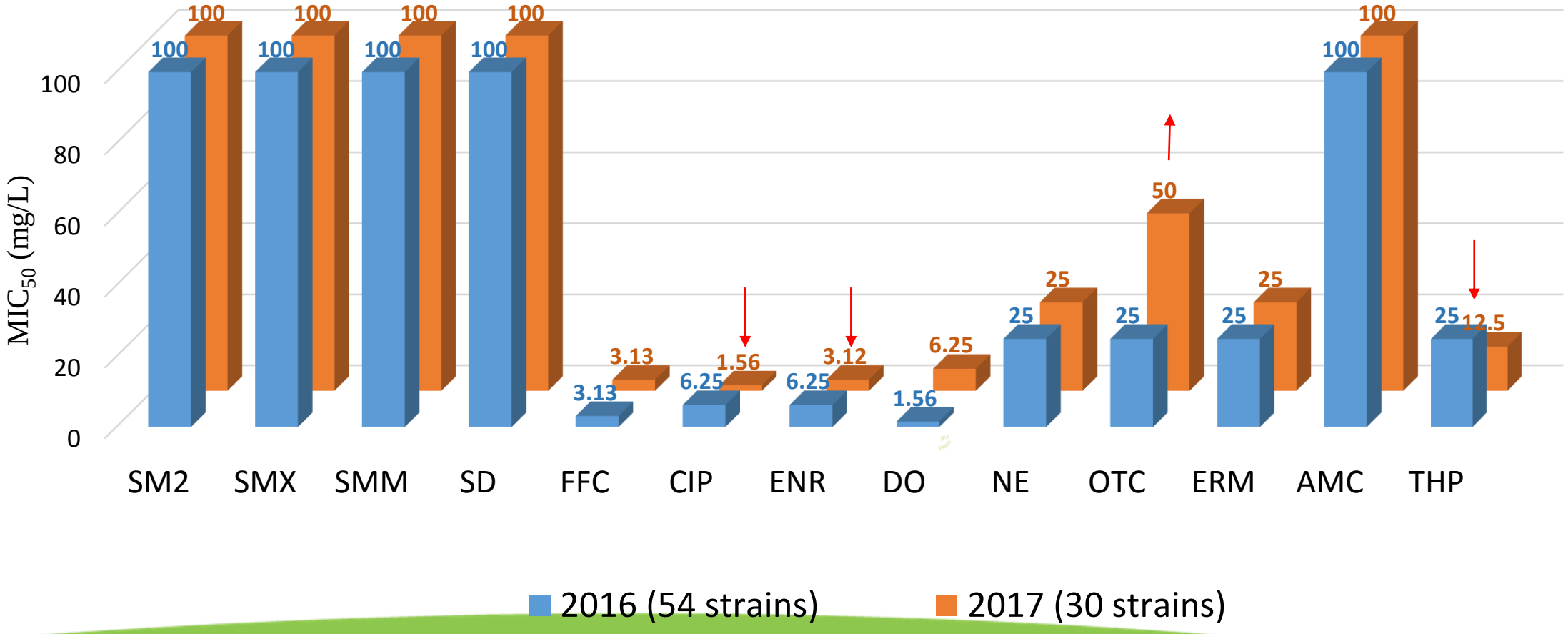
-Jiang YH,et al, 2015

PART 5

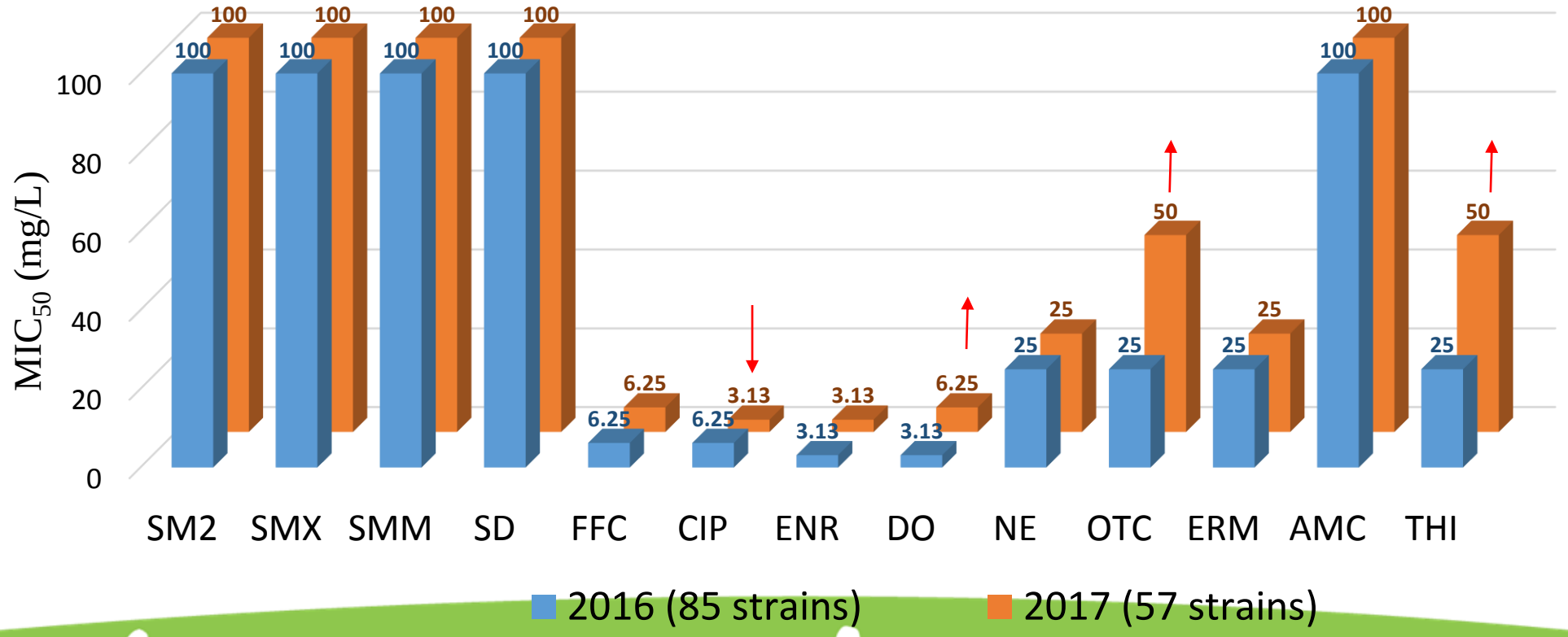
Annual Change of AMR in aquaculture in a specific region of China

- The host fish are pond-cultured common carp and channel catfish, and the isolation organs were liver and kidney.
- The dominant isolates are *Aeromonas* spp., *Acinetobacter* spp., *Morganella* spp. and *Bacillus* spp

Comparison of AMR of pathogenic *Aeromonas* strains isolated in 2016 and 2017



Comparison of AMR of miscellaneous fish pathogenic bacteria isolated in 2016 and 2017



Conclusions

- There are differences in AMR between cultured species and regions. Proportion of drug-resistant bacteria originating from fish were less than those from reptiles.
- Sulfonamides were least sensitive antimicrobials in most cases. AMR in cold fish and shellfish were better than that in other aquatic animals.
- Integron mediated AMR plays a very important role in the population of aeromonads and vibrios. Class I integron was more common than class II, and class III has not been detected so far.
- Gene cassette,*dfrA12*, *aadA2* and *aac(6')-Ib-cr*, encoding resistance to aminoglycosides, trimethoprim and quinolones, respectively, were the most common ones in the variable region of class I integron.

Conclusions (cont.)

- ARGs can be enriched in the pond sediment despite the lack of contemporaneous antibiotic use at the pond.
- In order to curb the deterioration of bacterial resistance in aquaculture, we need to develop more strategies in addition to the need for prudent and responsible use of antibiotics. For example, determining the possible sources of ARGs, especially mobilized ARGs, is essential for controlling the occurrence and spread of ARGs at fish farming facilities and for lowering the risk of ARG spread from the farms to surrounding environments.

Thank you very much for your attention！

感谢您的关注！