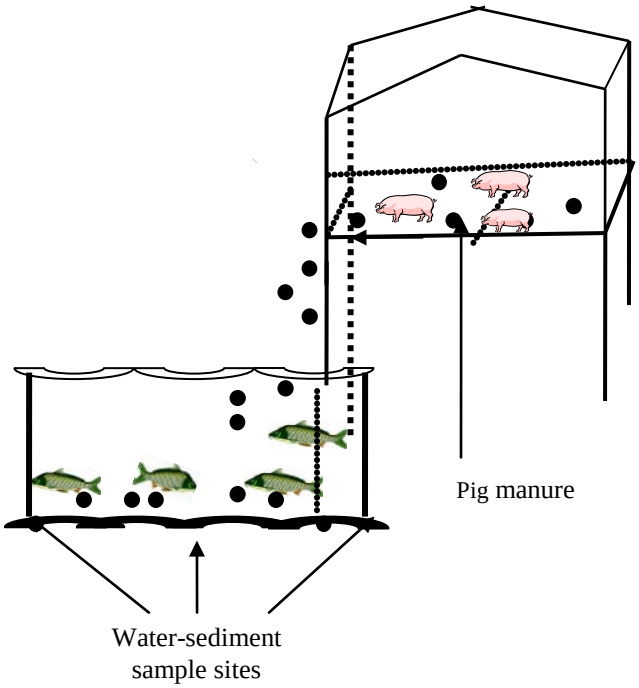
SUSANE - Sustainable, sanitary and efficient management of animal manure for plant nutrition

Impact of medicated feed on the development of antimicrobial resistant bacteria in integrated pig-fish farms in Vietnam.

Summary

1. Introduction

Use of animal manure as fertilizer of aquaculture ponds is practiced widely in Southeast Asia. Pigs are raised in houses on the edge of ponds and pig manure is discharged directly into the fish pond to support growth of algae and aquatic plants consumed by fish.



Aquaculture, including integrated systems, is of increasing economical importance in many provinces in Vietnam. Fish farms using untreated livestock wastes as fertilizers are widespread. Moreover, development and spread of antimicrobial resistance has become a health problem in Vietnam as well as globally; a problem that is impacted by human and non- human antimicrobial usage. Antimicrobials are commonly used as feed growth promoters to improve pig productivity. The pig growth promoters are also used in integrated pig-fish farms, and this use may also increase the antimicrobial resistant level of bacteria in the harvested fish products which are used for human consumption.

Unfortunately, there are very few evaluations of the resistance level of bacteria in fish product in Vietnam. Therefore, the sanitary component of SUSANE has carried out studies to throw light on the risk for increasing resistance of bacteria to medicine due the use of antibiotics and other antimicrobials in the swine production. This Newsletter presents a study of the impact of integrated pig-fish farming on the development of antimicrobial

resistant bacteria in the aquatic pond environment. In coming newsletters we will present results from an ongoing study of resistant bacteria in fish from these fish ponds.

2. Design of the experiment

Manure from pigs was added to fish ponds in a controlled experiment where pigs were fed antimicrobials as growth promoters added to the feed (Fig. 1, sampling periods 2 and 4), and the antimicrobial résistance of bacteria in the manure and mixed water sediment samples were examined. The results of this study was compared with the antimicrobial resistance of bacteria from manure and mixed water sediments from a trial where the manure used was collected from pigs feed commercial feed without antimicrobial (Fig. 1, sampling periods 1 and 3). Resistance of strains of *E. coli* and *Enterococcus* spp. isolated from the samples was examined. These bacteria were selected for the study because both are internationally used as indicators of fecal pollution and in surveillance programs of development of antimicrobial resistance.

3. Resistance of *E. coli*

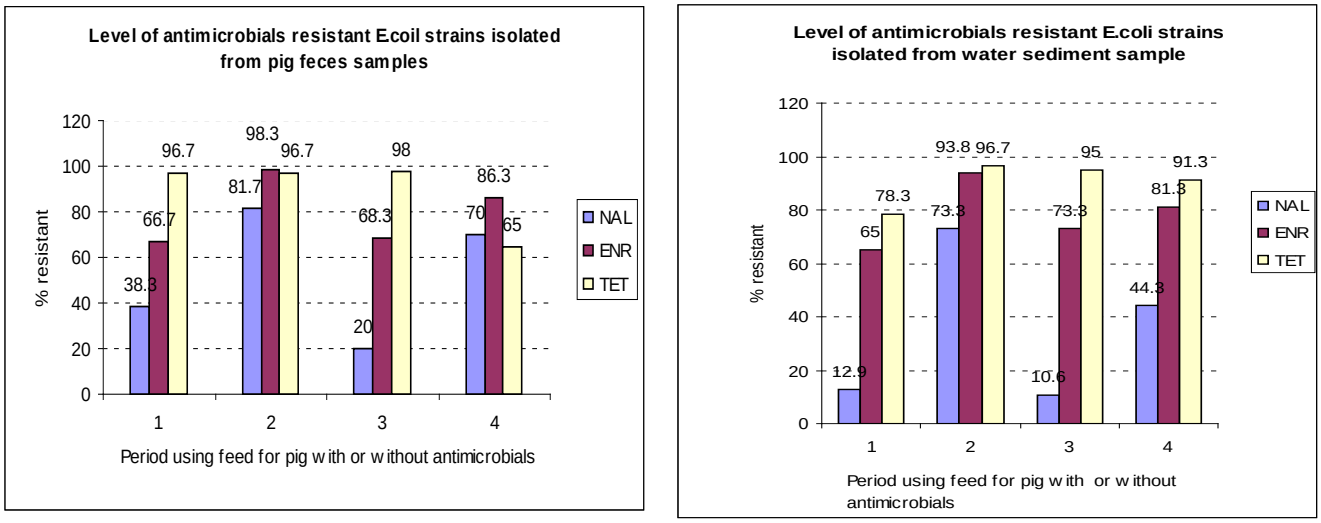


Figure 1: Level of antimicrobials resistant *E. coli* strains isolated from manure samples (left) and water sediment samples of fish pond (right)

NAL: Nalidixix acid; ENR: Enrofloxacin; TET: Tetracyclin

Period 1, 3: Using feed for pig without antimicrobials

*Period 2, 4: Adding tetracycline (*5 µg/kg pig weight/day) *and enrofloxacin (*0.45 µg/kg pig weight/day) *in to feed for pig*

During four month from February to April 2009, water sediment samples of fish pond and pig manure samples were obtained a total of 13 times. In total, 634 *Enterococcus* spp.

and 520 *E. coli* were obtained from all above pig manure samples (MS) and mixed water sediment samples (WS) for antimicrobial resistance testing. A significant temporal increase in nalidixic acid (NAL) and enrofloxacin (ENR) resistance were found for *E. coli* isolates obtained from two sample types (WS: NAL from 12.9% to 73.3% and ENR from 65% to 93.8%; MS: NAL from 38.3% to 81.7% and ENR from 66.7% to 98.3%). Consequently, the using antimicrobials in fish farming may represent a route of transmission of antimicrobial resistant bacteria and antimicrobial resistance genes from livestock to humans, e.g from pig, and fish products.

4. Resistance of enterococci

Period	Antimicrobial	Prevalence (%) of resistance of enterococci isolated from manure samples			Prevalence (%) of resistance of enterococci isolated water sediment samples		
		E. faecium	***E. faecalis***	Other E***nterococcus*** spp.	***E. faecium***	***E. faecalis***	Other E***nterococcus*** spp.
1	**Enrofloxacin**	-	-	-	-	-	-
2		-	19,4	-	-	16,7	-
3		-	5,9	-	-	6,3	3,7
4		-	4,8	-	-	9,1	-

Table 1: Prevalences of resistance to enrofloxacin of enterococci isolated from manure samples and water sediment samples

The tetracycline resistance prevalence among *Enterococci* spp. was 100% in isolates recovered from both sample types. The resistance prevalence for other antimicrobials shown by *Enterococci* spp. varied and was associated with the use or non-use of ENR in the pig feed (ENR resistance varied between 0% to 19.4% in manure samples and was 0% to 16,7% in water-sediment samples (table 1)); the MIC value of NAL varied from 61 µg/ml to 256 µg/ml).

Species typing of all *Enterococci* spp strains was done by PCR to determine species of strictly fecal (*E. faecalis* and *E. faecium*) i.e. from pigs as well as species originating from the "aquatic environment. Species from the pigs and from the aquatic environment may show and develop different patterns of antimicrobial resistance. A total of 43 *E. faecalis*, 62 *E. faecium* and 199 strains of other *Enterococcus* spp. were obtained from water sediment samples; In pig manure samples, a total of 78 *E. faecalis*, 48 *E. faecium* and 204 strains of

other *Enterococcus* spp. were recovered. Further analyses will be carried out to determine the resistance development of *E. faecalis* and *E. faecium* strains and to compare their pato- and genotypes with similar species isolates recovered from human specimens.

5. Conclusion

The conclusion of the project is that the use of antimicrobials as feed growth promoters for pigs raised in integrated pig- fish farming systems increases the prevalence of antimicrobial resistant bacteria in the pig manure as well as in the water and sediment of fish pond. The impact of such resistance development on food safety of fish produced and public health in general is uncertain, but is currently being investigated.

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