P17 Minimum Inhibitory Concentrations of Antimicrobials against Vibrio spp. and Streptococcus spp. Obtained from Clinical Isolates

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Introduction

Bacterial disease is considered to be the most important cause of mass mortality and economic loss when present in intensive fish farming. Antimicrobial therapy is one of the strategies frequently applied for bacterial disease control. As a part of the prudent use of animal antimicrobials, antimicrobial susceptibility testing is strongly recommended prior to treatment. Thus, this study is to determine the Minimum Inhibitory Concentrations (MICs) for 4 antimicrobial agents: Amoxicillin, Oxytetacycline, Sulphadiazine/Trimethoprim (SXT) and Sulfadimethoxine/Ormetoprim (ORS) against the pathogenic vibrios isolates from farmed Black Tiger shrimp (Penaeus monodon) and Pacific White shrimp (Litopenaeus vannamei) and pathogenic streptococcus isolated from farmed tilapia (Oreochromis nilotica) in Thailand. The MIC tests reveal antimicrobial susceptibility of bacterial pathogens associated with the aquaculture industry of Thailand and hence, provide data to direct the prudent use of antimicrobials in aquaculture production.

Materials and Method

Minimum Inhibitory Concentration (MIC):

The procedures are in accordance with the international recommendations provided by the Clinical and Laboratory Standards Institute (CLSI). Mueller Hinton Agar (MHA) plates with two-fold agents are inoculated with the standardized inoculums of the isolate to be tested. After a 18-20 hour incubation, the MIC is recorded as the lowest concentration of the compound with no visible growth of bacteria.

Antibacterial Agent: The antimicrobials that were tested were Amoxicillin, Oxytetacycline, Sulphadiazine, Trimethoprim (Sigma Chemical Co., USA.), Sulfadimethoxine and Ormetoprim (PHARMAQ, Norway). A serial two fold dilution of antimicrobials dissolved in the appropriate solvent was processed as described by CLSI.

Bacterial strains: All tested isolates were obtained from the culture collection of the Department of Medicine, Faculty of Veterinary Science, Chulalongkorn University, Thailand. The collection has culture specimens from clinical cases occurring between 2004 and 2006. The identification of the isolates was previously performed using conventional biochemical methods described in the API system (BioMerieux, France). All bacteria strains were stored in maintenance broth at -70°C. Before each experiment for the MIC was carried out, the stored bacteria strains were transferred to Tryptic Soy Agar (TSA, Difco Lab, USA) supplemented with 10% sheep blood (for vibrio) or 1% NaCl (for vibrio). After incubation at 30°C for 18-24 hr, the inocula were transferred to TSB and the cell density was adjusted to McFarland standard 0.5 or approximately 10⁷ CFU/ml. The inocula were then diluted ten-fold in sterile normal saline, giving a final cell density of approximately 10⁸ CFU/ml.

Results and Discussion

The distribution of the MICs of Amoxicillin, Oxytetacycline, SXT and ORS against 50 streptococcus isolates associated with the diseased tilapia are presented in Figure 1. The data shows that Amoxicillin, SXT and ORS were effective against the streptococcus isolates being tested. MIC testing results of 50 vibrio isolates associated with the diseased shrimp indicated that most of the tested vibrio isolates were susceptible to ORS and moderately susceptible to SXT and Oxytetacycline, while being resistant to Amoxicillin. Our observation that ORS did not show a significant increase in MIC values when vibrio strains were tested on a seawater based medium, compared to 1% NaCl supplemented medium, is necessary for optimal therapeutic application of the compound, particularly when the compound is used in a marine environment. The changes in MIC values due to the addition of seawater to the medium have been reported for several antimicrobial agents, resulting in implications for MIC testing. In conclusion, MIC data obtained in this study suggests the susceptibility of tilapia streptococcus pathogens and penaeid shrimp vibrio pathogens to ORS and SXT. The minimal influence of seawater components on the antimicrobial activity of ORS, in addition to the legitimate dossiers of this compound for therapeutic use in food fish species of many countries, make ORS a preferential antimicrobial compound for optimal therapeutic procedures in aquaculture.

Figure 1

Frequencies of MICs observed for 4 Antimicrobials against 50 streptococcal isolates associated with tilapia disease (left) and 50 vibrio isolates associated with shrimp disease (right).

References

1. API Company. https://apiweb.biomerieux.com