



Prevalence of chloramphenicol-resistant gene in *Escherichia coli* from water sources in aquaculture farms and rivers of Kuching, Northwestern Borneo

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Abstract

Antibiotic resistant *Escherichia coli* cases are increasing high especially in Southeast Asia. Illegal use of the antibiotic in the aquaculture farming may become the culprit of the outbreak and spread into environmental source. A study was conducted to: 1) detect the chloramphenicol (CAL)-resistant gene in *E. coli* isolated from three aquaculture farms and six rivers of northwestern Borneo and 2) investigate the correlation between *cat* gene with five common antibiotics used. Isolation of *E. coli* was done on Eosin methylene blue agar and characterized using indole, methyl red, Voges-Proskauer, citrate tests. *E. coli* isolates were subsequently tested for their susceptibility to five antibiotics commonly used in aqua-farming. The CAL-resistant *E. coli* were further analyzed for the presence of resistant genes (*cat I*, *cat II*, *cat III*, *cat IV*) using multiplex polymerase chain reaction. 42 bacterial colonies were isolated from a total of 80 individual water samples, 34 of which were identified as *E. coli*. Result showed 85.3% of the *E. coli* isolates were resistant to amoxicillin, 35.3% were resistant to tetracycline, 29.4% were resistant to CAL, 17.6% were resistant to nitrofurantoin and 8.8% were resistant to nalidixic acid. All of the 10 CAL resistant *E. coli* isolates were detected with *cat II* genes; five isolates detected with *cat IV* genes; three isolates detected with *cat III* genes; and another two detected with *cat I* genes. Pearson correlation coefficient shows highly significant relationship between resistance pattern of CAL with amoxicillin; and CAL with tetracycline. Our findings provide the supplementary information of the CAL resistance gene distribution, thereby improving our understanding of the potential risk of antibiotic resistance underlying within this microbial ecosystem.

Keywords: Chloramphenicol, *cat* genes, *Escherichia coli*, Aquaculture farms, Rivers

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Introduction

The alarming situation of chloramphenicol (CAL) antibiotic resistant has become a global concern, especially in the aquatic environment. The situation is worsened by pollution from industry, agriculture, or domestic life, hence, making the aquatic environment an ideal setting for the transmission of antibiotic resistance genes in the ecosystem. Water sources are closely related to human life's daily activities. Direct or indirect contact with water contaminated by such antibiotic resistant bacteria could harm and infect the human populace. Global dissemination of increasingly antibiotic resistant bacteria in developing countries is caused by the uncontrolled fecal disposal to surface water as mentioned by Manyi-Loh et al. (2018). The resistant gene carried by the bacteria can also migrate to microorganisms in humans via horizontal gene transfer (Bello-López et al., 2019).

CAL is a broad-spectrum antibiotic effective against both gram-positive and gram-negative bacteria (Lorenzo, 2019). It is commonly apply in aquaculture as a disinfectant or chemotherapeutic agent in treating fish diseases. The use of CAL in treating bacterial infection in food-producing animals has been prohibited in Malaysia since 1998 as stated in Malaysia Food Act 1983 (Act 281) & Regulations (Ministry of Health, 2014) as it can cause detrimental effects to human health. The European Union had ceased all aquaculture food imports from Asian countries in 2001 due to illegal CAL. CAL can cause thrombocytopenia, bone marrow depression, and aplastic anemia and produce positive results for genotoxicity in consumers (Keen & Montforts, 2012).

Shade et al. (2012) found that there is a fundamental relationship between the level of antibiotic characteristics and the distance from the disturbed stream. At the discharge site of the sewage treatment facility, it was found that an abundance of antibiotic resistant bacteria was present, but the concentration decreases gradually as the distance from the discharge site increased (Lupan et al., 2017). The water used in aquaculture is often discharged into the sea or river along with other pollutants from aquaculture farms. The contamination from sewage combined with the use of CAL will encourage the development of high resistance bacteria. If the food source is to be contaminated with CAL resistant bacteria, it may increase the risk of the microflora bacteria population inside the consumers developing antibiotic resistance. Bacteria typically develop resistance to CAL in the presence of plasmid-encoded or chromosomally

encoded enzyme named chloramphenicol acetyltransferases or CAT. Huang et al. (2017) stated that CAT inactivates the effect of CAL by acetylation. The bacteria can develop CAL resistance by two separate events. Firstly, CAT catalyzes the transfer of an acetyl group from acetyl-coenzyme A to the 3-hydroxyl group of CAL (Biswas et al., 2012) to generate 3-O-acetyl-CAL, preventing it from binding to ribosomes (Huang et al., 2017). Secondly, a non-enzymatic event will occur, which begins with subsequent transfer of the acetyl group of acetyl-coenzyme A to the neighboring 1-hydroxyl group to generate 1-O-acetyl-CAL, resulting in a di-acetylated CAL (Kleanthous et al., 1984).

The CAT enzymes are divided into three types, namely *cat* I, *cat* II, and *cat* III. Genomic analysis of *cat* I, which is present in most pathogens, displays a modest sequence identical to *cat* II (46%) and *cat* III (32%–47%) (Biswas et al., 2012). A quadruple mutant of *cat* III named *cat* IV was introduced, *cat* IV has mutated catalytic pocket residues to mimic *cat* I reactions (Biswas et al., 2012). *cat* I was well documented and reported as a proteomic tool in a common CAL-resistance marker encoded in plasmids (Li et al., 2011a; Li et al., 2011b; Speck et al., 2011). The *cat* II enzyme is the only enzyme with extreme susceptibility to thiol-modifying agents compared with other *cat* enzymes (Murray et al., 1990) and can be found in many transposable elements (Roberts & Schwarz, 2017). The *cat* III enzyme is responsible for conferring resistance of the antibiotic CAL to the cell. Structural and biochemical studies of *cat* III were well studied to understand the general features of *cat* I proteins (Barsukov et al., 1996). The sequence differences between *cat* I, *cat* II, *cat* III, and *cat* IV include several substitutions in the binding site which may cause positional differences of CAL bound to these two proteins (Biswas et al., 2012). Studies have shown that *E. coli* bacteria are more prompt to develop antibiotic resistance if they are exposed to an increasingly high concentration of antibiotics in the surrounding environment (Tonoyan et al., 2019).

Sarawak, a state in Malaysia, is one of the most developed states. The Rajang River serves the cities on the northwest coast of Borneo. Unfortunately, the rivers and streams are dangerously polluted from the cities' discharges (Leong et al., 2018). One of the many pollutants seeping into the waters is antibiotics via animal waste (Leong et al., 2020) or manure fertilization (Zhang et al., 2017). In Malaysia, manure is often recycled as cheap fertilizers for plantations. There are two antibiotics CAL and nitrofurans that are currently banned in Malaysia for use in agriculture since 1998 (Ministry of Health Malaysia, 2014). Farmers still illegally purchase and use CAL in most meat production