RESISTOME ANALYSIS OF *AEROMONAS VERONII* STRAINS ISOLATED FROM DISEASED OUTBREAK IN TILAPIA FARMS IN THAILAND

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Veterinary Science and technology
Common Course
Faculty of Veterinary Science
Chulalongkorn University
Academic Year 2019

การวิเคราะห์องค์ประกอบในจีโนมที่เกี่ยวข้องกับการดื้อยา ของเชื้อ*แอโรโมนาส เวโรนีไอ* สายพันธุ์ที่แยกได้จากโรคระบาดในฟาร์มปลานิล ในประเทศไทย

น.ส.รุ่งนภา สกุลวรกานต์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาวิทยาศาสตร์ทางการสัตวแพทย์และเทคโนโลยี ไม่สังกัดภาควิชา/เทียบเท่า
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2562

Thesis Title	RESISTOME ANALYSIS OF *AEROMONAS VERONII* STRAINS ISOLATED FROM DISEASED OUTBREAK IN TILAPIA FARMS IN THAILAND
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รุ่งนภา สกุลวรกานต์ : การวิเคราะห์องค์ประกอบในจีโนมที่เกี่ยวข้องกับการดื้อยา ของเชื้อ*แอโรโมนาส เวโรนีไอ* สายพันธุ์ที่แยกได้จากโรคระบาดในฟาร์มปลานิล ในประเทศไทย. (RESISTOME ANALYSIS OF *AEROMONAS VERONII* STRAINS ISOLATED FROM DISEASED OUTBREAK IN TILAPIA FARMS IN THAILAND) อ.ที่ปรึกษาหลัก : ผศ.ชาญณรงค์ รอดคำน.สพ., อ.ที่ปรึกษาร่วม : พัฒนพล ขยันสำรวจน.สพ.

การระบาดของ *Aeromonas veronii* ก่อให้เกิดการตายระหว่างการเลี้ยงปลานิล ซึ่งส่งผลกระทบต่อฟาร์มเลี้ยงปลานิลในประเทศไทยเป็นอย่างมาก นอกจากนี้ยังพบเชื้อที่ดื้อต่อยาต้านจุลชีพที่ใช้ในประเทศไทยหลายชนิด การประเมิณประสิทธิภาพของการใช้ยาต้านจุลชีพนั้นยังมีข้อจำกัดเนื่องจากยังไม่มีการกำหนดค่ามาตรฐานจาก CLSI ทำให้การวิเคราะห์ทางด้านจีโนไทป์มีความจำเป็น งานวิจัยนี้มีวัตถุประสงค์เพื่อนำเทคโนโลยีการหาลำดับเบสทั้งหมดของจีโนม(WGS) มาใช้วิเคราะห์ปัจจัยความต้านทานของเชื้อ *A. veronii* ทั้ง 12 ตัวอย่าง ได้ทำการจัดเก็บมาจากจังหวัด ชัยนาท หนองคาย และอุตรดิตถ์ และทำการระบุสายพันธุ์ด้วยการวิเคราะห์ลำดับเบสของยีน *qyr*B จากนั้นทำการตรวจวัดปริมาณของยาต้านจุลชีพที่ต่ำที่สุดที่สามารถยับยั้งเชื้อได้ในยา 8 ชนิด ได้แก่ AMP, AML, GEN, ENR, OXO, OXY, SXT, และ FFC จากเชื้อทั้งหมด 12 ตัวอย่าง จากนั้นทำการคัดเลือกเชื้อ 5 ตัวอย่างที่มีรูปแบบการดื้อยาที่น่าสนใจไปทำ WGS และการวิเคราะห์รีซิสโตม (Resistome analysis) ร่วมกับ 15 ตัวอย่างจากฐานข้อมูล NCBI ผลการศึกษาพบว่าเชื้อจากปลานิลในไทยมีความไวต่อ FFC แต่ดื้อกับ AML และ AMP และนอกจากนี้ยังว่าเชื้อส่วนใหญ่ดื้อต่อ OT หลังจากทำ WGS พบว่า *A. veronii* มีขนาดประมาณ 4.5 ล้านคู่เบส และจากการวิเคราะห์ทาง Resistome ตรวจพบ 19 ยีนที่เกี่ยวข้องกับการดื้อยา นอกจากนี้ 14 ยีนยังสามารถพบได้ในตัวอย่างอื่นๆที่นำมาจาก NCBI เช่นกัน ท้ายที่สุด *A. veronii* สายพันธุ์ที่แยกได้จากปลานิลนั้นมีความดื้อต่อยาต้านจุลชีพหลายชนิด ซึ่งสัมพันธ์กับการตรวจพบยีนดื้อยาจำนวนมากจากจีโนม ทั้งยังสัมพันธ์กับตัวอย่างในหลายประเทศ ซึ่งน่าจะเกี่ยวข้องกับกระบวนการรับเข้าของยีนดื้อยาจากแหล่งอื่น หรือการส่งผ่านระพว่างพลาสมิด

สาขาวิชา	วิทยาศาสตร์ทางการสัตวแพทย์และเทคโนโลยี	ลายมือชื่อนิสิต .....
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6075404031 : MAJOR VETERINARY SCIENCE AND TECHNOLOGY

KEYWORD: Aeromonas veronii, Antimicrobial resistance, Comparative genomics, Resistome analysis, Tilapia, Whole genome sequencing

Rungnapa Sakulworakan : RESISTOME ANALYSIS OF *AEROMONAS VERONII* STRAINS ISOLATED FROM DISEASED OUTBREAK IN TILAPIA FARMS IN THAILAND. Advisor: Asst. Prof. CHANNARONG RODKHUM, D.V.M., Ph.D.,D.T.B.V.P. Co-advisor: Pattanapon Kayansamruaj, D.V.M., Ph.D.

Aeromonas veronii outbreaks in tilapia farming caused relatively high mortality. Moreover, it was resistant to many kinds of antimicrobial used in Thailand aquaculture. According to no CLSI standard, the determination of antimicrobial efficacy has been limited phenotypically; the genomics study is required. This research aims to analyze the resistome of *A. veronii* isolated from diseased Tilapia in Chainat, Nong Khai and Uttaradit province, Thailand. Twelve isolates of *A. veronii* were identified base on *gyrB* sequencing then determined the MIC value to eight antimicrobials (AMP, AML, GEN, ENR, OXO, OXY, SXT, and FFC). According to MIC patterns, five representatives were performed the whole genome sequencing (WGS) and resistome analysis, including 15 isolates from NCBI. All Tilapia isolates are susceptible to FFC but resistant to AML and AMP; OT resistance is the most dominant resistance. For WGS analysis, 4.5 Mbp of *A. veronii* was characterized, 19 ARGs were detected by resistome analysis and 14 genes were shared among *A. veronii* population. In conclusion, *A. veronii* strains isolated from Tilapia exhibit resistant to several antimicrobials and multidrug resistance (MDR) which related to the presence of multiple ARGs. *A. veronii* shared the ARGs in their population worldwide with the possibility of acquisition and plasmid-mediated.

Field of Study:	Veterinary Science and technology	Student's Signature .....
Academic Year:	2019	Advisor's Signature .....
		Co-advisor's Signature .....

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my advisor Asst. Prof. Dr. Channarong Rodkhum and my co-adviser Dr. Pattanapon Kayansamruaj for all the support of my master study, also his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis.

Besides my advisor, I would like to thank the rest of my thesis committee: Assoc.Prof.Dr. Nopadon Pirarat, Assoc.Prof.Dr. Rungtip Chuanchuen and Dr. Teerapong Yata for their insightful comments and encouragement, but also for the hard question which incented me to widen my research from various perspectives. Moreover, I would like to say thank you to Prof.Dr. Ikuo Hirono, who represented as the second co-adviser in Japan, I appreciated your kindness and welcoming to me while I stayed there.

My sincere thanks also go to members in Fish Infectious Diseases Research Unit (FID-RU), all postgraduate students and the scientist in Department of Microbiology, Faculty of Veterinary Science, Chulalongkorn University who have so much kind, sharing research experiences as well as mental support.

Last but not least, I would like to thank the Overseas Research Experience Scholarship for Graduate Students of Chulalongkorn University for financial support me to research in Japan.

Rungnapa Sakulworakan

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CHAPTER I
Importance and Rationale

Aquaculture is considered an economically significant activity worldwide and fish remains as a good source of protein and essential nutrients particularly in developing countries (FAO, 2016). Top yield of world's fish production is freshwater fish; therefore, freshwater aquaculture is of significant importance. Thailand is one of the top fish producer with the continual increase of exportation of freshwater aquaculture product gradually (FAO, 2017b).

Nile tilapia (*Oreochromis niloticus*) has been crucial freshwater fish with the highest value of production among other freshwater species (DOF, 2018). However, when the demand of fish consumption was more elevated, intensive farming gains more popular also the risk of disease outbreaks due to the uncontrollable condition of the environment leading to the stressful rearing of Tilapias (Turnbull, 2012). This condition increase the risk of Tilapia stock to get a disease by infected with multiple pathogens (Dong et al., 2015).

One of the most critical pathogen often outbreaks in Tilapia farming in Thailand is *Aeromonas veronii* (*A. veronii*), it is a cause of high mortality in every stage of Tilapia during cultivation (Dong et al., 2017). *Aeromonas veronii* is a Gram-negative opportunistic bacteria which is commonly found and wildly spread in aquatic environment across continents (Igbinosa et al., 2012). Moreover, It has been reported as an important pathogen in other fish species (carp; catfish; and salmon), avian, amphibian (frogs; snakes and lizards) and mammalian (dogs; calves or bulls) including human (Martino et al., 2016). Several antimicrobials are used to control the bacterial diseases outbreak in aquaculture system (McNevin, 2017). On the other hand, their residue can shade through the environment and difficult to degrade nature.

This property would trigger antimicrobial resistance (AMR) which can be horizontally distributed to other pathogens (Subramani and Michael, 2017). Moreover, the resistant bacteria contaminate in the environment of livestock and agricultural settings carry antimicrobial resistance genes (ARGs) that could potentially be distributed along the food production chains and eventually be transferred to the consumers (Citarasu, 2012).

Aeromonas veronii becomes resistant to many kinds of antimicrobials which are typically used in Thailand aquaculture and some are commonly used in human medication (McNevin, 2017). There are limitations of antimicrobial susceptibility standard procedure to determine the resistance, *A. salmonicida* is the only species in *Aeromonas* genus having a standard MIC (minimum inhibitory concentration), cut-off value and zone diameter available (CLSI, 2014). Further, some of ARGs are hidden naturally without the expression related to their phenotypic resistance. Bacterial genomics study is necessary to gain more information of AMR and develop a comprehensive susceptibility testing, this application also useful for gaining an effective prevention and bacterial disease treatment strategies (Crofts et al., 2017).

Whole genome sequencing is the massively parallel sequencing technology for sophisticated genomic study base on Next generation sequencing. This high-throughput approach has a potency to determine the certain of resistance determinants with massive datasets for utilized the resistome analysis (Wright, 2007). The resistance outputs from resistome analysis are crucial in acknowledgment and helpful as the in silico evidence for prediction of antimicrobial resistance also prevent the emergence of antimicrobial resistance (Ellington et al., 2017). Therefore, this study aims to perform resistome analysis among *A. veronii* isolated from a disease outbreak in Tilapia farms in Thailand and compare with the *A. veronii* other isolates from diseased freshwater fish in the different sources from a genome database using genomics approaches.

CHAPTER II
Literature Review

1. Freshwater Aquaculture

Freshwater aquaculture is distributed around the world for the human diet and non-food purposes. The distribution of world's fish production as a source of human nutrition has significantly increased; being a nutritious source of high-quality proteins with essential amino acids, essential fats, vitamins and minerals (FAO, 2016).

Thailand has been ranked as the top aquaculture producer and main exporting country of aquaculture products in the world (FAO, 2018). Since 1993, the growth of freshwater culturing is gradually growing as high as the growth of fisheries products exportation. In addition to the production of freshwater animals in Thailand was accounted for a million tons, these gains more income and affects to Thailand economy significantly (DOF, 2018).

2. Tilapia (*Oreochromis* sp.) culturing

Tilapia has been cultured commercially and accounted as a major farm fish in global production; Asia is the leading producer in the world (FAO, 2018). Five decades ago, Nile tilapia (*Oreochromis niloticus*) was introduced to Thailand (FAO, 2017a). "Tilapia is the fish for next-generation aquaculture" It has a good adaptive ability and growing well in freshwater, brackish water, and seawater (El-Sayed, 2006).Tilapia culturing becomes popular, there are many breeds were adapted and distributed to many regions particularly in the central plain and the territory of Bangkok Metropolitan (FAO, 2017b). Inland Tilapia aquaculture is popular with a variety of on-growing techniques such as ponds, tanks, and raceways, recirculation systems or floating cages along with water resources (FAO, 2017a).

Pond cultures are extensively for Tilapia culturing with the advantage of feed supply management and aeration, on the other hand, the major drawback of pond culturing is water quality management; a limitation of water circulation system (James and Andrew, 1989). Upscale to tanks and raceways system, it is a water flow-through system which more effective in maintenance a water quality. The operation requires much less time and labor compared to those reared in ponds. Further, this technique increase production costs in part of a complete diet and the cost of pumping water and aeration, moreover, the risk of high mortality can encountered during mechanical or electrical failure (James, 1989). Recirculating culture system has been developed to solve several problems encountered in recycled or closed systems that utilize filtration and recycling techniques; the quality of water can be restored while filtration from solid wastes utilize with UV-sterilization and aeration (El-Sayed, 2006). Floating cage is a popular intensive culturing, it has been widely used for mesh size production with several advantages such as maintenance of free water circulation and multi-unit production with low capital investment (El-Sayed, 2006). On the other hand, this technique would also have its disadvantages like the uncontrollable condition of the environment including predators, and disasters, and poor water quality with a greater risk of disease outbreaks leading to a high mortality rate of fish and economic losses (Dong et al., 2015).

3. Aeromonas veronii and Disease

Gram-negative opportunistic bacteria *A. veronii* is commonly found and widely spread in the aquaculture system (Janda and Abbott, 2010). It has been reported as contaminant in food, vegetables also naturally abundant in soil and water. Moreover, there are many case-reports from *A. veronii* as food born pathogen in human with the various virulence-associated factors such as enterotoxin, cytotoxin, hemolysins and proteases production (Sylvia, 1993). Recently, *A. veronii* has been detected as an aerobic microbe contaminated in urban hospitals in China (Gao et al., 2018).

Aeromonas veronii outbreaks in Tilapia farming has been published globally also be an important pathogen in Thailand aquaculture (Rahman et al., 2002; Castro-Escarpulli et al., 2003; Manna et al., 2013; Abu-Elala et al., 2015; Eissa et al., 2015; Hassan et al., 2017).

Motile Aeromonas Septicemia (MAS) is the most common problem in freshwater fish caused by motile *Aeromonas* species (Camus et al., 1998). *Aeromonas veronii* has been described as highly pathogenic to Tilapia (Dong et al., 2017). After infected by *Aeromonads*, several clinical signs from fish will appear such as lethargic swimming, swollen abdomen and enteritis. The infection also reveals typical hemorrhage lesions both on external and internal organs (Dong et al., 2017). The experimental challenge of Ha Thanh Dong by the inoculation of *A. veronii* strains into Nile tilapia (*O. niloticus*) showed the typical clinical signs and resulted into high mortality of fish; proving the Koch's postulates (Dong et al., 2015). *Aeromonas veronii* was identified as a microflora in the gastrointestinal tract of Nile tilapia with 0.6 percent of the predominant gut microbiome (Molinari et al., 2003). A number of publications have described the relationship between stress-mediated immune reduction and increasing of disease susceptibility of the host (Turnbull, 2012). The immunocompromised Tilapia is easily susceptible to various pathogen not only *Aeromonas* sp. but also *Flavobacterium columnare, Plesiomonas shigeloides, Streptococcus agalactiae, Vibrio cholera* and Tilapia lake virus as well (Dong et al., 2015; Amal et al., 2018).

4. Antimicrobials use and the resistance

After antimicrobials were discovered, these chemotherapeutic agents have been distributed for therapeutic purposes as prophylaxis (prevent the infections) and metaphylaxis (treatment of diseases) which widely practices in human medication, livestock, agriculture and aquaculture (Romero et al., 2012). Important antimicrobials in veterinary for food animal were categorized by the Office International des Epizooties (OIE), twenty-seven drugs are listed using in fish therapeutic with a specific purpose (prevention or treatment) and particular species of the pathogen (OIE, 2007). The Food and Drug Administration (FDA) of Thailand announced the twelve licensed antimicrobials for the therapeutic purposes in aquaculture which are amoxicillin, enrofloxacin, oxytetracycline, sarafloxacin, oxolinic acid, toltrazuril, sulfamonomethoxine sodium, sulfadiazine + trimethoprim, sulfadimethoxine sodium + trimethoprim, sulfadimethoxine sodium + ormetoprim, sulfamonomethoxine + trimethoprim and sulfadimidine + trimethoprim (FCSTD, 2012). Due to the overuse or/and misuse of antimicrobials, more than 70 percent of the mixing between fish diet and antimicrobials have wasted through the water or sunk into the sediment while feeding (G, 2016). These antimicrobial residues are remain in the aquatic system and contaminate the environment including the agriculture, livestock, and human food chain (Cabello, 2006).

Regarding to the mechanism of antimicrobial resistance, the next generation of resistant bacteria has been evolved under selective pressure from the antimicrobial residue; there are four mechanisms that play a role to induce the AMR. Firstly, antimicrobial molecule alteration by enzymatic producing is mainly for acquired AMR (Munita and Arias, 2016). Many chemical groups were transferred to inhibit the action of acyl, phosphate, or nucleotidyl groups result in steric hindrance that prevent the binding of antimicrobial to target (Blair et al., 2015). Moreover, some bacterial enzyme can be produced to inactivate many classes of antimicrobial compound directly such as beta-lactams, aminoglycosides, and macrolides by breaking the amide bond of the beta-lactam ring (Munita and Arias, 2016). The second mechanism is the prevention of target accession, focusing on permeability reduction and Increased efflux activity (Blair et al., 2015). The outer membrane of Gram-negative bacteria is formed with the less permeability barrier compares to membrane of Gram-positive (Zgurskaya et al., 2015).

Hydrophilic antimicrobial easily passes through porin channel inside the bacterial cell; however, the emergence of mutations in porin genes can downregulate the permeability of bacterial cell as described in *ompK*36 porin gene of *Klebsiella pneumoniae* strains (Clancy et al., 2013). Meanwhile, the intracellular antimicrobial can be bound with transcriptional repressor protein, then transported out by bacterial efflux pumps commonly (Blair et al., 2015). Antimicrobials bind specifically to the targets; therefore, the mutation affects to target changing, the efficiency of specific-binding will be decreased and contributes the high expression of efflux genes to become multidrug-resistant bacteria (Ogawa et al., 2012). The last mechanism is alteration of antimicrobial targets, the bacterial target cell can be modified or protected by a chemical group that binds to inactivate antimicrobial activity at the binding site (Blair et al., 2015). Target protection model has been well described in tetracycline resistance determinants, *TetM* and *TetO* are compete with tetracycline bind to the same ribosomal space (Li et al., 2013). The most common mechanisms of changing the target sites is modification which consists of I) point mutations in target gene result in decreased affinity of the drug for its target II) enzymatic alterations by catalyzing methylation result to biochemical change and target impairment and III) replacement of the original target site by evolving a new target such as methicillin resistance in *Staphylococcus aureus* (Munita and Arias, 2016).

The occurrence of multidrug-resistant bacteria has been distributed in aquaculture settings, it has becomes a serious public health concern with a several reports worldwide. Multidrug-resistant *A. veronii* was indicated in many countries. Antimicrobials-resistant *A. veronii* from India, it was demonstrated by disc diffusion method with eight antimicrobials ampicillin; penicillin; vancomycin; kanamycin; polymyxin B; rifampicin; erythromycin and streptomycin (Rawal et al., 2016). In China, multidrug-resistant *A. veronii* isolated from Channel Catfish (*Ictalurus punctatus*) show 100% resistance rates to oxacillin and penicillin G also resistant to other beta-lactams, tetracycline, doxycycline, chloramphenicol, florfenicol and first-generation cephalosporins; based on MICs determination (Yang et al., 2017).

Recently, the antibiogram testing of *A. veronii* infection among farmed *Oreochromis niloticus* was reported in Egypt, it revealed resistance to amoxicillin + clavulanic acid and ampicillin (Hassan et al., 2017). The antimicrobial resistant bacteria in the aquaculture environment and their resistance gene can be transmitted to the terrestrial bacteria through horizontal gene transfer and reach to be more resistance in human. As a result of this exposure, humans risk to develop resistance as well; the certain medications containing the same ingredient while some antimicrobials used in human medicine are applied in aquaculture practices (Heuer et al., 2009). The occurrence of resistant bacteria plays a vital role in antimicrobial susceptibility expression by the resistance genes. Phenotypically, the susceptibility test can be interpret into sensitivity or resistance, by the way, the sensitive isolate also carries resistance genes and possible to gain more resistance. Phenotypic sensitivity to antimicrobial may resulted from the lack of silence-gene expression as though proto-gene promotes a little/no activity against antimicrobials; the default in opportunistic pathogens. However, these two resistance genes could gain resistance phenotypic activity if mutations occur (Wright, 2007). Phenotypic resistance is an antimicrobial-resistant bacteria that can reveal their phenotype under the expression of intrinsic or acquired resistance gene. Intrinsic ARGs play a role to make the antimicrobial target ineffective and acquired ARGs can be occurred by point-mutation or horizontal transfer activity (Perry et al., 2014). The mechanism related to antimicrobial resistance are genetic drift among the population of bacteria and the selection of AMR mutants under selective events (González-Candelas et al., 2017). The selective pressure from antimicrobial residues combined with the potential of acquired ARGs, bacteria can gain their resistance ability from genotype and express phenotypically.

5. Determination of antimicrobial efficacy

Nowadays, to determine the active antimicrobial agents for bacterial treatment the information of standard antimicrobial susceptibility procedures are recommended. The guidelines of *Aeromonas* antimicrobial susceptibility testing from The Clinical and Laboratory Standards Institute (CLSI), the performance standards for antimicrobial susceptibility testing of bacteria isolated from aquatic animals are a vital source of information (CLSI, 2014). The breakpoint data of zone diameter and MIC cut-off value are available only for *Aeromonas salmonicida*, while, no data available for other *Aeromonas* sp. collected from aquatic species. As crucial as phenotypic susceptibility testing, the genotypic study is also required for improve the knowledge of antimicrobials resistance in *Aeromonas* sp. (Wright, 2007).

Resistome analysis is the investigation of all ARGs collection in microorganisms which is essential for epidemiology surveillance. Since several genes play an important role in AMR, this analysis investigates the comprehensive mechanism of AMR and support the phenotypic susceptibility test also acknowledge the information of resistance-associated gene (Zankari et al., 2012). Likewise, set of *tet* and *qnr* genes were successfully amplified in *A. salmonicida* isolated from salmonid farms in Korea (Kim et al., 2011). The tetracycline resistance sequence (*tetA* and *tetE* genes) have shown the homology with *Escherichia coli*. Moreover, point mutations in both *gyrA* and *parC* codon affects the increasing of quinolone resistance rate (Kim et al., 2011). However, molecular characterization using low-throughput techniques (PCR and Sanger sequencing) are hardly to identify the whole of ARGs, due to the hidden resistance genes may not express or weakly express their susceptible phenotypically; it may misidentify of some ARGs (Crofts et al., 2017).

6. Whole genome and Resistome analysis

The new era of antimicrobial resistance research is the utilization of resistome analysis, it has been improved based on high-throughput sequencing techniques with more efficiency and accuracy to characterize the AMR (Zankari et al., 2012). Whole genome sequencing (WGS) approach is shaping up a new dimension for resistance analysis (Lopez-Causape et al., 2018). Currently, the databases about resistance analysis are available online for freely access such as ARDB; Antibiotic Resistance Genes Database, ARG-ANNOT; Antibiotic Resistance Gene - ANNOTation, CARD; Comprehensive Antibiotic Resistance Database and ResFinder. These web portals are provided for a catalog of antimicrobial resistance genes identification also support the point mutation and SNPs information. Instance for the analysis of multidrug-resistance in five *A. hydrophila* with reference strains in Genbank using pan-genome approach, the core genes of five mutant genomes and references revealed closely related, moreover, plenty of unique genes have been identified which may associated with horizontal gene transfer events (Zhang et al., 2018). In addition to the information of Versatile Mutational Resistome in *Pseudomonas* (*P.*) *aeruginosa*, the next-generation approach has expanded the potentials of resistome study and extensive knowledge of AMR. The mutational study is useful for AMR monitoring in a part of understanding the classical resistance pathways (Jaillard et al., 2017). The whole genome sequencing (WGS) mutational resistome data provided the evolutionary of AMR in *P. aeruginosa*, the mutation of genes are associated-antipseudomonal classes including b-lactams, aminoglycosides, fluoroquinolones or polymixins which are consequenced from antimicrobial exposure. Those resistance determinants showed correlation with MIC value, support the association between genotypic and phenotypic antimicrobial susceptibility in *P. aeruginosa* (Jaillard et al., 2017).

CHAPTER III
Materials and Methods

1. Sample collection

This study was conducted using 12 isolates of *A. veronii* (table1). The first group previously isolated from diseased Nile tilapia (*Oreochromis niloticus*; n=7) from Nong Khai province, which have been published as an important Nile tilapia pathogen in Thailand (Dong et al., 2015). The second group was obtained from Hybrid red tilapia farm (*Oreochromis niloticus X mossambicus*; n=5) during the disease outbreak in 2018 from Chainat and Uttaradit province, Thailand.

Table 1: Details of A. veronii 12 isolates in this study.

Collection period	Location	Host	Health status	AMU	Organ	Isolate	Reference
2015	Nong Khai	Nile tilapia	NA	NA	NA	NK01 NK02 NK03 NK04 NK05 NK06	(Dong et al., 2015)
Feb 2018	Uttaradit	Hybrid red tilapia	Hemorrhage on skin kidney and fin	OXY and Vit. C	Kidney	UDRT09	This study
Feb 2018	Chainat	Hybrid red tilapia	Hemorrhage on skin and kidney enlargement with fin rot	OXY	Kidney	CNRT07 CNRT11 CNRT12 CNRT13	This study

* NA; not available, OXY; oxytetracyclin, Vit.C; vitamin C

2. **Bacterial isolation**

Initially, the moribund fish were euthanized with over dosage of clove oil before dissecting. The internal organs (liver, kidney, and spleen) were collected and did the bacteria culture on Tryptic Soy Agar (TSA) supplemented with 5% sheep blood then incubated at 28°C for 24h. (Skwor et al., 2013; Hassan et al., 2017). After incubation period and sub-culturing, a single colony was picked and inoculated into 5ml of Tryptic Soy Broth (TSB) follow incubated with constant shaking at 160rpm under 28°C for 24h. The bacterial suspension has been kept as 1ml stock in TSB mixed with 20% glycerol and stored in -80°C for further experiment.

3. **Bacterial identification**

3.1 Biochemical test

The bacterial isolate from glycerol stocks were recovered onto TSA following the condition as described before. The biochemical tests were prepared to characterize their phenotype include Gram's staining, Oxidase test, Catalase test, Oxidation-fermentation test, Indole test, Motility test, Decarboxylase test, MR-VP test and Salt tolerant test (Cowan, 2003). The result was checked after 24h incubation under 28°C, suspected *Aeromonas* sp. were selected to perform the *gyrB* sequencing for species identification afterward.

3.2 *gyrB* sequencing

Ten microliters (10µl) of each putative *A. veronii* glycerol stocks were inoculated into TSB separately and incubated at 28°C for 24h in shaking incubator (160 rpm). After incubation, a milliliter (1ml) of each inoculum were subjected for DNA extraction by using Wizard Genomic DNA purification kit (Promega Corporation, Madison, WI, USA) by following the instruction. The suspected *Aeromonads* DNA were amplified with *gryB* universal primer for species identification, *gryB*3F: TCC GGC GGT CTG CAC GGC GT and *gryB*14R: TTG TCC GGG TTG TAC TCG TC with PCR condition as shown in table 2 (Hoel et al., 2017). Then PCR products were processed the electrophoresis by loaded into 1% agarose gel stained with Red Safe™ staining solution (Intron, Korea) and run in TBE with 100 V for 30 min; the amplicons were observed under UV light. The DNA was purified from agarose gel using NucleoSpin® Gel and PCR clean-up (Mache rey-Nagel, USA) then subjected to perform the Sanger sequencing.

The data from sequencing were blasted through NCBI nucleotide database using BLASTn with ≥99 percent identity for species confirmation (Hoel et al., 2017). All *A. veronii* isolated from Hybrid red tilapia have been kept in 20% glycerol stock with TSB under -80 °C.

Table 2: PCR condition of gyrB amplification.

Stage	Temperature	Duration	Cycle	Product size
Pre denaturation	94°C	2min		
Denature	94°C	30s		
Annealing	56°C	30s	30 cycles	1100bp
Extension	72°C	2min		
Post extension	72°C	5min		

4. Determination of minimum inhibitory concentrations (MICs)

The broth microdilution assay was used to determine MIC value of eight antimicrobials (Amoxicillin; AMC, Ampicillin; AMP, Enrofloxacin; ENR, Florfenicol; FFC, Gentamicin; GEN, Oxolinic acid; OXO, Oxytetracycline; OXY and Sulfamethoxazole + trimethoprim; SXT) according to CLSI guideline VET04 (CLSI, 2014). Before the experiment, stock solution (concentration is 1024 mg/L) of eight antimicrobials were prepared and kept at −20°C. Next, each of antimicrobial stocks were two-fold diluted with cation-adjusted Mueller-Hinton broth (CAMHB) into 10 concentration, AMC (256 – 1 mg/L); AMP (256 – 1 mg/L); ENR (16 – 0.03 mg/L); FFC (256 – 0.5 mg/L); GEN (256 – 0.5 mg/L); OXO (128 – 0.25 mg/L); OXY (256 – 0.5 mg/L); SXT (256 – 0.5 mg/L). Twelve isolates of *A. veronii* were recovered on TSA for MIC determination, after 24h incubation at 28°C, a single colony of each were sub-cultured onto MHA and incubated again with same condition (Baron et al., 2017).

The day after, all incubates were adjusted the concentration by turbidity equal to 0.5 McFarland standard then diluted into 1:100 with CAMHB final concentration is 1.5 x 10^6 CFU/mL (Baron et al., 2017). The *Aeromonads* suspension were inoculated into 96-well plate with diluted antimicrobial solutions in 1:1 proportion (final concentration is 7.5 x 10^5 CFU/ml). The suspension without antimicrobial was used as positive control, and antimicrobial solution without *Aeromonads* suspension was utilized as negative control; MICs were tested duplicate in each antimicrobials. The MICs value was interpreted by observe the growth of bacteria visibly after 24h incubation at 28°C; *Escherichia coli* ATCC® 25922 was used as reference strains for a standard control (Mata et al., 2018). According to the MICs value, the degree of antimicrobial susceptibility was categorized each isolate into either resistant, sensitive, or multidrug resistant isolate base on epidemiology cut-off values of *Aeromonas* sp. (Baron et al., 2017).

5. **Whole Genome sequencing**

The representative *A. veronii* isolates based on susceptibility pattern, sensitive; intermediate or resistance and multidrug resistance (resistance more than three antimicrobial from three different classes) were selected and extracted the DNA using Wizard Genomic DNA purification kit with RNase A treatment (Promega Corporation, Madison, WI, USA). The DNA qualities were checked by DNA loading to 1% of agarose gel and run through 100V 20min in TBE; The good quality was observed by unsmear single band of DNA without RNA contamination. Next, the DNA ratio was check by Nanodrop, 260/280ratio was shown in a range of 1.8-2.0. To quantify the extracted DNA, its concentration was checked by Qubit Fluorometric Quantitation with Qubit® dsDNA BR Assay Kit (Invitrogen, Carlsbad, CA, USA).

The qualified samples were submitted to Next-generation sequencing, the libraries were constructed with NEBNext® Ultra™ DNA Library Prep Kit for Illumina® and run with Illumina HiSeq instrument according to manufacturer's instructions (Illumina, San Diego, CA, USA) with 150 paired-end run length.

6. Genome assembly and annotation

Raw reads from WGS were checked their quality using FastQC ver. 0.11.8 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and trimmed the low-quality bases and WGS-adapters out by Trimmomatic ver. 0.32 to get the base at Q25 (Bolger et al., 2014). Then, SPAdes ver. 3.13.0 software with default settings was used to assembly the reads into contigs (Bankevich et al., 2012).Next, the contigs were qualified again with QUAST web base (http://quast.bioinf.spbau.ru/) and combined with the reference genome for scaffold construction using Medusa server (http://combo.dbe.unifi.it/medusa). Reads were mapped against the obtained scaffold scaffolds were mapped again with reference genome by BWA software (http://bio-bwa.sourceforge.net/) and closed the gaps with Pilon and GMcloser, sequentially (Li and Durbin, 2010; Walker et al., 2014; Kosugi et al., 2015). Assembled genomes were uploaded to the NCBI whole genome shotgun (WGS) submission portal, then the web tools Rapid Annotation using Subsystem Technology (RAST; http://rast.nmpdr.org/) was applied for scaffolds annotation and genome characterization of *A. veronii* Tilapia isolates (Overbeek et al., 2014).

7. Genome characterization

Average Nucleotide Identity (ANI) calculation was performed to define species identity and similarity of genomic nucleotide – level among representative of *A. veronii* Tilapia isolates and other reference *Aeromonas* species as describes in table 4; The species ANI cut-off value is ≥ 95 % (Chun, 2017). The sequence of the *gyrB* gene from *Aeromonas* genome was retrieved from features in subsystems of SEED viewer to construct the phylogenetic tree including *A. veronii* Tilapia isolates, *A. veronii* strain B565 as the in-group control and other species of *Aeromonas* as the outgroup, details

have been described in table 3. The Molecular Evolutionary Genetic Analysis version X (MEGA X) was used for multiple sequence alignment and generated the phylogenetic tree by the neighbor-joining method with 1000 replicates of bootstrap test. The substitution model were chosen based on the lowest BIC scores, the evolutionary distances were computed using the Maximum Composite Likelihood (Kumar et al., 2018).

Table 3: Details of Aeromonas sp. references from NCBI genome database.

Isolates	GenBank assembly accession	Host
A. veronii B565	GCA_000204115.1	Pond sediment
A. hydrophila subsp. *hydrophila* ATCC 7966	GCA_000014805.1	Milk
A. salmonicida subsp. *salmonicida* A449	GCA_000196395.1	NA
A. jandaei CECT 4228	GCA_000819955.1	NA
A. caviae CECT 838	GCA_000819785.1	NA
A. schubertii ATCC 43700	GCA_001481395.1	*Homo sapiens*

*NA; Not available

8. Resistome analysis

This analysis was conducted base on two kinds of bioinformatics tool, CARD and Resfinder. Firstly, Comprehensive Antibiotic Resistance Database (CARD; http:// arpcard.mcmaster.ca/) was used for antimicrobial resistance gene identification of the assemblies (Lomonaco et al., 2018). Initially, the FASTA file of representative assemblies were submitted to the Resistance Gene Identifier (RGI) on web resource, data type was set as DNA sequence with high quality/ coverage setting. According to the CARD system, perfect or strict match are high identical to the reference sequence. By the way, loose hit is the matched sequence less than the curated BLASTn bitscore, it provides a

novel ARGs but it may not have a role in AMR. The output for resistome analysis was required in perfect and strict-hits only and the query sequences lower than 96% nucleotide identity were excluded. Besides, ResFinder V3.1 (http://cge.cbs.dtu.dk/services/ResFinder) was used to identify the acquired ARGs located on *A. veronii* genome. The assembled genome/ contigs were uploaded to the web portal, the process of analysis was configured with all antimicrobials as default, then, the threshold of sequence identity was set at 95% with 80% minimum alignment length (Lomonaco et al., 2018).

9. **Comparative resistome analysis**

Fifteen assemblies of *A. veronii* from diseased freshwater fish which have been published in NCBI genome database were retrieved to compare their resistome data with the isolates from Tilapia in Thailand. The selected sequences from NCBI were submitted to screen for ARGs presented in genome, the process as previously described in resistome analysis section. The outputs from resistome of NCBI group (CARD and Resfinder) were compared to the group of ARG sequences from Tilapia assemblies in Thailand; details of 15 assemblies are shown in table 4. In addition, the query amino acid sequences of ARGs (Tilapia isolates and NCBI isolates) resulted from 2 database were operated as a local blast to evaluate the similarity of the ARG sequences against *A. veronii* genomes by Blast2GO; the bioinformatics platform for functional annotation and analysis (Conesa et al., 2005). All assemblies were imported to the software and used tBLASTn to the customized local database, the result was presented in a percent identity of each genes; details are described as below with the flowchart (figure 1).

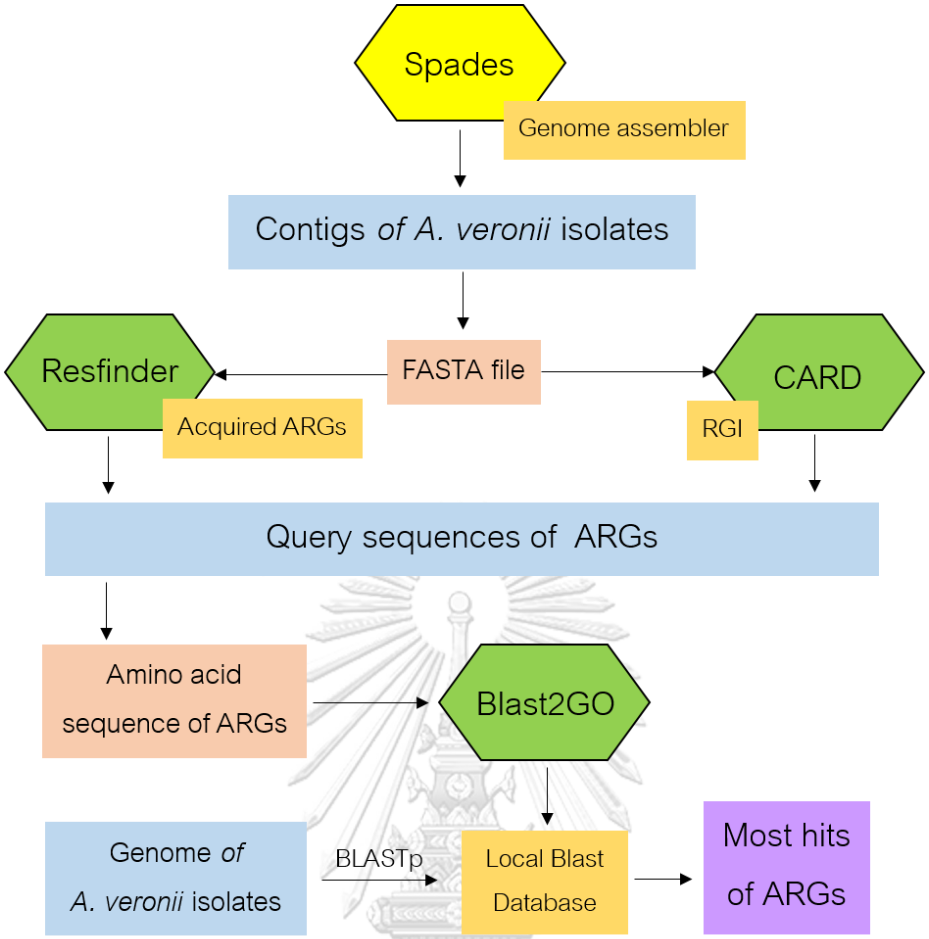

Figure 1: Comparative resistome analysis flowchart.

The flowchart shows the process of resistome analysis consist of two database and software, Resfinder (acquired ARGs identifier), CARD (ARGs and SNPs identifier) and Blast2GO software (local blast database).

Table 4: List of the assemblies from the NCBI genome database used in this study.

Type of assemble	Accession No.	Strain	Host	Country	Year
Complete Genome	GCA_001634345.1	CB51	Grass carp; *Ctenopharyngodon idella*	China	2016
	GCA_001593245.1	TH0426	Yellowhead catfish; Tachysurus fulvidraco	China	2016
	GCA_002803925.1	X11	Wuchang bream; *Megalobrama amblycephala*	China	2017
	GCA_002803945.1	X12	Wuchang bream; *Megalobrama amblycephala*	China	2017
	GCA_003722175.1	MS1837	Catfish; ***Siluriformes sp.***	USA	2018
	GCA_003491365.1	17ISAe	Discus; *Symphysodon discus*	Korea	2018
Scaffold	GCA_002339005.1	UBA1835	European eel; *Anguilla anguilla*	Spain	2017
	GCA_003345755.1	XHVA2	Channel catfish; *Ictalurus punctatus*	China	2018
Contigs	GCA_000409545.1	PhIn2	Unpublished	India	2013
	GCA_001748325.1	Ae52	Gold fish; *Carassius auratus*	Sri Lanka	2016
	GCA_002906945.1	ML09123	Catfish; ***Siluriformes sp.***	USA	2018
	GCA_003611985.1	MS1788	Catfish; ***Siluriformes sp.***	USA	2018
	GCA_003367145.1	NS	European bass; *Dicentrarchus labrax*	Greece	2018
	GCA_003367095.1	VCK	Unpublished	Greece	2018
	GCA_003036425.1	XHVA1	Channel catfish; *Ictalurus punctatus*	China	2018

CHAPTER IV
Results

1. Isolation and identification

Five putative *A. veronii* (UDRT09, CNRT07, CNRT11, CNRT12, and CNRT13) were successfully isolated from an internal organ of diseased Hybrid red tilapia and characterized into *Aeromonas* sp. through the biochemical tests (table 5). Additionally, all five putative isolates were identified as *A. veronii* base on *qyrB* sequencing. Approximately, 1000 bp of *qyrB* sequence from each isolates were blasted through NCBI nucleotide database using BLASTn; all isolates share 98 % to 99 % similarity with *A. veronii* strains published in the NCBI database; the details are shown in table 6.

2. MICs Determination

The susceptibility of 12 *A. veronii* to eight antimicrobials was evaluated by the broth microdilution method; MIC titer of all isolates are shown in table 7. All of the *A. veronii* isolated in this study are beta-lactams resistant (ampicillin and amoxicillin; MIC >256 mg/L), but susceptible to phenicol (florfenicol; MIC <1 mg/L). Oxytetracycline resistance can be detected in eight out of twelve isolates with 67 percent of resistance rate. However, almost a half of samples were intermediated resistance in enrofloxacin and oxolinic acid; only one isolate notes as gentamicin resistant and Trimethoprim/ sulfamethoxazole resistant (NK02; MIC >256 mg/L and NK07; MIC >256 mg/L respectively). The observation of multidrug resistance (MDR) in this study reveals; NK07 is resistant to six drugs in five classes of antimicrobial. Additionally, four classes of antimicrobial resistance were detected UDRT09 (beta-lactams; fluoroquinolone and tetracycline) and three classes of resistance in NK01 and NK03 (beta-lactams; quinolone and tetracycline).

Lastly; NK02, NK07, NK01, UDRT09 and CNRT12 were selected as representative isolates for whole-genome sequencing according to aminoglycoside resistant, multidrug resistance in five classes, multidrug resistance in four classes and sensitive to all antimicrobials (except beta-lactams class) respectively.

Table 5: Phenotype of five A. veronii isolated from diseased Hybrid red tilapia with two reference strains.

Characteristics	This study isolates					Reference isolate (Abbott et al., 2003)	
	CNRT07	CNRT11	CNRT12	CNRT13	UDRT09	*A. veronii*	*A. hydrophilla*
Morphology	Gram negative short rod-shape						
Growth on BTSA, 28°C	+	+	+	+	+	ND	ND
Hemolysis	-	β	β	β	β	+	+
Oxidase	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+
O/F	F	F	F	F	F	ND	ND
Decarboxylase							
Arginine	+	+	+	+	+	V	+
Lysine	-	+	+	+	+	+	+
Ornithine	-	-	-	-	-	V	-
Indole	+	+	+	+	+	+	+

Characteristics	This study isolates					Reference isolate (Abbott et al., 2003)	
	CNRT07	CNRT11	CNRT12	CNRT13	UDRT09	*A. veronii*	*A. hydrophilla*
MR (methylred)	+	+	+	+	+	ND	ND
VP (Voges - Proskauer)	+	+	+	+	+	+	+
NaCl 1%	+	+	+	+	+	+	+
NaCl 6%	-	-	-	-	-	ND	ND

* The result was interpreted after incubation at 28˚C for 24h, (-); negative, (+); positive,

F; Fermentation reaction, β; beta-hemolysis, V; vary, ND; non-determine

Table 6: Result from qyrB sequencing of A. veronii Tilapia isolates blasted through NCBI nucleotide database.

Isolates	Most closely related species	Query coverage	Identity (%)	Accession No.
CNRT07	*A. veronii* strain FZG1	100%	99.9%	KY767547.1
CNRT11	*A. veronii* strain K30	100%	99.44%	MK548536.1
CNRT12	*A. veronii* strain FZG1	100%	99.03%	KY767547.1
CNRT13	*A. veronii* strain K30	100%	99.52%	MK548536.1
UDRT09	*A. veronii* bv. veronii strain NJ1	100%	98.85%	MK898824.1

Table 7: MIC pattern of A. veronii 12 isolates with eight antimicrobials.

Antimicrobial			MIC (mg/L)										
Class	Drug	Cut-off [a] value	NK01	NK02	NK03	NK04	NK05	NK06	NK07	CNRT07	CNRT11	CNRT12	CNRT13	UDRT09
Beta-lactams	Amoxicillin	NA	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256
	Ampicillin	NA	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256
Aminoglycosides	Gentamicin	2/4	2	>256	2	8	4	2	4	2	4	2	2	4
Fluoroquinolones	Enrofloxacin	0.125	2	0.5	2	1	<0.03	1	4	0.5	0.5	<0.03	0.5	>16
Quinolone	Oxolinic acid	0.031	64	1	32	1	<0.25	8	32	2	8	<0.25	4	64
Tetracyclines	Oxytetracyclin	0.25	>256	1	>256	>256	1	128	>256	1	128	1	128	>256
Sulfonamides	Sulfamethoxazole/ Trimethoprim	0.25	4	2	2	2	2	2	>256	2	2	2	2	2
Phenicol	Flofenicol	2/4	1	1	1	1	1	1	1	1	1	1	1	1
Interpretation			3MDR		3MDR		Sense		5MDR			Sense		4MDR

* (a) the cut-off are referred to the generic epidemiological cut-off values of *Aeromonas* sp. in freshwater (Baron et al., 2017), NA; not available, 3MDR; resistance to three drug classes, 4MDR; resistance to four drug classes, 5MDR; resistance to five drug classes Sense; almost susceptible to every drug class

3. Genomic identification of *Aeromonas veronii* Thailand isolates

3.1 Genome annotation

Five representatives of *A. veronii* Tilapia isolates (NK01, NK02, NK07, UDRT09, and CNRT12) were sequenced by the Illumina HiSeq platform. The information of five genomes used in this study are shown in table 8. Size of *A. veronii* genome from Tilapia isolates are vary ranged from 4.56 Mbp to 4.83 Mbp (around 58.4% GC-content) which approximately consists 4,383 of coding sequences (CDSs) and number of RNAs was accounted from 78 to 133. The size of the genome from all isolates are larger than *A. veronii* type strain B565 (4,551,783 bp include plasmid) but still smaller than *A. salmonicida* subsp. *salmonicida* A449 (5,040,536 bp). CNRT12 is the biggest genome among all Tilapia isolates; it was sequenced by 4,835,067 bp with the highest number of N50 (265,081) and the lowest number of L50 (5). Among all isolate, 343-520 subsystems were characterized, the top 3 of subsystem features were counted into amino acid and derivatives; carbohydrate; and protein metabolism association by 31 – 57% coverage of subsystem (figure 2).

Table 8: General genomic information of five representative A. veronii obtained from RAST Annotation Server.

Genome	AMR Labeling	Size (bp)	GC Content (%)	N50	L50	Number of Contig (with PEGs)	Number of Subsystems	Subsystem coverage	Number of Coding Sequences	Number of RNAs
NK01	5MDR	4,559,863	58.5	171,547	9	95	520	57%	4,097	133
NK02	GEN	4,717,439	58.4	110255	16	134	352	31%	4560	81
NK07	7MDR	4,787,406	58.6	214996	6	86	529	54%	4317	114
CNRT12	Sense	4,835,067	58.3	265081	5	324	348	31%	4611	78
UDRT09	6MDR	4,598,294	58.4	192429	7	125	343	33%	4329	99
A. veronii B565	NA	4,551,783	58.7	NA	1	1	363	35%	4,187	133
A. salmonicida subsp. *salmonicida* A449	NA	5,040,536	58.51	NA	1	1	541	35%	5,180	147

* 3MDR; resistance to three drug classes, 4MDR; resistance to four drug classes, 5MDR; resistance to five drug classes, GEN; resistance to gentamycin, Sense; almost susceptible to every drug class, NA; not available, PEGs; protein-encoding genes

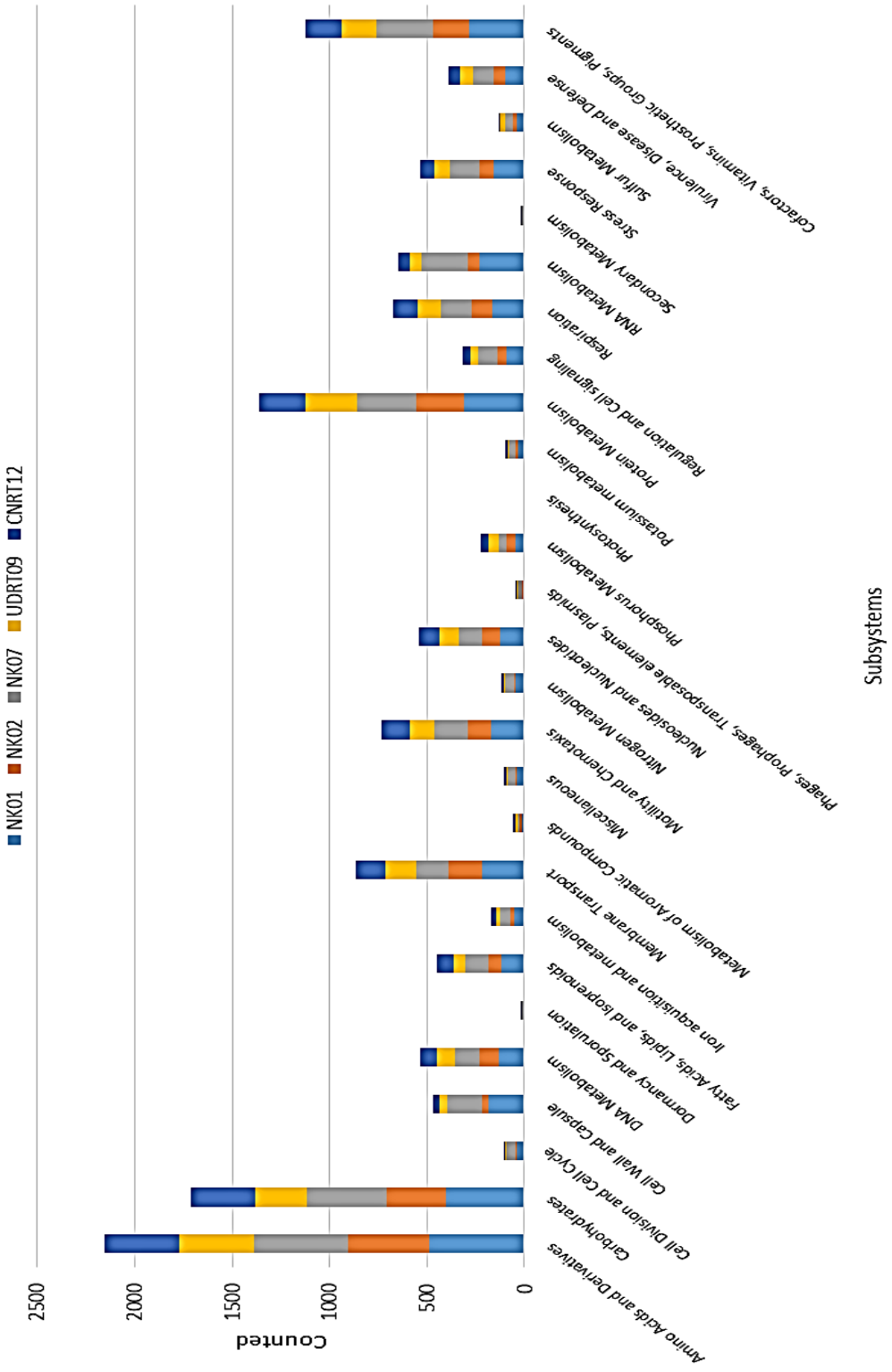

Figure 2: The chart illustrates the subsystem among five isolate of A. veronii isolated from Thailand.

3.2 Genomic identity and similarity

Species identity and similarity base on genomic nucleotide–level of five representative *A. veronii* were compared with their type strain and outgroup by ANI calculator. The similarity resulted to 96.1-100% among members in *A. veronii* group (ANI species cut-off is ≥ 95 %); in contrast, percent similarity is lower than 90.4 when compared with outgroup (figure 3).

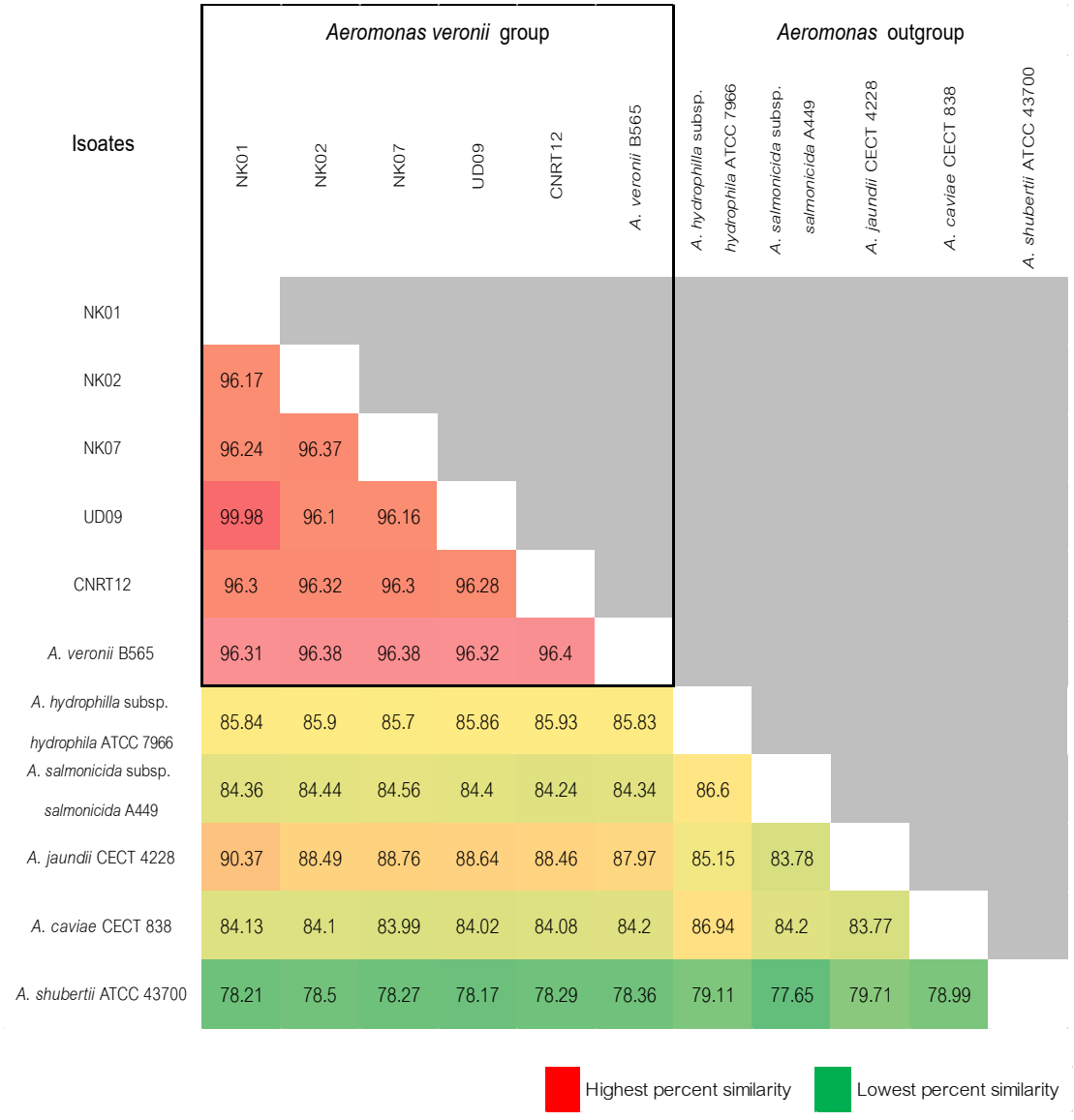

Figure 3: Heat map illustrates the species identity and similarity of genomic nucleotide – level among A. veronii *isolates and outgroup.*

The phylogenetic tree was constructed base on 2,415 bp of the gyrB gene (obtained from the genome) to visualized and supported the ANI result, it generated two clusters that divides *A. veronii* group and the outgroup (Figure 4). The cluster I contained five sequences of Tilapia isolate (NK01, NK02, NK07, UDRT09, and CNRT12) and a sequence of *A. veronii* type strain B565; bootstrap value is 83, 83, 100, and 68 respectively. The tree revealed *A. veronii* Tilapia isolates are closely relate to *A. veronii* type strain B565, which significantly differs from the outgroup (Cluster II). The cluster II contains five reference species of *Aeromonas* (*A. hydrophila* subsp. *hydrophila* ATCC 7966, *A. salmonicida* subsp. *salmonicida* A449, *A. jandaei* CECT 4228, *A. caviae* CECT 838, and *A. schubertii* ATCC 43700) which are retrieved from NCBI genome database; bootstrap value are 100, 69, 94 and 49 respectively.

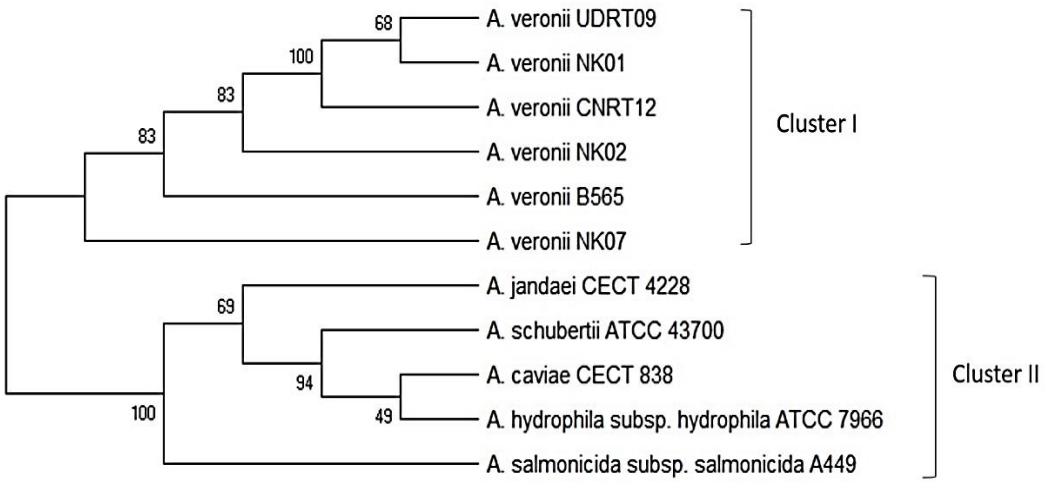

Figure 4: Phylogenetic tree based on gyrB gene of Aeromonas sp. generated by Mega X software with neighbor-joining method.

Aeromonas veronii B565; reference strain of A. veronii group. Aeromonas hydrophilla subsp. hydrophila ATCC 7966, A. salmonicida subsp. salmonicida A449, A. jaundii CECT 4228, A. caviae CECT 838, and A. shubertii ATCC 43700 are represented as outgroup.

4. Resistome analysis

Two web portals; CARD and Resfinder were mainly used for antimicrobial resistance gene determination. According to the CARD database, only the perfect and strict hit of ARGs of *A. veronii* Tilapia isolates were identified with ≥96% identity; detail of loose matches also provide in the appendix D. Likewise, acquired ARGs were analyzed by Resfinder web tool with 95% identity and 80% of minimum length. The samples were divided into 2 groups: *A. veronii* isolated from Tilapia (Tilapia group) and isolates retrieved from NCBI (NCBI group).

4.1 Tilapia group

Aeromonas veronii Tilapia isolates contained 20 ARGs (14 genes were found in acquired AGR database) from eight antimicrobial classes Aminoglycoside, beta-lactams, Elfamycin, Macrolide, Organic compound, Quinolone, Sulfonamide, and Tetracycline; the details are shown in table 9. Among five isolates in Tilapia group; NK07, NK02 and UDRT09 contain the highest number of ARGs (11 genes) follow by NK01 and CNRT12 with six ARGs. Meanwhile, *adeF, ampS,* $bla_{\text{CEPH-A3}}$, *cphA5*, and *OXA-12* gene were detected in all isolates.

4.2 NCBI group

Aeromonas veronii has been noted as an important aquatic pathogen worldwide; the relationship of AMR pattern of *A. veronii* in many country should be concerned. Fifteen genomes of *A. veronii* isolated from freshwater fish were retrieved from the NCBI genome database (NCBI group) to perform the resistome analysis as five current isolates of Tilapia group. Sixteen ARGs were detected in the group related to six antimicrobial classes Aminoglycoside, Beta-lactams, Elfamycin, Organic compound, Sulfonamide, and Tetracycline; nine genes were identified as acquired ARGs. *OXA-12* gene is commonly found among all isolate follow by *adeF* and *Elfamycin resistant EF-Tu* genes which can be detected in 13 and 11 isolates, respectively. In addition, MS1837 isolated from Catfish (USA) and 171SAe isolated from Discus (Korea) carry the majority of ARGs; nine ARGs in six antimicrobial classes. The details of ARGs in this group are shown in table 9.

Table 9: The pattern of ARGs share among Aeromonas veronii isolates from CARD and Resfinder.

Country		USA		Greece		Spain	Korea	Sri Lanka	India	China					Thailand				Type of ARG
Host		Catfish		European bass		European eel	Discus	Gold fish	NA	Yellowhead catfish	Grass carp	Wuchang bream		Channel catfish	Tilapia				
ARGs	Isolates	ML09123	MS1788	MS1837	NS	VCK	UBA1835	171SAe	Ae52	Phlh2	TH0426	CB51	X11	X12	XHVA1	XHVA2	NK01	NK02	NK07	UDRT09	CNRT12	
Aminoglycoside	*aac(3)-IIb*																					acquired
	aac(6')-Ib-cr						***											***				acquired
	aadA																		**			acquired
	aadA2			*																		acquired
Beta-lactams	$bla_{CEPH-A3}$																**	**	**	**	**	acquired
	$bla_{TEM-116}$																			**		acquired
	cphA3		*	*	*	*	*			*					*	*						
	cphA5						***	***		***		***	***	***			***	***	***	***	***		
	OXA-12	***	***	***	***	***	***	***	***	***		***	***	***	***	***	***	***	***	***	***	
	TEM-1								*													
	TEM-81																			**		
	TRU-1					*																
Elfamycin	Elfamycin resistant EF-Tu	***	***	***			***	***		***		***	***	***	***	***	***	***	***	***	***	
Macrolides	*mphA*																	**				acquired
	mrx																	**				
Organic compounds	*arr-3*						*															acquired
	catA1																			**		acquired
	dfrA12			***															***			acquired
	mcr-3						*															acquired
Quinolone	*qnrS2*																	**		**		acquired
Sulfonamide	*sul1*		***				***												***			acquired
Tetracycline	*tetA*																		**			acquired
	tetC						***												***			acquired
	tetD	*	*																			acquired
	tetE			***														***				acquired
Multidrug	*adeF*	***	***	***	***	***		***	***	***			***	***	***	***	***	***	***	***	***	
	ampS																**	**	**	**	**	acquired

* Gene present in genome from database ** Gene present in genome of Thailand isolates *** Gene shares among *A. veronii*

5. Comparative resistome analysis of *Aeromonas veronii* isolated from diseased freshwater fish

According to the result from CARD and Resfinder as previously described (table 9), twenty-seven ARGs with 18 acquired genes were detected from eight antimicrobial classes. Eight genes were shared among *A. veronii* population (*aac(6')-Ib-cr*, *cphA5*, *OXA-12, Elfamycin resistant EF-Tu, dfrA12, sul1*, *tetC, tetE* and *adeF*), eleven genes were detected only in Tilapia group and others were specifically found in NCBI group. Query sequence of 27 ARGs were translated into amino acid sequence and blasted (tBLASTn) against *A. veronii* 14 isolates (MS1837, 171SAe, TH0426, CB51, X11, and X12 are not included due to the limitation of Blast2GO; the complete genome cannot be blasted); results are illustrated in table 10. As can be seen, twenty-seven ARGs were filtered into nineteen homologous sequences with 45.52 to 100% identity. Most of the gene in aminoglycoside and beta-lactams class show a high percent identity over 65%. In contrast, the percent identity exhibit lower in a class of organic compound, quinolone, sulfonamide and tetracycline except in a few isolates. Firstly, seventeen ARGs were detected from NCBI group compare to Tilapia group (18 homolog ARGs). Besides, there are 16 ARGs shares among NCBI group and Tilapia group, $bla_{\text{CEPH-A3}}$ and *qnrS2* were found in every isolates followed by a group of *adeF; OXA-12; dfrA12*; and *sul1* which were found in most of 14 isolates. Among the presence of multiple ARGs in each isolates, the presence of $bla_{\text{TEM-116}}$ gene is specifically found in UDRT09 isolate from Tilapia group and *tetD* gene was found in Phln2 from NCBI group only. Moreover, there are ARGs associated - specific isolate from Thailand and NCBI group were identified; *aac(3)-Ib* in Phln2 and NK02; *catA1* in Ae52 and UDRT09 isolate; *ampS* only found in Tilapia group.

Table 10: Heat map illustrates the identity of ARGs and phenotypic resistance pattern among Aeromonas veronii isolates from Tilapia and NCBI genome database.

Country		USA		Greece		Spain	India	Sri lanka	China		Thailand				
Phenotypic resistance		Unpublished		AMP	Unpublished			MDR	Unpublished		OXO, OXY AMP, AMC	GEN AMP, AMC	OXY, SXT ENR, OXO AMP, AMC	OXY ENR, OXO AMP, AMC	AMP, AMC
Antimicrobial resistance genes		ML09123	MS1788	NS	VCK	UBA1835	PhIn2	Ae52	XHVA1	XHVA2	NK01	NK02	NK07	UDRT09	CNRT12
Amino glycoside	*aac(3)-IIb*						73.86					87.64			
	aac(6')-Ib-cr					52.38	48.94					100			
β-lactams	*ampS*										83.57			83.57	83.85
	$bla_{CEPH-A3}$	66.82	74.31	98.29	97.38	98.43	98.16	84.79	85.09	85.09	84.22	98.29	80	84.22	98.03
	$bla_{TEM-116}$													99.82	
	CEPH-A3	64.13	69.19	97.64	80.38				80.38	80.38	84.22				
	OXA-12	84.25	67.86	100	99.56	99.56	99.19	83.87	84.25	84.25	76.18	83.96		99.56	76.11
	TEM-1						100				51.2	51.6	92.09	51.2	
	TRU-1					89.14								68.13	
Organic compounds	*catA1*							68.75						97.92	
	dfrA12	58.9		58.28	59.51	59.51	60.9	59.88	59.51	59.51	59.51	60.12		59.51	58.9
	mcr-3	60.85		75.05		73.99	75.05	98.91			60.79	75.05		60.79	
Quinolone	*qnrS2*	61.95	62.93	62.93	62.93	61.46	62.44	61.95	62.44	62.44	61.46	100	62.93	100	62.93
Sulfonamide	*sul1*	52.02		50.4	52.53		50.8	98.56	52.53	52.53	51.2	51.6	93.08	51.2	51.2
Tetracycline	*tetA*			47.49				91.33	46.65	46.65	47.54	47.46	94.47		47.49
	tetC			47.31	45.52	58.73	49.45		45.52	45.52	47.48	46.27	74.98	79.63	47.48
	tetD						68								
	tetE			45.86	45.86		75.86	74.45			74.59			74.59	49.76
Multidrug	*adeF*	65.64	76.11	65.74	70.44	97.64	97.64		70.44	70.44	65.64	65.64	65.64	94.12	65.64

*Aeromonas genomes were blasted against the amino acid of ARGs received from CARD and Resfinder by using tBLASTn through Blast2GO.

CHAPTER V

Discussion and Conclusion

This resistome analysis evaluated antimicrobials resistance among Tilapia isolates of *Aeromonas* and determined the resistance genes from the whole genome. The presence of ARGs were applied for resistance phenotypic prediction, the relation of resistance genes to MIC value was also investigated. Our findings identified resistant *A. veronii* and multi-drug resistance isolates, since some drugs for *A. veronii* treatment in veterinary and human medication have been shared. The effective of antimicrobial should be more concerned, the resistance from animal may relevant to human health (Romero et al., 2012). The epidemiological cut-off values from *Aeromonas* diversity and antimicrobial susceptibility in freshwater - An attempt to set generic epidemiological cut-off values in France was used for interpretation (Baron et al., 2017). The MIC value of *A. veronii* in this study were evaluated with eight antimicrobials, some of antimicrobials resulted in seriously higher than the cut-off from previous publication. Florfenicol showed the best activity against *A. veronii* isolated from Tilapia followed by gentamicin and sulfamethoxazole/trimethoprim, which showed resistance in only one isolate, these are similar to the previous study from Australia; *Aeromonas* sp. are susceptible to gentamicin and sulfamethoxazole/trimethoprim more than 98 and 99 percent respectively (Aravena-Roman et al., 2012). Therefore, gentamicin resistant and sulfamethoxazole/trimethoprim resistant were determined in a high level of MIC without the evidence of drug used before; gentamicin is an active against Gram-negative bacterial infection widely used in medical and veterinary (CVMP, 2015). Their resistance should be aware as a public health consideration, the resistance may be acquired from the other pathogens in the environment (H. Heuer 2002).

Contrast to three drugs mentioned above, all isolates were resistant to beta-lactams agents (amoxicilin and ampicilin) with a high level of MIC as well reported in the previous publication (Janda and Abbott, 2010; Yang et al., 2017). According to the licensed antimicrobial allowed to use in Thailand (FCSTD, 2012), oxytetracyclines are popular used in Tilapia farm lead to high in MIC value and more higher when compare to previous report (Troy Skwor et al., 2014). In addition to oxolinic acid and enrofloxacin, both are less ability against *A. veronii* contrast to the study from China; *A. veronii* isolated from Chinese long-snout catfish, it was susceptible to those antimicrobials (Shuang-Hu Cai, 2012). Besides, *A. veronii* isolated from Tilapia revealed resistant to multiple antimicrobials similar to the study in Channel Catfish (Yang et al., 2017). Multidrug resistance was observed mainly in NK07 with resistant to six out of eight antimicrobials in this study followed by UDRT09, NK01 and NK03 which are five, four and four drugs resistance respectively.

Regarding to the ARGs, their presences and transmission are implicated the efficacy of human and animal diseases treatment caused by the resistant *A. veronii* (Yang et al., 2017). The resistome analysis of *A. veronii* was performed by the consideration of ARGs associated with the phenotypic resistance expression. Five isolates of *A. veronii* were analyzed and revealed 20 ARGs, which belongs to seven antimicrobial classes (Aminoglycoside, Beta-lactams, Elfamycin, Macrolide, Organic compound, Quinolone, Sulfonamide, and Tetracycline) as shown in table 10.

7. Aminoglycoside resistome

According to the ARGs found in this study, *aac(3)-IIb* and *aac(6')-Ib-cr* were detected in NK02 which is gentamicin-resistance phenotypically; these genes are aminoglycoside acetyltransferase encoded on the mobile genetic element (plasmid). Notable, the reports of aminoglycoside resistance in *Aeromonas sp*. remain rare worldwide, most publications study in *A. hydrophila* (Shak et al., 2011; Po-Lin Chen, 2019); only 2 publications have reported about gentamicin resistant *A. veronii* which were isolated from Channel catfish and Discus (Yang et al., 2017; Roh et al., 2019). , In any case, gentamycin is an uncommon drug use in aquaculture especially in the farm that NK02 isolate was isolated. This evidence support the fact that gentamicin resistance in *A. veronii* even was not directly induced from the antimicrobial use, but it can transferred via mobile genetic element, such as plasmids, transposons or integrons from other microorganisms in the environment (Wang et al., 2017). Referred to the MIC result and the presence of related ARGs, we assume that gentamicin resistance genes were transferred into NK02 then facilitate the resistance.

8. Beta-lactams resistome

Beta-lactams are broadly used worldwide, this class of antimicrobials has been improved their efficiency in bacteria targeting (Bush and Bradford, 2016). *Aeromonas veronii* in this study all resistant to amoxicillin and ampicillin both are categorized as a broad spectrum beta-lactams, set of beta-lactam resistance genes were detected in all isolates including *ampS,* $bla_{\text{CEPH-A3}}$, $bla_{\text{TEM-116}}$, *cphA3, OXA-12, TEM-1* and *TRU-1* which are beta-lactamase encoded gene located on chromosome typically found in *Aeromonas* species (Po-Lin Chen, 2019). Previously, *A. hydrophila* and other species of *Aeromonas* have been reported as intrinsic ampicillin-resistant, however, there is no officially report of intrinsic resistance in *A. veronii* until now (S. W. JOSEPH, 1979; N. YUCEL, 2005).

Refer to the high MIC value supported by the presence of beta-lactams resistance genes including the report from previous publications, *A. veronii* should be noted as a broad spectrum beta-lactams intrinsic resistance (Baron et al., 2017). In addition, *A. veronii* isolates carry at least two beta-lactam associated genes, most of genes were identified in UDRT09 (six genes) as seen on the table 10. Here in, percent identities are vary and the pattern of each genes are different in each isolates; this may related to the system of gene expression and related resistance mechanism to beta-lactams.

The distribution of beta-lactams resistance has affected not only aquaculture setting but also global activities especially spreading of ESBL producing microorganism. According to resistome analysis, *A. veronii* isolates carry four classes of beta-lactamase resistance genes. Class A beta-lactamase is the most commonly found in Gram-negative bacteria especially in *Escherichia coli, m*ore than a single amino acid substitution can responsible for the extended-spectrum beta lactamase (ESBL) phenotype (Shaikh et al., 2015). Recently, there is no reported about ESBL drug group was used in Thailand aquaculture. By the way, TEM-1 and $bla_{\text{TEM-116}}$ gene (ESBL related gene) were detected, its occurrence may receive from agricultural, human medication or others source as reported from previous publication (Piotrowska et al., 2017). In addition to class B metallo-carbapenemase superfamily, *cphA3 and* $bla_{\text{CEPH-A3}}$ are members of plasmid mediated carbapenemase. These genes can be detected similar to the previous study in blood sample (Wu et al., 2012; Sinclair et al., 2016). Next is class C cephalosporinase, previously TRU-1 was identified from *Aeromonas enteropelogenes*, it was only one species in *Aeromonas* that produces beta-lactamase belonging to molecular class C (De Luca et al., 2010). Notable, first detection of TRU-1 in A. *enteropelogenes* was isolated from stool in human, however, this resistance gene also can be detected in *A. veronii* isolated from fish; these is an evidence support the transferable AMR among human and animal. Lastly, class D oxacillinase with chromosomally located and naturally occurring.

OXA-12 and *ampS* are generally found in *Aeromonas jandaei* confer to ampicillin and cephalosporin resistance related to the phenotypic resistance testing in this study (Poirel et al., 2010). In conclusion, the presence of beta-lactams resistance genes are support the ESBL-producing, phenotype detection of ESBL should be further survey.

9. Quinolone and Fluoroquinolone resistome

The presence of *QnrS2* gene in this study refer to quinolone resistance by protection of DNA gyrase binding to quinolones. *QnrS2* is a plasmid-mediated quinolone resistance protein which originally found in *Salmonella enterica* and plays a role on horizontal gene transfer (Jia et al., 2017). As displayed on table 10, *QnrS2* was detected in all isolates but only show perfect identity in NK02 and UDRT09; likewise, there is a few publications have been reported *A. veronii* encoded *qnrS2* gene on a plasmid since the first report in 2008 (Sanchez-Cespedes et al., 2008). Generally, the missense mutations in DNA gyrase (*gyrA* or *gyrB*) and topisomerase IV (*parC* or *parE*) are the common mechanism to enable fluoroquinolone or quinolone resistance (Redgrave et al., 2014). On the other hand, there is a study about *qnrS2* expression confer to MIC, *qnrS2* also plays a role for quinolone and fluoroquinolone resistance as a supportive resistance gene (Sanchez-Cespedes et al., 2008).

10. Tetracycline resistome

Four ARGs were blasted against isolates; *tetA, tetC, tetE* and *adeF*. As previously described, *adeF* gene works as a secondary resistance gene enhance the tetracycline and fluoroquinolone resistance (Mobasseri et al., 2018). The presence of the *adeF* gene also found in multidrug-resistant *A. veronii* strain MS-1837 isolated from diseased catfish (Abdelhamed et al., 2019). In addition to set of *tet* genes, it located on a plasmid and functionally for tetracycline resistance (Jia et al., 2017). Similar to the previous study, *A. veronii* resistant to tetracycline and their associated genes have been reported worldwide (Troy Skwor et al., 2014; Baron et al., 2017; Yang et al., 2017). As

seen in NK01, NK07, and UDRT09 isolate, the high similarity of the resistance genes sequence showed higher MICs; this involvement of *tet genes* to the tetracycline resistance has been mention in the previous publication (Ilana Teruszkin Balassiano, 2007).

11. Other resistance genes

To the group of class 1 integron resistance association, the list of an antimicrobial resistance gene from *A. veronii* genomes consists of the member of organic compound and class of sulfonamide; *sul1, dfrA12,* and *catA1*. These genes are differently acquired from other pathogens; *catA1* is a gene encoded chloramphenicol acetyltransferase from *Shigella flexneri* 2a*, dfrA12* is a gene encoded dihydrofolate reductase from *Vibrio cholera*, and *sul1* is a gene encoded dihydropteroate synthase from *Escherichia coli* (Jia et al., 2017). Similar to this study, set of *sul1, and dfrA12* have been detected and reported as multidrug resistance mediated by class 1 Integrons in *Aeromonas* Isolates (Deng et al., 2016). In case of *catA1*, it has been previously detected in *Salmonella* sp.*, A. salmonicida* and recently in *A. veronii* (Aarestrup et al., 2003; Tanaka et al., 2016; E. Syrova, 2018). The high similarity of the *sul1* gene (93%) to the genome of NK07 related to the high phenotypic resistance itself (>256 mg/l). However, the presence or absent of *dfrA12* and *catA1* seem not to affect to the MIC level in this study.

Lastly, *mcr-3* gene was detected in three isolates from Thailand with the high percent similarity in NK02. The *mcr-3* is a transferable colistin resistance gene, the first reported was in China isolated from pWJ1 plasmid of *Escherichia coli* (Wenjuan Yin, 2017). According to the previous study, the amino acid sequence of *mcr-3* also close to *Aeromonas* species.

Herein, we do not have the result of MIC in *A. veronii*, but we believe that the presence of *mcr-3* in NK02 may affect to the MIC value of colistin same as the previous study (Xu et al., 2018).

The ARGs were mainly divided into two group; Sensitive group and MDR group. As shown in figure 5, many of ARGs were detected in sensitive group and shared a part of MDR group. The presence of *ampS, blaCEPH-A3,* and *OXA-12* gene supported the beta-lactams resistance in all isolates of this study; on the other hand, $bla_{\text{TEM-116}}$, *CEPH-A3, TEM-1* and *TRU-1* were found only in MDR group. Beta-lactam and chloramphenicol are well showed synergistic action, their resistance associated genes are located on MDR cassettes of mobile genetic element including ARGs in aminoglycosides, macrolides and sulfonamides class; similar to the presence of ARGs in this study (Wilke et al., 2005). In addition, *adeF* plays a role in multidrug efflux complex for tetracycline and fluoroquinolone. As shown in UDRT09 which resistant to oxolinic acid, enrofloxacin, and oxytetracyclin, this isolate revealed 94% similarity of *adeF* in the genome. The mutation at the adeFGH complex may inactivated the function of this gene in sensitive isolate as reported in *Acinetobacter baumannii*, which related to the low percent similarity of this gene (Coyne et al., 2010).

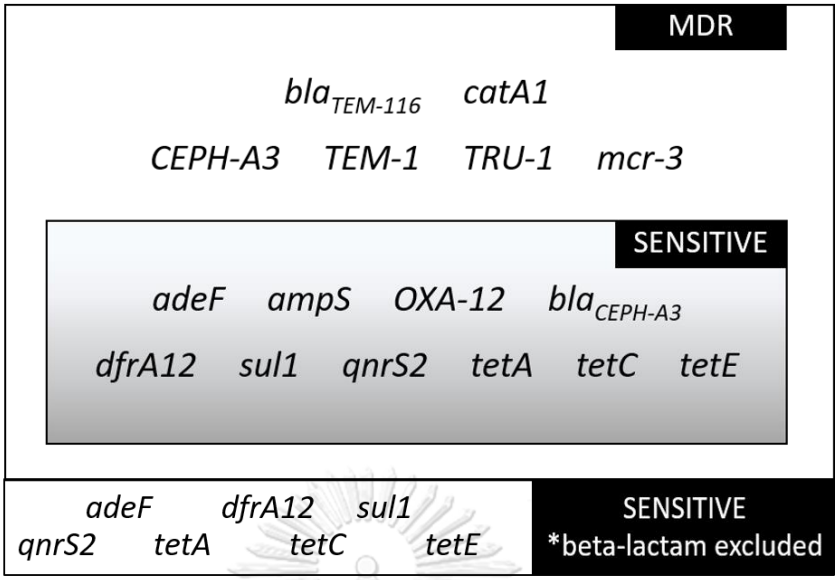

Figure 5: The figure illustrates the presence of ARGs in sensitive isolate are part of MDR isolate with a presentation of unique ARGs in MDR isolate.

The Comparative resistome analysis of *A. veronii* in freshwater fish, 28 ARGs were identified among 20 isolates of *A. veronii* from freshwater fish (Table 9). The multidrug-resistant isolate MS1837, 171SAe, and MS1788 are exhibits diverse of ARG with the highest number (nine genes) among NCBI group (Abdelhamed et al., 2019; Roh et al., 2019), by the way, it was lower than the number of ARG from Tilapia group; 11 genes from NK02, NK07 and UDRT09. According to the homolog sequence of 19 genes blasted from Blast2GO in table 10, *bla* $_{CEPH-A3}$ and *qnrS2* gene were detected in all isolates; while, 14 ARGs share among Tilapia and other NCBI isolates. These show the similarity of resistance gene generally found among *A. veronii* population worldwide, which is beneficial for resistance prediction also useful for design a further treatment program. On the contrary, the specific isolate ARGs were characterized; *blaTEM-116* and *tetD*.

The presence of *blaTEM-116* in *A. veronii* from Thailand compare to the study in *A. hydrophila* and *A. jandaei* from Brazil, this gene has been noted as the most frequently detected ARG (Balsalobre Livia Carminato, 2010); by the way, it was only found in UDRT09 isolate from Thailand. Refer to *tetD* gene was mainly localized in PhIn2 isolate from India. There is not much of the research about *tetD* has been published, once reported in 2005 as a transcriptional activator in a subset of genes of the *Escherichia coli* (Griffith et al., 2005). Then in 2006, *tetD* was molecularly characterized for tetracycline-resistant *A. veronii* Isolates from Catfish (Nawaz et al., 2006). For instance, there are three resistance genes shared among one Tilapia isolate and one from NCBI isolate; *catA1* and *TRU-1* from UDRT09 share to Ae52 and UBA1835 respectively. The presence of *TRU-1* is referred to the previous study in *A. enteropelogenes* by characterized TRU-1 associated with the Endogenous Class C beta-Lactamase (De Luca et al., 2010). *CatA1* encoded chloramphenicol acetyltransferase is originally in *Shigella flexneri* 2a. Lastly, *aac (3)-IIb*, this gene shares between PhIn2 isolate from India and NK02 from Thailand. As mention before, these three genes located on integrons and plasmid; the horizontal gene transfer event may support and share the genes through the environment.

12. Conclusion

Resistome analysis of *A. veronii* isolated from Tilapia in Thailand provided evidences that conventional antimicrobials used in aquaculture are going to lack of effectiveness. According to the licensed antimicrobials allowed to use in Thailand, amoxcilin, oxytetracyclin and oxolinic acid may not recommend for longer use, likewise, enrofloxacin have to use in high dosage (more than 16 mg/L) but should concern the effect about resistant *A. veronii* in human medication. The last choice of recommend antimicrobial use is sulfamethoxazole/ trimethoprim and florfenicol (after license announcement by FDA). In this study, *A. veronii* isolates are evolved into multidrug-resistance which related to the presence of multiple ARGs; several of genes are shared in the aquatic system among *A. veronii* population worldwide. Therefore, the series of ESBL, beta-lactam and colistin were found, *A. veronii* should be noted as a broad spectrum beta-lactams intrinsic resistance and the possibility of resistance gene acquisition with plasmid-mediated especially in gentamicin, sulfamethoxacin, tetracycline and colistin should be concern; these can affect the human and other animal health care. The outcomes of this study are useful for AMR prediction and further treatment plan.

13. Future research direction

Present study provides the information of *A. veronii* isolated from Tilapia resistant to multiple of antimicrobials in Thailand. As well as, the data of ARGs related to the MIC level of each antimicrobials also the pattern of AGRs share among *A. veronii* population isolated from freshwater fish. Therefore, further study should be carried out to determine the localization of ARGs and their mobile genetic elements. Moreover, ESBL producing *A. veronii* should be concern and more study as same as the spread of colistin and aminoglycoside resistance. Finally, new techniques for treatment or prevention of *Aeromonads* infection are importance such as phage therapy or reverse vaccination development.

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APPENDIX A
Reagents formula

Glycerol preservation

Sterile glycerol 50%	400 ml
Bacterial culture in TSB	600 ml

Cation Adjust Muller Hinton Broth

Muller Hinton	21 g
Distilled water	1000 ml
CaCl	20mg/L
MgCl	10mg/L

TBE electrophoresis buffer (10X)

Tris base	108 g
Boric acid	55 g
EDTA (0.5 M)	40 ml
Distilled water	1000 ml

Antimicrobials	solvent
Amoxicilin	1M NaOH
Ampicillin	1M NaOH
Enrofloxacin	1M NaOH
Florfenicol	Ethanol
Gentamicin	Ultrapured water
Oxolinic acid	1M NaOH
Oxytretracyclin	Ultrapured water
Sulfamethoxazole/trimethoprim	DMSO

APPENDIX B
Determination of minimum inhibitory concentrations

Amoxicillin	Ampicillin	Enrofloxacin	Florfenicol	Gentamicin
Oxolinic acid	Oxytetracycline	Sulfamethoxazole + trimethoprim		

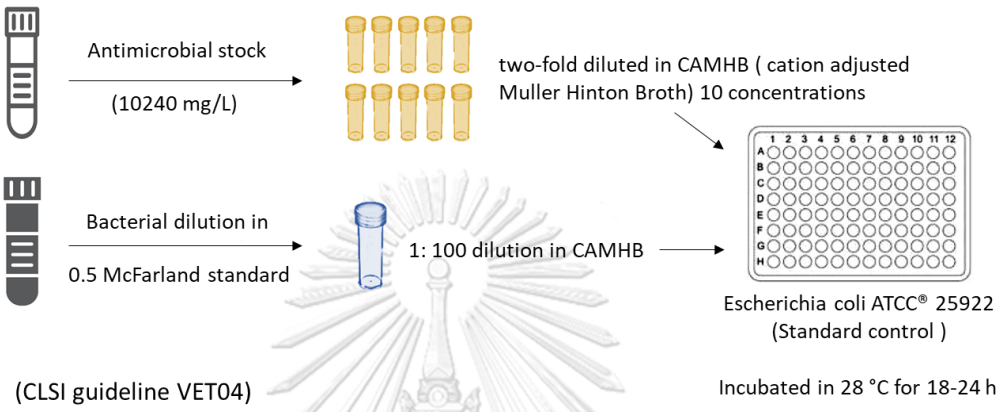

Figure 6: Flowchart for MIC determination in this study

Antimicrobials	NK01	NK02	NK03	NK04	NK05	NK06	NK07	CNRT07	CNRT11	CNRT12	CNRT13	UDRT09
	3MDR		**3MDR**				**5MDR**					**6MDR**
AMC	R	R	R	R	R	R	R	R	R	R	R	R
AMP	R	R	R	R	R	R	R	R	R	R	R	R
GEN	S	R	S	I	S	S	S	S	S	S	S	S
ENR	I	I	I	S	S	S	R	I	I	S	I	R
OXO	R	I	R	I	S	I	R	I	I	S	I	R
OXY	R	S	R	R	S	R	R	S	R	S	R	R
SXT	I	S	S	S	S	S	R	S	S	S	S	S
FFC	S	S	S	S	S	S	S	S	S	S	S	S

Figure 7: MIC interpretation

APPENDIX C
Genomics workflow

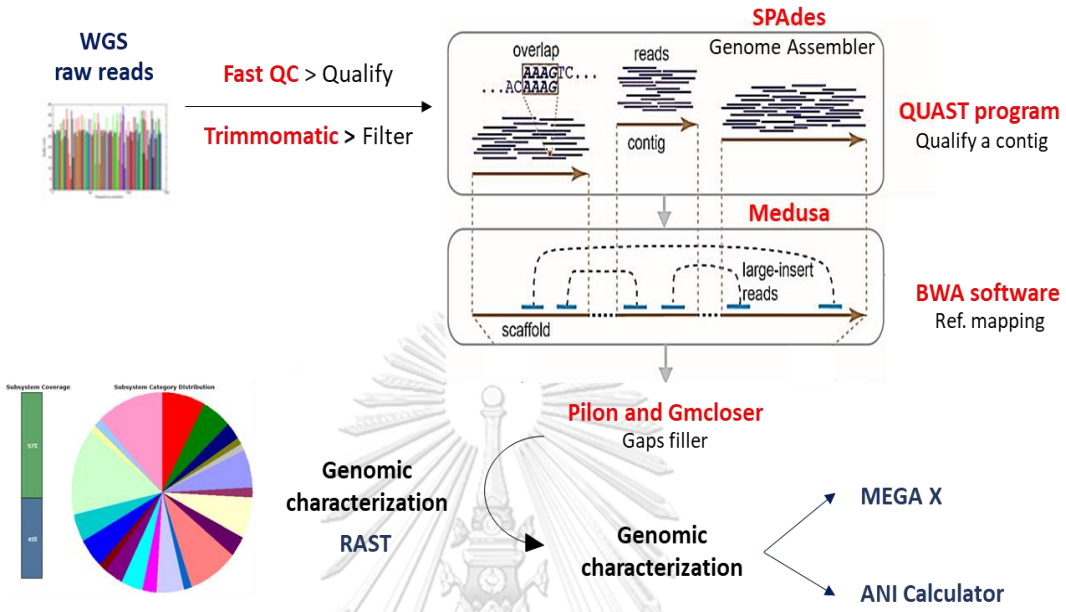

Figure 8: Comparative resistome analysis flowchart

APPENDIX D
Antimicrobial resistance genes data

- Perfect and Strict hit ARGs of Tilapia isolates from CARD
- Loose hit ARGs of Tilapia isolates from CARD
- Acquired ARGs of Tilapia isolates from Resfinder
- Amino acid sequence for local blast by Blast2GO
- The most hit of ARGs resulted from Blast2GO

Table 11: Antimicrobial resistance genes of Tilapia isolates from CARD

Isolate	Contig	Start	Stop	Cut off	Pass Bitscore	Best Hit Bitscore	Best Hit ARO	Best Identities	ARO	Model type	Drug Class	Resistance Mechanism	AMR Gene Family
NK01	scaffold_7_11	12749	13513	Strict	450	487.6	*cphA5*	90.16	3003101	homolog	beta-lactam	ABO inactivation	CphA beta-lactamase
	scaffold_1_296	331962	335111	Strict	750	800	*adeF*	43.89	3000777	homolog	tetracycline, fluoroquinolone	efflux pump	resistance-nodulation-cell division (RND) antibiotic efflux pump
	scaffold_14_118	129737	132841	Strict	750	902.5	*adeF*	48.56	3000777	homolog	tetracycline, fluoroquinolone	efflux pump	resistance-nodulation-cell division (RND) antibiotic efflux pump
	scaffold_8_21	25085	26302	Strict	750	771.9	*tet(E)*	99.75	3000173	homolog	tetracycline	efflux pump	major facilitator superfamily (MFS) antibiotic efflux pump
	scaffold_16_12	11351	12145	Strict	500	515	*OXA-12*	94.7	3001407	homolog	beta-lactam	ABO inactivation	OXA beta-lactamase
NK02	NODE_18	19602	22712	Strict	750	897.9	*adeF*	48.65	3000777	homolog	tetracycline, fluoroquinolone	efflux pump	resistance-nodulation-cell division (RND) antibiotic efflux pump
	NODE_2	62265	65414	Strict	750	798.5	*adeF*	43.71	3000777	homolog	tetracycline, fluoroquinolone	efflux pump	resistance-nodulation-cell division (RND) antibiotic efflux pump
	NODE_14	133	1317	Strict	700	720.7	*E. coli EF-Tu mutants*	89.06	3003369	variant, SNP: R234F	elfamycin	target alteration	elfamycin resistant EF-Tu
	NODE_14	19336	20520	Strict	700	720.7	*E. coli EF-Tu mutants*	89.06	3003369	variant, SNP: R234F	elfamycin	target alteration	elfamycin resistant EF-Tu
	NODE_82	1121	1981	Strict	300	425.6	*AAC(3)-IIb*	77.15	3002534	homolog	aminoglycoside	ABO inactivation	AAC(3)
	NODE_10	121261	122055	Strict	500	518.8	*OXA-12*	95.44	3001407	homolog	beta-lactam	ABO inactivation	OXA beta-lactamase
	NODE_9	9169	9933	Strict	450	488	*cphA5*	90.16	3003101	homolog	beta-lactam	ABO inactivation	CphA beta-lactamase
	NODE_77	737	1393	Perfect	400	459.1	*QnrS2*	100	3002791	homolog	fluoroquinolone	target protection	quinolone resistance protein (qnr)
	NODE_77	2698	3297	Perfect	275	405.2	*AAC(6')-Ib-cr*	100	3002547	homolog	aminoglycoside, fluoroquinolone	ABO inactivation	AAC(6')

Isolate	Contig	Start	Stop	Cut off	Pass Bitscore	Best Hit Bitscore	Best Hit ARO	Best Identities	ARO	Model type	Drug Class	Resistance Mechanism	AMR Gene Family
NK07	Scaffold_1_pilon_583	640087	643191	Strict	750	899	*adeF*	48.46	3000777	homolog	tetracycline, fluoroquinolone	efflux pump	resistance-nodulation-cell division (RND) antibiotic efflux pump
	Scaffold_4_pilon_516	546032	546796	Strict	450	492.7	*cphA5*	91.34	3003101	homolog	beta-lactam	ABO inactivation	CphA beta-lactamase
	Scaffold_2_pilon_885	982925	984163	Perfect	600	740	*Mrx*	100	3003839	homolog	macrolide	ABO inactivation	macrolide phosphotransferase (MPH)
	Scaffold_2_pilon_1172	1281921	1283105	Strict	700	720.7	*E. coli EF-Tu mutants*	89.06	3003369	variant, SNP: R234F	elfamycin	target alteration	elfamycin resistant EF-Tu
	Scaffold_2_pilon_1189	1301124	1302308	Strict	700	720.7	*E. coli EF-Tu mutants*	89.06	3003369	variant, SNP: R234F	elfamycin	target alteration	elfamycin resistant EF-Tu
	Scaffold_2_pilon_891	988239	989078	Perfect	500	549.7	*sul1*	100	3000410	homolog	sulfonamide antibiotic; sulfone antibiotic	target replacement	sulfonamide resistant sul
	Scaffold_2_pilon_884	982023	982928	Strict	500	594.7	*mphA*	100	3000316	homolog	macrolide	ABO inactivation	macrolide phosphotransferase (MPH)
	Scaffold_2_pilon_150	169801	172950	Strict	750	796.2	*adeF*	43.71	3000777	homolog	tetracycline, fluoroquinolone	efflux pump	resistance-nodulation-cell division (RND) antibiotic efflux pump
	Scaffold_2_pilon_893	989583	990362	Strict	450	458	*aadA*	87.11	3002601	homolog	aminoglycoside	ABO inactivation	ANT(3'')
	Scaffold_2_pilon_894	990782	991279	Perfect	300	332.4	*dfrA12*	100	3002858	homolog	diaminopyrimidine	target replacement	trimethoprim resistant dihydrofolate reductase dfr
	Scaffold_2_pilon_839	935949	936743	Strict	500	519.6	*OXA-12*	95.44	3001407	homolog	beta-lactam	ABO inactivation	OXA beta-lactamase
	Scaffold_40_pilon_3	1318	2517	Strict	500	621.3	*tet(C)*	79.39	3000137	homolog	tetracycline	efflux pump	major facilitator superfamily (MFS) antibiotic efflux pump

Isolate	Contig	Start	Stop	Cut off	Pass Bitscore	Best Hit Bitscore	Best Hit ARO	Best Identities	ARO	Model type	Drug Class	Resistance Mechanism	AMR Gene Family
UDRT 09	Scaffold_15_pilon_198	208845	210029	Strict	700	720.7	*E. coli EF-Tu mutants*	89.06	3003369	variant, SNP: R234F	elfamycin	target alteration	elfamycin resistant EF-Tu
	Scaffold_1478_pilon_1	1	432	Strict	350	297.4	*catA1*	96.53	3004459	homolog	phenicol	ABO inactivation	chloramphenicol acetyltransferase (CAT)
	Scaffold_2055_pilon_1	2	844	Strict	500	556.2	*TEM-81*	98.57	3000948	homolog	beta-lactam	ABO inactivation	TEM beta-lactamase
	Scaffold_16_pilon_11	14557	15321	Strict	450	487.6	*cphA5*	90.16	3003101	homolog	beta-lactam	ABO inactivation	CphA beta-lactamase
	Scaffold_17_pilon_412	442313	445462	Strict	750	800	*adeF*	43.89	3000777	homolog	tetracycline, fluoroquinolone	efflux pump	resistance-nodulation-cell division (RND) antibiotic efflux pump
	Scaffold_1734_pilon_4	1768	2424	Perfect	400	459.1	*QnrS2*	100	3002791	homolog	fluoroquinolone	target protection	quinolone resistance protein (qnr)
	Scaffold_15_pilon_181	189642	190826	Strict	700	732.6	*E. coli EF-Tu mutants*	91.09	3003369	variant, SNP: R234F	elfamycin	target alteration	elfamycin resistant EF-Tu
	Scaffold_13_pilon_13	11142	11936	Strict	500	515	*OXA-12*	94.7	3001407	homolog	beta-lactam	ABO inactivation	OXA beta-lactamase
	Scaffold_15_pilon_334	353423	354640	Strict	750	771.9	*tet(E)*	99.75	3000173	homolog	tetracycline	efflux pump	major facilitator superfamily (MFS) antibiotic efflux pump
	Scaffold_17_pilon_841	936425	939529	Strict	750	902.5	*adeF*	48.56	3000777	homolog	tetracycline, fluoroquinolone	efflux pump	resistance-nodulation-cell division (RND) antibiotic efflux pump
CNRT 12	NODE_5	44312	47422	Strict	750	897.9	*adeF*	48.65	3000777	homolog	tetracycline, fluoroquinolone	efflux pump	resistance-nodulation-cell division (RND) antibiotic efflux pump
	NODE_8	196163	197347	Strict	700	720.7	*E. coli EF-Tu mutants*	89.06	3003369	variant, SNP: R234F	elfamycin	target alteration	elfamycin resistant EF-Tu
	NODE_8	176960	178144	Strict	700	728	*E. coli EF-Tu mutants*	90.84	3003369	variant, SNP: R234F	elfamycin	target alteration	elfamycin resistant EF-Tu
	NODE_15	12141	12905	Strict	450	491.5	*cphA5*	91.34	3003101	homolog	beta-lactam	ABO inactivation	CphA beta-lactamase
	NODE_1	418370	421516	Strict	750	799.3	*adeF*	43.74	3000777	homolog	tetracycline, fluoroquinolone	efflux pump	resistance-nodulation-cell division (RND) antibiotic efflux pump
	NODE_14	122670	123464	Strict	500	518.1	*OXA-12*	94.7	3001407	homolog	beta-lactam	ABO inactivation	OXA beta-lactamase

Table 12: Loose hit ARGs of Tilapia isolates from CARD

Best hit ARO	Identities				
	NK01	NK02	NK07	UDRT09	CNRT12
AAC(2')-Ib	39.29	38.5	-	39.29	42.86
AAC(3)-Xa	-	-	89.15	-	-
AAC(6')-Iad	40.91	40.9	-	-	-
AAC(6')-Iak	-	33.7	83.33	-	32.53
AAC(6')-Ib8	-	-	-	73.68	-
AAC(6')-Isa	32.14	32.1	71.88	32.14	30.68
AAC(6')-Iy	-	-	-	55.4	-
abcA	30.61	47.1	-	-	28.26
abeM	40.92	40.7	-	-	41.61
acrB	22.6	-	-	30.77	23.75
acrF	-	-	-	-	33.33
acrS	33.33	-	71.5	33.33	-
act-27	-	-	-	31.94	-
adeA	22.67	23.4	70.52	44.95	-
adeB	-	26.8	67.37	62.35	-
adeF	-	-	62.94	-	-
adeG	-	23.8	-	-	23.08
adeH	-	20.7	-	-	21.67
adeI	-	-	-	-	27.01
adeJ	-	21.2	-	-	20.79
adeL	38.03	37.7	62.55	38.03	38.03
adeN	40	40	-	-	40
adeR	29.96	30.1	45.71	50.67	30.13
adeS	31.09	31.1	44.95	38.35	31.09
Agrobacterium fabrum chloramphenicol acetyltransferase	-	33.3	44.67	43.65	-
aim-1	27.42	26.6	-	-	-
amrA	27.27	30.7	43.71	29.19	30.71
amrB	29.43	-	-	-	-
apmA	49.06	49.1	-	-	49.06
arlR	-	-	43.45	57.58	-

Best hit ARO	Identities				
	NK01	NK02	NK07	UDRT09	CNRT12
arlS	24.4	20.5	-	-	20.49
arnA	32.14	32.1	43.08	32.14	32.14
AxyX	-	24.4	-	-	20.83
AxyY	29.45	29.5	-	-	29.72
bacA	72.16	72.2	42.32	72.16	72.16
baeR	30.53	34.9	42.01	44.34	-
baeS	-	-	40.44	32.41	30.53
basR	25.14	26	-	-	26.02
basS	-	-	39.65	-	-
bcr-1	-	-	39.11	42.57	-
bcrA	-	-	38.79	39.76	52.63
bcrC	44.29	44.3	38.24	44.29	45.71
Bifidobacterium ileS conferring resistance to mupirocin	25.4	24.7	38.05	28.49	24.66
blt	19.78	-	-	-	23.15
Burkholderia pseudomallei Omp38	28.32	-	-	28.32	26.67
carA	-	26.6	-	-	-
catB2	37.21	37.2	-	48.33	37.21
catB9	-	-	37.8	43.4	-
catI	-	-	-	99.31	-
CAU-1	-	-	-	37.5	-
cdeA	-	-	37.74	24.77	-
ceoB	-	-	37.64	25.49	-
Chlamydia trachomatis intrinsic murA conferring resistance to fosfomycin	32.87	32.6	-	-	38.58
chrB	-	-	-	40	
clbA	30.21	30.8	-	-	30.21
clbB	-	-	37.62	34.51	-
Clostridium difficile gyrA conferring resistance to fluoroquinolones	-	-	-	53.25	-
cmeA	24.49	22.8	-	-	24.02
cmeB	19.96	-	-	-	-
cmeR	35.09	35.1	-	37.25	35.09

Best hit ARO	Identities				
	NK01	NK02	NK07	UDRT09	CNRT12
cmlB1	-	25.1	-	-	-
cmlv	-	-	37.33	35.49	-
cmrA	-	-	37.08	48.72	-
cpxA	31.68	31.7	37.02	37.64	31.68
CRP	89.15	89.2	36.67	89.15	89.15
D-Ala-D-Ala ligase	-	-	36.21	34.49	-
dfrA1	35.29	35.3	-	-	35.29
dfrA3	-	-	36.14	62.58	-
dfrG	-	-	-	34.55	-
efrA	-	27.4	-	-	-
efrB	24.1	24.1	-	52.38	24.1
emeA	19.8	19.8	-	-	20.11
emrB	-	-	36.1	31.05	-
emrR	25.2	26	-	-	26.02
emrY	23.53	23.5	-	-	23.53
Enterobacter cloacae acrA	-	-	36.04	55.25	-
Enterococcus faecium cls conferring resistance to daptomycin	27.5	27.5	36.02	27.92	27.5
Enterococcus faecium liaR mutant conferring daptomycin resistance	-	-	-	34.93	-
Enterococcus faecalis liaS mutant conferring daptomycin resistance	26.67	26.7	35.98	26.67	26.67
ErmE	-	-	35.81	29.03	-
ErmO	26.15	26.2	-	-	26.15
Escherichia coli acrR with mutation conferring multidrug antibiotic resistance	34.62	34.6	35.71	37.62	34.62
Escherichia coli EF-Tu mutants conferring resistance to kirromycin	27.34	27.3	35.05	30.99	27.34
Escherichia coli EF-Tu mutants conferring resistance to Pulvomycin	32.14	-	-	-	-
Escherichia coli emrE	-	-	34.91	32.08	-
Escherichia coli gyrA conferring resistance to fluoroquinolones	32.48	-	34.9	74.42	-
Escherichia coli gyrB conferring resistance to aminocoumarin	-	-	-	40.85	-

Best hit ARO	Identities				
	NK01	NK02	NK07	UDRT09	CNRT12
Escherichia coli marR mutant conferring antibiotic resistance	-	-	34.85	32.06	-
Escherichia coli parC conferring resistance to fluoroquinolone	-	-	34.75	67.11	-
Escherichia coli parE conferring resistance to fluoroquinolones	38.98	39	-	-	38.98
Escherichia coli soxR with mutation conferring antibiotic resistance	31.48	31.5	-	-	31.48
Escherichia coli soxS with mutation conferring antibiotic resistance	29.29	36	34.71	37.5	36
Escherichia coli UhpA with mutation conferring resistance to fosfomycin	26.61	26.6	-	-	26.61
Escherichia coli UhpT with mutation conferring resistance to fosfomycin	83.33	83.3	34.51	83.33	83.33
evgA	32.76	32.8	34.49	36.67	32.76
evgS	33.91	33.1	34.25	42.53	33.91
facT	-	24.7	33.85	30.91	25.29
farA	31.85	23.1	33.78	37.43	22.91
fexA	32.26	32.3	-	-	32.26
FosA	-	-	-	60.31	-
FosA2	-	-	-	-	29.37
FosB	25.42	25.4	33.73	25.42	25.42
FosB3	32.56	-	-	-	-
FosC2	39.58	39.6	33.72	39.58	35.42
gadW	-	28.3	-	-	28.28
gadX	36.96	26.1	33.71	36.96	34.78
golS	32.84	24.1	33.62	50	27.19
hmrM	-	-	33.33	47.39	-
H-NS	55.88	55.9	33.05	55.88	55.15
ICR-Mc	30	29.6	-	-	29.63
iri	27.47	27.5	33.03	27.47	27.47
kdpE	35.9	31.7	32.99	46.22	31.68
Klebsiella pneumoniae acrA	24.18	-	-	-	24.73
Klebsiella pneumoniae OmpK37	36.08	36.7	32.65	36.08	36.98
LlmA 23S ribosomal RNA	36.94	36.9	32.64	36.94	35.29

Best hit ARO	Identities				
	NK01	NK02	NK07	UDRT09	CNRT12
lmrB	-	43.8	-	-	-
lmrC	23.96	24	-	-	19.83
lmrD	28.57	-	32.61	23.78	24.18
LpeA	-	23.2	-	-	23.46
LpeB	-	-	32.59	24.93	-
LRA-2	25.7	25.7	32.58	25.7	-
lrfA	-	-	-	36.23	23.97
lsaA	-	24.5	-	-	-
lsaB	27.42	21.7	-	-	24.22
lsaC	26.11	28.3	32.5	-	26.34
lsaE	25.29	28.1	-	-	25.29
macA	25.91	-	32.39	39.24	-
macB	28.39	27.8	32.26	48.31	28.13
marA	25.53	-	31.45	25.51	-
MCR-7.1	-	-	31.36	70.15	-
mdsB	24.34	-	-	-	-
mdtA	26.77	-	-	57.14	25.67
mdtE	28.85	28.2	-	42	-
mdtH	-	-	31.19	60.82	-
MdtK	23.2	23.7	31.18	25.75	23.2
mdtM	-	22	-	-	-
mdtN	25.44	-	31.09	29.59	-
mdtP	22.54	22.3	-	-	21.67
mecA	22.18	22.2	-	-	22.18
mecC	-	-	31.01	29.18	-
mel	-	-	31.01	30.99	-
mepA	21.9	21.9	-	-	23.28
mepR	-	22.6	-	-	-
MexA	-	-	-	-	22.93
MexD	23.39	-	-	-	24.58
MexF	26.37	-			26.56
MexH	-	-	31	30.5	-

Best hit ARO	Identities				
	NK01	NK02	NK07	UDRT09	CNRT12
MexI	-	-	-	37.74	-
MexJ	-	24.2	-	-	-
MexK	-	-	30.95	69.3	-
MexL	-	-	30.92	38.69	-
mexM	-	-	30.91	31.86	-
mexN	-	31	-	37.04	31
mexQ	-	-	30.8	26.73	-
MexR	37.5	37.5	30.77	-	37.5
MexS	38.37	38.4	30.71	70	38.37
MexT	32.21	32.2	30.65	75.56	32.21
MexV	-	-	30.3	38.46	-
MexW	-	-	30.23	48.51	-
MexZ	46.81	46.8	30.18	66.67	46.81
mgrA	-	-	-	27.47	-
Morganella morganii gyrB conferring resistance to fluoroquinolone	-	-	30.15	39.78	-
mprF	-	-	37.88	35.87	-
msbA	-	-	30.15	59.72	-
msrA	30.77	30.8	-	-	25
msrC	31.54	25.8	-	-	25.95
msrE	41.3	41.3	-	-	41.3
mtrA	28.57	32	29.79	40.44	31.07
mtrD	-	23.5	-	-	-
mtrR	-	-	29.62	42.5	-
MuxA	27.12	27.1	29.53	68.97	-
MuxB	29	29.9	-	-	29.11
Mycobacterium tuberculosis gyrB mutant conferring resistance to fluoroquinolone	40.91	38.5	-	-	40.64
MuxC	-	-	-	57.14	-
Mycobacterium tuberculosis katG mutations conferring resistance to isoniazid	55.87	56.1	29.5	55.87	56.22

Best hit ARO	Identities				
	NK01	NK02	NK07	UDRT09	CNRT12
Mycobacterium tuberculosis ndh with mutation conferring resistance to isoniazid	28.03	28	-	28.03	28.03
Mycobacterium tuberculosis pncA mutations conferring resistance to pyrazinamide	26.9	26.9	29.44	26.9	26.9
Mycobacterium tuberculosis rpoB mutants conferring resistance to rifampicin	-	-	29.41	56.29	-
Mycobacterium tuberculosis thyA with mutation conferring resistance to para-aminosalicylic acid	67.42	67.1	29.28	67.42	67.05
Mycoplasma hominis parC conferring resistance to fluoroquinolone	33.43	33.6	-	45.88	33.57
myrA	-	-	29.26	28.71	-
nalC	37.25	-	29.21	31.25	37.25
nalD	32.91	34.6	-	-	34.18
NmcR	51.02	38.4	29.2	51.02	38.36
novA	45.83	-	28.69	60.49	-
oleB	42.03	42	-	-	42.03
oleC	33.94	33.9	28.57	47.83	33.94
OpmD	22.32	-	-	-	-
OpmH	-	-	28.49	30	-
OprJ	-	-	-	42.5	-
OprM	22.74	23.5	28.43	81.44	23.46
optrA	30.77	22.8	28.38	32.26	30.77
oqxA	26.52	25.4	-	-	25.41
otr(A)	-	-	28.21	43.45	-
otr(B)	-	-	-	33.01	-
otrC	-	31.5	-	-	31.48
OXA-12	94.7	-	-	-	-
OXA-240	30.08	30.1	-	-	-
OXA-74	-	-	-	-	28.46
patA	34.58	34.4	-	54.84	36.33
patB	31.4	31.1	-	39.68	31.82

Best hit ARO	Identities				
	NK01	NK02	NK07	UDRT09	CNRT12
PDC-73	-	-	-	63.33	-
Planobispora rosea EF-Tu mutants conferring resistance to inhibitor GE2270A	29.96	30	-	30.21	29.96
PmpM	-	-	28.19	23.76	-
pmrA	31.91	31.9	28.19	31.91	31.91
PmrF	29.38	29.7	28.17	67.12	29.69
poxtA	30.34	30.3	-	-	30.34
pp-flo	-	-	28.14	-	-
Pseudomonas aeruginosa catB7	27.19	-	28.12	56.88	38.18
Pseudomonas aeruginosa CpxR	28.16	28.2	28.03	69.9	28.16
Pseudomonas aeruginosa soxR	31.33	32.9	27.92	30.91	31.33
qacH	29.13	29.1	27.85	-	29.13
QepA1	22.6	-	-	-	22.6
QepA2	29.73	-	-	-	30.27
QepA4	23.1	23.9	27.85	30.29	23.1
QnrB17	44.93	-	-	-	-
QnrB57	-	-	-	-	44.93
QnrB66	-	48	-	-	-
QnrVC1	-	-	-	47	-
QnrVC5	-	-	27.83	-	-
ramA	25.93	25.9	27.82	34.07	-
RlmA(II)	28.8	28.8	-	39.77	28.8
rosB	27.58	27.9	27.77	27.58	27.77
rphA	36.81	36.8	-	-	36.81
rphB	-	38.2	27.65	38.24	38.24
rpoB2	56.41	56.4	-	-	56.41
salA	23.88	23.6	-	-	27.21
Salmonella enterica ramR mutants	-	-	27.56	39.39	-
SAT-2	-	-	27.47	-	-
SAT-4	-	37.3	-	-	37.29
sdiA	32.81	32.8	27.4	31.63	32.81
SMB-1	-	-	27.34	27.52	-

Best hit ARO	Identities				
	NK01	NK02	NK07	UDRT09	CNRT12
smeB	-	22.4		37.5	-
smeR	22.99	23	27.27	38.99	33.33
smeS	25.37	25.4	27.24	33.62	25.37
srmB	28.36	28.4	-	-	-
Staphylococcus aureus fusA with mutation conferring resistance to fusidic acid	-	-	27.2	58.12	-
Staphylococcus aureus fusE with mutation conferring resistance to fusidic acid	42.86	42.9	-	42.86	42.86
Staphylococcus aureus murA with mutation conferring resistance to fosfomycin	-	-	27.05	52.14	30.67
Staphylococcus mupA conferring resistance to mupirocin	31.58	30.7	27.03	33.82	-
Staphylococcus mupB conferring resistance to mupirocin	-	-	-	21.36	-
Streptococcus agalactiae mprF	29.5	28.3	-	-	28.29
Streptomyces lividans cmlR	-	21.1	-	-	21.03
Streptomyces rishiriensis parY mutant conferring resistance to aminocoumarin	-	-	27.01	40.89	-
sul1	31.6	32	-	-	32
sul4	-	-	26.95	42.38	-
TaeA	23.78	23.8	26.92	46.43	23.65
tap	-	-	26.87	-	-
tcr3	-	25.6	26.8	-	-
tet(33)	28.09	28.1	-	-	28.09
tet(35)	50.57	50.6	26.77	50.57	-
tet(41)	-	-	26.68	30.22	-
tet(43)	-	27.2	-	47.5	27.23
tet(A)	-	27.5	-	-	23.64
tet(B)	23.32	-	-	-	-
tet(C)	24.01	24	-	91.36	23.89
tet(D)	30.69	35.4	-	-	35.42

Best hit ARO	Identities				
	NK01	NK02	NK07	UDRT09	CNRT12
tet(H)	23.86	23.5	-	-	-
tet(J)	25.14	25.1	-	-	25.14
tet(Z)	26.09	26.1	-	-	26.09
tet32	39.23	39.2	-	-	39.23
tet36	32.84	-	-	-	-
tet44	-	-	26.5	32.11	-
tet44	36.55	36.6	-	-	36.55
tetA(46)	26.36	26.4	26.5	24.89	31.33
tetA(48)	26.85	26.9	26.36	40.99	-
tetA(60)	-	-	25.89	33.92	-
tetB(46)	22.69	-	25.81	37.33	-
tetB(60)	26.32	23.6	25.8	36.1	27.27
tetB(P)	-	-	25.7	39.07	-
tetM	29.35	29.4	-	-	29.35
tetQ	-	-	-	-	30.46
tetR	51.74	-	25.68	51.74	-
tetS	32.26	-	-	-	32.26
tetT	-	-	-	-	35.96
tetW	-	28.2	-	31.63	-
tetX	40.74	40.7	-	-	48.48
tlrC	25.13	25.1	-	32.81	25.13
tmrB	-	34.3	-	-	-
TolC	-	-	25.49	48.75	-
TriA	28.03	29.3	25.42	78.05	28.23
TriB	23.86	-	25.17	28.03	-
TriC	-	-	-	84.44	-
tsnR	27.96	28	25.09	27.96	27.01
ugd	28.76	31.7	25	28.76	30.64
vanB	26.22	26.2	-	-	26.22
vanE	-	33	-	-	-
vanHA	-	26.8	25	32.62	-
vanHB	-	-	24.89	33.71	-

Best hit ARO	Identities				
	NK01	NK02	NK07	UDRT09	CNRT12
vanHD	27.27	33.5	-	-	26.26
vanHM	29.58	29	-	-	29.58
vanHO	32.5	32.5	24.81	34.59	32.5
vanL	-	-	-	47.62	-
vanRA	29.17	29.2	-	-	29.17
vanRB	31.67	31.7	-	-	31.67
vanRC	27.52	27.5	24.8	24.55	27.52
vanRD	38.79	38.8	-	-	33.33
vanRF	33.66	29.3	24.77	38.81	32.67
vanRI	-	-	24.12	39.11	-
vanRL	26.83	-	-	-	27.64
vanRM	33.93	33.9	24.03	44.76	33.93
vanRN	28.85	28.9	-	-	28.85
vanRO	32.61	32.6	24	38.07	32.61
vanSA	27.84	26.6	23.98	26.24	27.84
vanSB	24.54	-	-	-	-
vanSC	23.97	24.4	-	22.82	24.38
vanSD	-	19	-	-	-
vanSE	20	20	-	-	20
vanSL	24.13	23.8	-	-	25
vanSM	24.88	24.9	-	48.72	24.88
vanSN	23	33.3	23.92	23.92	24.66
vanSO	28.14	27.6	23.76	-	28.14
vanTE	26.07	-	23.69	-	-
vanTG	-	-	23.58	32.43	-
vanTN	30.68	31	23.42	29.49	31.04
vanXYE	-	28.6	-	-	-
vanYA	29.63	-	-	-	29.63
vanYM	-	-	22.99	25.64	-
vatE	26.32	-	-	-	41.51
vatF	-	-	22.86	71.98	-
vatH	-	-	22.11	-	-

Best hit ARO	Identities				
	NK01	NK02	NK07	UDRT09	CNRT12
VatI	-	33.3	-	-	-
vgaA	26.37	33.3	-	-	28.23
vgaALC	31.25	31.3	-	-	31.25
vgaB	24.02	24.1	-	-	27.86
vgaD	33.33	33.3	-	-	33.33
vgaE	26.26	23.3	-	-	26.09
Vibrio cholerae varG	31.18	-	21.91	31.18	-
YojI	22.63	22.6	20.89	63.12	22.63

Table 13: Acquired antimicrobial resistance genes of Tilapia isolates from Resfinder

Isolate	Resistance gene	Accession no.	Identity	Query/HSP	Contig	Position in contig	Phenotype
NK01	*ampS*	X80276	95.21	795/773	scaffold_16	11351..12123	Beta-lactam resistance
	bla$_{CEPH-A3}$	AY112998	95.03	765/765	scaffold_7	12749..13513	Beta-lactam resistance
	tet(E)	L06940	99.92	1218/1218	scaffold_8	25085..26302	Tetracycline resistance
NK02	*aac(3)-IId*	EU022314	99.88	861/861	NODE_82_length_4687	1121..1981	Aminoglycoside resistance
	aac(6')-Ib-cr	EF636461	100	519/519	NODE_77_length_6196	2698..3216	Fluoroquinolone and aminoglycoside resistance
	ampS	X80276	95.34	795/773	NODE_10_length_133171	121283..122055	Beta-lactam resistance
	bla$_{CEPH-A3}$	AY112998	95.82	765/765	NODE_9_length_142416	9169..9933	Beta-lactam resistance
	aac(6')-Ib-cr	EF636461	100	519/519	NODE_77_length_6196	2698..3216	Fluoroquinolone and aminoglycoside resistance
	qnrS2	DQ485530	100	657/657	NODE_77_length_6196	737..1393	Fluoroquinolone and aminoglycoside resistance
NK07	*aadA2*	JQ364967	100	792/792	Scaffold_2_pilon/0009	65030..65821	Aminoglycoside resistance
	ampS	X80276	95.34	795/773	Scaffold_2_pilon/0009	11396..12168	Beta-lactam resistance
	bla$_{CEPH-A3}$	AY112998	95.56	765/765	Scaffold_4_pilon/0007	299281..300045	Beta-lactam resistance
	mph(A)	D16251	100	906/906	Scaffold_2_pilon/0009	57470..58375	Macrolide resistance
	sul1	U12338	100	840/840	Scaffold_2_pilon/0009	63686..64525	Sulphonamide resistance
	tet(A)	AJ517790	100	1200/1200	Scaffold_40_pilon/0001	1318..2517	Tetracycline resistance
	dfrA12	AM040708	100	498/498	Scaffold_2_pilon/0009	66229..66726	Trimethoprim resistance
UDRT09	*ampS*	X80276	95.21	795/773	Scaffold_13_pilon/0001	11142..11914	Beta-lactam resistance
	bla$_{CEPH-A3}$	AY112998	95.03	765/765	Scaffold_16_pilon/0006	12801..13565	Beta-lactam resistance
	bla$_{TEM-116}$	AY425988	99.88	861/844	Scaffold_2055_pilon/0001	1..844	Beta-lactam resistance
	qnrS2	DQ485530	100	657/657	Scaffold_1734_pilon/0001	1768..2424	Fluoroquinolone resistance
	catA1	V00622	99.77	660/432	Scaffold_1478_pilon/0001	1..432	Phenicol resistance
	tet(E)	L06940	99.92	1218/1218	Scaffold_15_pilon/0003	142282..143499	Tetracycline resistance
CNRT12	*bla*$_{CEPH-A3}$	AY112998	95.42	765/765	NODE_15_length_77837	12141..12905	Beta-lactam resistance
	ampS	X80276	95.18	795/767	NODE_14_length_134531	122698..123464	Beta-lactam resistance

Table 14: Amino acid sequence for local blast by Blast2GO

Best Hit ARO	CARD Protein Sequence
AAC(3)-IIb	MNTIESITADLHGLGVRPGDLIMVHASLKAVGPVEGGAASVVSALRAAVGSAGTLMGYAS WDRSPYEETLNGARMDEELRRRWPPFDLATSGTYPGFGLLNRFLLEAPDARRSAHPDAS MVAVGPLAATLTEPHRLGQALGEGSPLERFVGHGGKVLLLGAPLDSVTVLHYAEAIAPIPN KRRVTYEMPMLGPDGRVRWELAEDFDSNGILDCFAVDGKPDAVETIAKAYVELGRHREGI VGRAPSYLFEAQDIVSFGVTYLEQHFGAP
AAC(6')-Ib-cr	MSNAKTKLGITKYSIVTNSNDSVTLRLMTEHDLAMLYEWLNRSHIVEWWGGEEARPTLAD VQEQYLPSVLAQESVTPYIAMLNGEPIGYAQSYVALGSGDGRWEEETDPGVRGIDQLLAN ASQLGKGLGTKLVRALVELLFNDPEVTKIQTDPSPSNLRAIRCYEKAGFERQGTVTTPYGP AVYMVQTRQAFERTRSDA
aadA	MREAVIAEVSTQLSEVVGVIERHLEPTLLAVHLYGSAVDGGLKPHSDIDLLVTVTVRLDETT RRALINDLLETSASPGESEILRAVEVTIVVHDDIIPWRYPAKRELQFGEWQRNDILAGIFEPA TIDIDLAILLTKAREHSVALVGPAAEELFDPVPEQDLFEALNETLTLWNSPPDWAGDERNVV LTLSRIWYSAVTGKIAPKDVAADWAMERLPAQYQPVILEARQAYLGQEEDRLASRADQLE EFVHYVKGEITKVVGK
adeF	MNISKFFIDRPIFAGVLSVLILLAGLLSVFQLPISEYPEVVPPSVVVRAQYPGANPKVIAETVA SPLEESINGVEDMLYMQSQANSDGNLTITVNFKLGIDPDKAQQLVQNRVSQAMPRLPEDV QRLGVTTLKSSPTLTMVVHLTSPDNRYDMTYLRNYAVLNVKDRLARLQGVGEVGLFGSG DYAMRVWLDPQKVAQRNLTATEIVNAIREQNIQVAAGTIGASPSNSPLQLSVNAQGRLTTE QEFADIILKTAPDGAVTRLGDVARVELAASQYGLRSLLDNKQAVAIPIFQAPGANALQVSDQ VRSTMKELSKDFPSSIKYDIVYDPTQFVRASIKAVVHTLLEAITLVVVVVILFLQTWRASIIPLL AVPVSIIGTFALMLAFGYSINALSLFGMVLAIGIVVDDAIVVVENVERNIEAGLNPREATYRA MREVSGPIIAIALTLVAVFVPLAFMTGLTGQFYKQFAMTIAISTVISAFNSLTLSPALAALLLK GHDAKPDALTRIMNRVFGRFFALFNRVFSRASDRYSQGVSRVISHKASAMGVYAALLGLT VGISYIVPGGFVPAQDKQYLISFAQLPNGASLDRTEAVIRKMSDTALKQPGVESAVAFPGL SINGFTNSSSAGIVFVTLKPFDERKAKDLSANAIAGALNQKYSAIQDAYIAVFPPPPVMGLG TMGGFKLQLEDRGALGYSALNDAAQNFMKAAQSAPELGPMFSSYQINVPQLNVDLDRVK AKQQGVAVTDVFNTMQIYLGSQYVNDFNRFGRVYQVRAQADAPFRANPEDILQLKTRNS AGQMVPLSSLVNVTQTYGPEMVVRYNGYTSADINGGPAPGYSSSQAEAAVERIAAQTLP RGIKFEWTDLTYQKILAGNAGLWVFPISVLLVFLVLAAQYESLTLPLAVILIVPMGILAALTGV WLTAGDNNIFTQIGLMVLVGLACKNAILIVEFARELEMQGATAFKAAVEASRLRLRPILMTSI AFIMGVVPLVTSTGAGSEMRHAMGVAVFFGMIGVTFFGLFLTPAFYVLIRTLNSKHKLHSA AVHEAPLASPHDH
arr-3	MVKDWIPISHDNYKQVQGPFYHGTKANLAIGDLLTTGFISHFEDGRILKHIYFSALMEPAVW GAELAMSLSGLEGRGYIYIVEPTGPFEDDPNLTNKRFPGNPTQSYRTCEPLRIVGVVEDW EGHPVELIRGMLDSLEDLKRRGLHVIED
catA1	MEKKITGYTTVDISQWHRKEHFEAFQSVAQCTYNQTVQLDITAFLKTVKKNKHKFYPAFIHI LARLMNAHPEFRMAMKDGELVIWDSVHPCYTVFHEQTETFSSLWSEYHDDFRQFLHIYSX DVACYGENLAYFPKXFXENMXFVSANPWVSFTSFDLNVANMDNFFAPVFTMGKYYTQGD KVLMPLAIQVHHAVCDGFHVGRMLNELQQYCDEWQGGA
cphA3	MMKGWIKCTLAGAVVLMASFWGGSVRAAGIELKQVSGPVYVVEDNYYVKENSMVYFGAK GVTVVGATWTPDTARELHKLIKRVSSKPVLEVINTNYHTDRAGGNAYWKSIGAKVVATRQ TRDLMKSDWAEIVAFTRKGLPEYPDLPLVLPNVVHDGDFTLQEGKVRAFYAGPAHTPDGI FVYFPDEQVLYGNCILKEKLGNLSFANVKAYPQTIERLKAMKLPIKTVIGGHDSPLHGPELID HYEELIKAVPQS
cphA5	MMKGWIKCGLAGAVVLVASFWGGSVHAAAISLTQVSGPVYVVEDNYYVKENSMVYFGAK GVTIVGATWTPDTARELHKLIKRVNNKPVLEVINTNYHTGQAGGNAYWKSIGAKVVSTRQT RDLMKSDWAEIVAFTRKGLPEYPDLPLVLPNVVHDGDFNLQEGKVRAFYAGPAHTPDGIF VYFPDQQVLYGNCILKEKLGNLSFADVKAYPQTLERLKAMKLPIKIVVGGHDSPLHGPELID HYQALIKAATHS
dfrA12	MNSESVRIYLVAAMGANRVIGNGPNIPWKIPGEQKIFRRLTEGKVVVMGRKTFESIGKPLP NRHTLVISRQANYRATGCVVVSTLSHAIALASELGNELYVAGGAEIYTLALPHAHGVFLSEV HQTFEGDAFFPMLNETEFELVSTETIQAVIPYTHSVYARRNG

Best Hit ARO	CARD Protein Sequence
Escherichia coli EF-Tu mutants	MLSPEGESTIVRNIAVSKEKFERTKPHVNVGTIGHVDHGKTTLTAAITTVLAKTYGGAARAFD QIDNAPEEKARGITINTSHVEYDTPTRHYAHVDCPGHADYVKNMITGAAQMDGAILVVAATD GPMPQTREHILLGRQVGVPYIIVFLNKCDMVDDEELLELVEMEVRELLSQYDFPGDDTPIVR GSALKALEGDAEWEAKILELAGFLDSYIPEPERAIDKPFLLPIEDVFSISGRGTVVTGRVERGI IKVGEEVEIVGIKETQKSTCTGVEMFRKLLDEGRAGENVGVLLRGIKREEIERGQVLAKPGTI KPHTKFESEVYILSKDEGGRHTPFFKGYRPQFYFRTTDVTGTIELPEGVEMVMPGDNIKMV VTLIHPIAMDDGLRFAIREGGRTVGAGVVAKVLG
MCR-3	MPSLIKIKIVPLMFFLALYFAFMLNWRGVLHFYEILYKLEDFKFGFAISLPILLVAALNFVFVPF SIRYLIKPFFALLIALSAIVSYTMMKYRVLFDQNMIQNIFETNQNEALAYLSLPIIVWVTIAGFIP AILLFFVEIEYEEKWFKGILTRALSMFASLIVIAVIAALYYQDYVSVGRNNSNLQREIVPANFVN STVKYVYNRYLAEPIPFTTLGDDAKRDTNQSKPTLMFLVVGETARGKNFSMNGYEKDTNPF TSKSGGVISFNDVRSCGTATAVSVPCMFSNMGRKEFDDNRARNSEGLLDVLQKTGISIFWK ENDGGCKGVCDRVPNIEIEPKDHPKFCDKNTCYDEVVLQDLDSEIAQMKGDKLVGFHLIGS HGPTYYKRYPDAHRQFTPDCPRSDIENCTDEELTNTYDNTIRYTDFVIGEMIAKLKTYEDKY NTALLYVSDHGESLGALGLYLHGTPYQFAPDDQTRVPMQVWMSPGFTKEKGVDMACLQQ KAADTRYSHDNIFSSVLGIWDVKTSVYEKGLDIFSQCRNVQ
mphA	MTVVTTADTSQLYALAARHGLKLHGPLTVNELGLDYRIVIATVDDGRRWVLRIPRRAEVSAK VEPEARVLAMLKNRLPFAVPDWRVANAELVAYPMLEDSTAMVIQPGSSTPDWVVPQDSEV FAESFATALAALHAVPISAAVDAGMLIRTPTQARQKVADDVDRVRREFVVNDKRLHRWQR WLDDDSSWPDFSVVVHGDLYVGHVLIDNTERVSGMIDWSEARVDDPAIDMAAHLMVFGEE GLAKLLLTYEAAGGRVWPRLAHHIAERLAFGAVTYALFALDSGNEEYLAAAKAQLAAAEAA E
Mrx	MSERRYSPLATLFAATFLFRIGNAVAALALPWFVLSHTKSAAWAGATAASSVIATIIGAWVG GGLVDRFGRAPVALISGVVGGVAMASIPLLDAVGALSNTGLIACVVLGAAFDAPGMAAQDS ELPKLGHVAGLSVERVSSLKAVIGNVAILGGPALGGAAIGLLGAAPTLGLTAFCSVLAGLLGA WVLPARAARTMTTTATLSMRAGVAFLWSEPLLRPLFGIVMIFVGIVGANGSVIMPALFVDAG RQVAELGLFSSMMGAGGLLGIAIHASVGARISAQNWLAVAFCGSAVGSLLLSQLPGVPVLM LLGALVGLLTGSVSPILNAAIYNRTPPELLGRVLGTVSAVMLSASPMVMLAAGAFVDLAGPL PGLVVSAVFAGLVALLSLRLQFATMAAAATASAPTHTEGEH
OXA-12	MSRLLLSGLLATGLLCAVPASAASGCFLYADGNGQTLSSEGDCSSQLPPASTFKIPLALMG YDSGFLVNEEHPALPYKPSYDGWLPAWRETTTPRRWETYSVVWFSQQITEWLGMERFQQ YVDRFDYGNRDLSGNPGKHDGLTQAWLSSSLAISPEEQARFLGKMVSGKLPVSAQTLQYT ANILKVSEVEGWQIHGKTGMGYPKKLDGSLNRDQQIGWFVGWASKPGKQLIFVHTVVQKP GKQFASIKAKEEVLAALPAQLKKL
QnrS2	METYRHTYRHHSFSHQDLSDITFTACTFIRCDFRRANLRDATFINCKFIEQGDIEGCHFDVA DLRDASFQQCQLAMANFSNANCYGIELRECDLKGANFSRANFANQVSNRMYFCSAFITGC NLSYANMERVCLEKCELFENRWIGTHLAGASLKESDLSRGVFSEDVWGQFSLQGANLCHA ELDGLDPRKVDTSGIKIASWQQEQLLEALGIVVFPD
sul1	MVTVFGILNLTEDSFFDESRRLDPAGAVTAAIEMLRVGSDVVDVGPAASHPDARPVSPADEI RRIAPLLDALSDQMHRVSIDSFQPETQRYALKRGVGYLNDIQGFPDPALYPDIAEADCRLVV MHSAQRDGIATRTGHLRPEDALDEIVRFFEARVSALRRSGVAADRLILDPGMGFFLSPAPE TSLHVLSNLQKLKSALGLPLLVSVSRKSFLGATVGLPVKDLGPASLAAELHAIGNGADYVRT HAPGDLRSAITFSETLAKFRSRDARDRGLDHA
TEM-1	MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEER FPMMSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSA AITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPNDERDTTMPAAM ATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIAA LGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW
TEM-81	MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEER FPMLSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAA VTMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPNDERDTTMPAAMA TTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIAAL GPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW

Best Hit ARO	CARD Protein Sequence
tet(C)	MKSNNALIVILGTVTLDAVGIGLVMPVLPGLLRDIVHSDSIASHYGVLLALYALMQFLCAPVLG ALSDRFGRRPVLLASLLGATIDYAIMATTPVLWILYAGRIVAGITGATGAVAGAYIADITDGED RARHFGLMSACFGVGMVAGPVAGGLLGAISLHAPFLAAAVLNGLNLLLGCFLMQESHKGE RRPMPLRAFNPVSSFRWARGMTIVAALMTVFFIMQLVGQVPAALWVIFGEDRFRWSATMI GLSLAVFGILHALAQAFVTGPATKRFGEKQAIIAGMAADALGYVLLAFATRGWMAFPIMILLA SGGIGMPALQAMLSRQVDDDHQGQLQGSLAALTSLTSIIGPLIVTAIYAASASTWNGLAWIV GAALYLVCLPALRRGAWSRATST
tet(D)	MNKPAVIALVITLLDAMGIGLIMPVLPSLLREYLPEADVANHYGILLALYAVMQVCFAPLLGR WSDKLGRRPVLLLSLAGAAFDYTLLALSNVLWMLYLGRIISGITGATGAVAASVVADSTAVS ERTAWFGRLGAAFGAGLIAGPAIGGLAGDISPHLPFVIAAILNACTFLMVFFIFKPAVQTEEK PAEQKQESAGISFITLLKPLALLLFVFFTAQLIGQIPATVWVLFTESRFAWDSAAVGFSLAGL GAMHALFQAVVAGALAKRLSEKTIIFAGFIADATAFLLMSAITSGWMVYPVLILLAGGGIALP ALQGIISAGASAANQGKLQGVLVSLTNLTGVAGPLLFAFIFSQTQQSADGTVWLIGTALYGL LLAICLLIRKPAPVAATC
tet(E)	MNRTVMMALVIIFLDAMGIGIIMPVLPALLREFVGKANVAENYGVLLALYAMMQVIFAPLLGR WSDRIGRRPVLLLSLLGATLDYALMATASVVWVLYLGRLIAGITGATGAVAASTIADVTPEE SRTHWFGMMGACFGGGMIAGPVIGGFAGQLSVQAPFMFAAAINGLAFLVSLFILHETHNA NQVSDELKNETINETTSSIREMISPLSGLLVVFFIIQLIGQIPATLWVLFGEERFAWDGVMVG VSLAVFGLTHALFQGLAAGFIAKHLGERKAIAVGILADGCGLFLLAVITQSWMVWPVLLLLA CGGITLPALQGIISVRVGQVAQGQLQGVLTSLTHLTAVIGPLVFAFLYSATRETWNGWVWII GCGLYVVALIILRFFHPGRVIHPINKSDVQQRI
TRU-1	MKQRIALSLLALGPLLLVPRVYAAADEPMANIVEKAVQPLLEEYRIPGMAVAVLKEGKPHYF NYGVANRESGRRISERTLFEIGSVSKTFTATLGTYAVVKGGFRLDDKVSQHAPWLQNSAF DRVTMAQLATYSAGGLPLQFPDAVDSNERMRQYYRQWSPLYAAGTHREYSNPSIGLFGH LAASTLGQPFRQLMSQTLLPKLDLQHTYLEVPDAAMVDYAYGYSKEDKPVRVNPGVLADE AYGIKTSAADLIKFVGANMTGSGDKAVQQALAMTRTGFYSVGEMTQGLGWESYAYPVTE QALLAGNSPAVSFKANPVKPFVAPRVMGNERLYNKTGSTNGFGAYVVFVPARGVGIVMLA NRNYPIEARVKAAYAIMRHLAP
Best Hit ARO	Resfinder Protein sequence
aadA2	MTIEISNQLSEVLSVIERHLESTLLAVHLYGSAVDGGLKPYSDIDLLVTVAVKLDETTRRALL NDLMEASAFPGESETLRAIEVTLVVHDDIIPWRYPAKRELQFGEWQRNDILAGIFEPAMIDID LAILLTKAREHSVALVGPAAEEFFDPVPEQDLFEALRETLKLWNSQPDWAGDERNVVLTLS RIWYSAITGKIAPKDVAADWAIKRLPAQYQPVLLEAKQAYLGQKEDHLASRADHLEEFIRFV KGEIIKSVGK
ampS	MSRLLLSSLLATGLLAALPASAASGCFLYADGNGQTLSSEGDCSSQLPPASTFKIPLALMG YDSGFLVDEEHPALPFKPGYDDWLPAWRETTTPRRWETYSVVWFSQQITEWLGMERFQ QYVDRFDYGNRDLSGNPGKHDGLTQAWLSSSLAISPEEQARFLGKMVSGKLPVSAQTLQ YTANILKVSEIDGWQIHGKTGMGYPKKLDGSLNRDQQIGWFVGWASKPGKQLIFVHTVVQ KPGKQFASLKAKEEVLAALPAKLKTL
$bla_{\text{CEPH-A3}}$	MMKGWIKCTLAGAVVLMASFWGGSVRAAGIELKQVSGPVYVVEDNYYVKENSMVYFGAK GVTVVGATWTPDTARELHKLIKRVSSKPVLEVINTNYHTDRAGGNAYWKSIGAKVVATRQT RDLMKSDWAEIVAFTRKGLPEYPDLPLVLPNVVHDGDFTLQEGKVRAFYAGPAHTPDGIFV YFPDEQVLYGNCILKEKLGNLSFANVKAYPQTIERLKAMKLPIKTVIGGHDSPLHGPELIDHY EELIKAVPQS]
$bla_{\text{TEM-116}}$	MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEER FPMMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSA AITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPNDERDTTMPVAM ATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIAA LGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW
tet(A)	VKPNRPLIVILSTVALDAVGIGLIMPVLPGLLRDLVHSNDVTAHYGILLALYALMQFACAPVL GALSDRFGRRPVLLVSLAGAAVDYAIMATAPFLWVLYIGRIVAGITGATGAVAGAYIADITDG DERARHFGFMSACFGFGMVAGPVLGGLMGGFSPHAPFFAAAALNGLNFLTGCFLLPESH KGERRPLRREALNPLASFRWARGMTVVAALMAVFFIMQLVGQVPAALWVIFGEDRFHWD ATTIGISLAAFGILHSLAQAMITGPVAARLGERRALMLGMIADGTGYILLAFATRGWMAFPIM VLLASGGIGMPALQAMLSRQVDEERQGQLQGSLAALTSLTSIVGPLLFTAIYAASITTWNG WAWIAGAALYLLCLPALRRGLWSGAGQRADR

Table 15: The most hit of ARGs resulted from Blast2GO

Isolate	Sequence name	Description	Length	Hits	E-Value	Sim mean
NK01	scaffold_16	*ampS*	128467	3	5.54E-149	83.57%
	scaffold_7	*bla*$_{CEPH-A3}$	178684	3	9.37E-168	79.62%
	scaffold_8	*tet(E)*	171547	8	0.00E+00	74.59%
	scaffold_14	*adeF*	138654	1	0	65.64%
	scaffold_9	*QnrS2*	162740	1	1.05E-48	61.46%
	scaffold_5	*dfrA12*	209230	1	5.07E-35	59.51%
	scaffold_3	*sul1*	238123	1	1.22E-28	51.20%
	scaffold_30	*tet(A)*	26176	8	4.87E-10	47.54%
	scaffold_16	*OXA-12*	128467	2	1.76E-146	7618.00%
	scaffold_30	*tet(C)*	26176	2	1.10E-07	4748.00%
NK02	NODE_77_length_6196	*QnrS2*	6196	2	2.40E-150	100
	NODE_9_length_142416	*bla*$_{CEPH-A3}$	142416	2	4.00E-171	97.64
	NODE_82_length_4687	*AAC(3)-IIb*	4687	1	3.86E-132	87.64
	NODE_10_length_133171	*OXA-12*	133171	3	3.58E-152	83.96
	NODE_18_length_91155	*adeF*	91155	1	0	65.64
	NODE_5_length_162208	*dfrA12*	162208	1	7.03E-34	60.12
	NODE_21_length_72884	*sul1*	72884	1	1.45E-29	51.6
	NODE_17_length_105323	*tet(A)*	105323	7	2.29E-09	47.46
	NODE_77_length_6196	*AAC(6')-Ib-cr*	6196	1	1.46E-135	100
	NODE_17_length_105323	*tet(C)*	105323	1	5.61E-07	46.27
NK07	Scaffold_40_pilon/0001	*tet(A)*	13006	8	0	94.47
	Scaffold_2_pilon/0009	*sul1*	140304	7	0	92.09
	Scaffold_4_pilon/0007	*bla*$_{CEPH-A3}$	438197	4	2.47E-169	75.19
	Scaffold_1_pilon/0006	*adeF*	214996	1	0	65.64
	Scaffold_5_pilon/0005	*QnrS2*	148742	1	9.05E-51	62.93
	Scaffold_1_pilon/0007	*tet(A)*	933637	7	1.94E-08	47.46
	Scaffold_4_pilon/0007	*CEPH-A3*	438197	4	4.92E-170	75.19
	Scaffold_40_pilon/0001	*tet(C)*	13006	3	0	74.98

Isolate	Sequence name	Description	Length	Hits	E-Value	Sim mean
	Scaffold_1734_pilon/0001	*QnrS2*	9247	1	1.13E-149	100
	Scaffold_2055_pilon/0001	$bla_{TEM-116}$	1779	2	0	99.82
	Scaffold_1478_pilon/0001	*catA1*	2156	2	2.39E-102	97.92
	Scaffold_2794_pilon/0001	*adeF*	246	1	1.50E-05	94.12
	Scaffold_13_pilon/0001	*ampS*	128277	3	5.53E-149	83.57
UDRT09	Scaffold_4653_pilon/0001	*tet(C)*	257	8	1.32E-47	79.63
	Scaffold_16_pilon/0006	$bla_{CEPH-A3}$	147800	3	7.75E-168	79.62
	Scaffold_15_pilon/0003	*tet(E)*	169007	8	0	74.59
	Scaffold_12_pilon/0003	*dfrA12*	162515	1	3.94E-35	59.51
	Scaffold_11_pilon/0001	*sul1*	169295	1	8.67E-29	51.2
	Scaffold_15_pilon/0003	*tet(E)*	169007	3	0	80.38
	Scaffold_13_pilon/0001	*OXA-12*	128277	2	1.76E-146	76.18
	Scaffold_4653_pilon/0001	*tet(C)*	257	3	8.97E-48	75.09
	NODE_15_length_77837	$bla_{CEPH-A3}$	77837	2	1.16E-171	97.44
	NODE_14_length_134531	*ampS*	134531	3	5.43E-150	83.85
	NODE_5_length_265081	*adeF*	265081	1	0	65.64
	NODE_11_length_168120	*QnrS2*	168120	1	1.08E-49	62.93
	NODE_7_length_226766	*dfrA12*	226766	1	5.88E-34	58.9
CNRT12	NODE_16_length_69939	*tet(E)*	69939	1	1.25E-10	49.76
	NODE_4_length_273348	*tet(A)*	273348	7	3.91E-09	47.49
	scaffold_8	*tet(E)*	171547	3	0	80.38
	scaffold_16	*OXA-12*	128467	2	1.76E-146	76.18
	scaffold_3	*sul1*	238123	1	8.29E-29	51.2
	scaffold_30	*tet(C)*	26176	2	1.10E-07	47.48
	BDGY01000067.1	*MCR-3*	94290	1	7.54E-55	98.91
	BDGY01000002.1	*sul1*	14082	5	0	98.56
	BDGY01000008.1	*tet(A)*	3809	9	0	91.33
	BDGY01000071.1	$bla_{CEPH-A3}$	261662	4	1.29E-170	84.79
Ae52	BDGY01000051.1	*OXA-12*	141592	3	1.34E-154	83.87
	BDGY01000045.1	*tet(E)*	158595	9	0	74.45
	BDGY01000010.1	*catA1*	1484	1	6.15E-71	68.75
	BDGY01000070.1	*QnrS2*	131681	1	3.29E-49	61.95
	BDGY01000039.1	*dfrA12*	167365	1	2.29E-34	59.88

Isolate	Sequence name	Description	Length	Hits	E-Value	Sim mean
ML09123	PPUW01000016.1	*OXA-12*	121614	3	3.08E-142	84.25
	PPUW01000005.1	*bla*$_{CEPH-A3}$	331025	4	1.80E-113	66.82
	PPUW01000004.1	*adeF*	401428	1	0	6.56E+01
	PPUW01000012.1	*QnrS2*	156161	1	3.65E-49	61.95
	PPUW01000006.1	*dfrA12*	327606	1	6.12E-34	5.89E+01
	PPUW01000001.1	*sul1*	464634	1	1.97E-24	52.02
	PPUW01000002.1	*MCR-3*	436648	8	0	5.10E+01
MS1788	RAWX01000003.1	*bla*$_{CEPH-A3}$	1025620	6	9.25E-172	7.43E+01
	RAWX01000001.1	*adeF*	1457362	10	0	73.34
	RAWX01000008.1	*QnrS2*	173782	1	5.73E-50	62.93
	RAWX01000004.1	*OXA-12*	371935	10	5.37E-147	5.95E+01
XHVA1	PZKL01000015.1	*bla*$_{CEPH-A3}$	319273	4	5.86E-170	85.09
	PZKL01000028.1	*OXA-12*	132182	3	1.08E-143	84.25
	PZKL01000010.1	*adeF*	216427	2	0	70.44
	PZKL01000024.1	*QnrS2*	156631	1	2.01E-49	62.44
	PZKL01000032.1	*dfrA12*	397861	1	1.37E-34	59.51
	PZKL01000012.1	*sul1*	549835	1	2.27E-25	52.53
	PZKL01000043.1	*tet(A)*	131832	7	1.22E-08	46.65
XHVA2	QQOQ01000004.1	*CEPH-A3*	319373	4	4.03E-170	85.09
	QQOQ01000015.1	*OXA-12*	132182	3	1.08E-143	84.25
	QQOQ01000009.1	*adeF*	216427	2	0	70.44
	QQOQ01000014.1	*QnrS2*	156631	1	2.01E-49	62.44
	QQOQ01000003.1	*dfrA12*	397628	1	1.37E-34	5.95E+01
	QQOQ01000001.1	*sul1*	549836	1	2.27E-25	52.53
	QQOQ01000016.1	*tet(A)*	132138	7	1.23E-08	46.65
NS	NMUR01000037.1	*OXA-12*	50760	2	1.28E-142	99.56
	NMUR01000047.1	*CEPH-A3*	39898	3	4.19E-172	98.29

Isolate	Sequence name	Description	Length	Hits	E-Value	Sim mean
NS	NMUR01000001.1	*MCR-3*	213985	1	0	75.05
	NMUR01000002.1	*adeF*	199757	1	0	6.57E+01
	NMUR01000005.1	*QnrS2*	160009	1	1.82E-50	62.93
	NMUR01000044.1	*dfrA12*	39171	1	5.41E-29	58.28
	NMUR01000009.1	*sul1*	92735	1	1.97E-24	5.04E+01
	NMUR01000039.1	*tet(A)*	40158	8	8.64E-10	47.49
	NMUR01000008.1	*tet(E)*	101291	1	1.21E-10	4.59E+01
PhIn2	ANNT01001425.1	*OXA-12*	2260	2	5.90E-164	98.97
	ANNT01000406.1	*CEPH-A3*	1750	3	0	98.16
	ANNT01001760.1	*TEM-1*	1777	10	0	89.63
	ANNT01000818.1	*tet(E)*	583	1	4.80E-06	75.86
	ANNT01000454.1	*MCR-3*	5686	1	0	75.05
	ANNT01001406.1	*tet(D)*	635	1	1.92E-05	68
	ANNT01001716.1	*adeF*	1729	1	2.02E-63	67.92
	ANNT01000940.1	*QnrS2*	3149	1	2.79E-57	62.44
	ANNT01000466.1	*dfrA12*	3645	1	4.08E-36	60.9
	ANNT01000272.1	*sul1*	2226	1	1.11E-30	50.8
	ANNT01000485.1	*tet(A)*	1987	9	2.35E-11	48.05
UBA1835	DDJB01000143.1	*OXA-12*	16222	2	1.16E-143	99.56
	DDJB01000183.1	*CEPH-A3*	15754	3	2.28E-173	98.43
	DDJB01000009.1	*TRU-1*	14766	1	0	89.14
	DDJB01000099.1	*MCR-3*	18962	1	0	73.99
	DDJB01000170.1	*adeF*	3723	1	1.27E-97	70.55
	DDJB01000314.1	*QnrS2*	15579	1	7.36E-51	61.46
	DDJB01000192.1	*dfrA12*	37694	1	1.46E-33	59.51
	DDJB01000145.1	*tet(C)*	12678	1	5.60E-04	49.45
VCK	NNSF01000040.1	*OXA-12*	46619	2	1.17E-142	99.56
	NNSF01000074.1	*CEPH-A3*	12716	3	1.53E-171	97.38
	NNSF01000001.1	*adeF*	247593	2	0	69.45
	NNSF01000009.1	*QnrS2*	124838	1	1.42E-50	62.93
	NNSF01000016.1	*dfrA12*	82899	1	4.19E-28	57.67
	NNSF01000035.1	*sul1*	43913	1	1.02E-24	50.4
	NNSF01000002.1	*tet(A)*	211931	7	4.09E-09	4.74E+01
	NNSF01000013.1	*tet(E)*	98640	1	1.18E-10	45.86

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PUBLICATION	
AWARD RECEIVED	-