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COMPUTATIONAL ANALYSIS OF *ANOPHELES GAMBIAE* METABOLISM TO FACILITATE INSECTICIDAL TARGET AND COMPLEX RESISTANCE MECHANISM DISCOVERY

BY

ADEBIYI, MARION OLUBUNMI

(CUGP060188)

DOCTOR OF PHILOSOPHY DEGREE IN COMPUTER SCIENCE

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CERTIFICATION

I hereby certify that this is an original research work carried out by Marion Olubunmi ADEBIYI in the Department of Computer and Information Sciences, School of Natural and Applied Sciences, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria, under my supervision.

1. Name: Professor Ezekiel Adebiyi
   (Supervisor)

   Signature: _____ Date: _____

2. Name: Dr. Jason Rasgon
   (Co-Supervisor)

   Signature: _____ Date: _____

3. Name: Dr. Nicholas Omoregbe
   (Head of Department)

   Signature: _____ Date: _____

4. Name: Professor Emmanuel Onibere
   (External Examiner)

   Signature: _____ Date: _____

DECLARATION

It is hereby declared that this research was undertaken by Marion Olubunmi Adebiyi. The thesis is based on her original study in the Department of Computer and Information Sciences, College of Science and Technology, Covenant University, Ota, under the supervision of Prof. Ezekiel Adebiyi and Dr. Jason Rasgon. Ideas and views of this research work are products of the original research undertaken by Marion Olubunmi Adebiyi and the views of other researchers have been duly expressed and acknowledged.

Prof. Ezekiel Adebiyi

(Supervisor)

Signature: _____ Date: _____

Dr. Jason Rasgon

(Co- Supervisor)

Signature: _____ Date: _____.

DEDICATION

I dedicate this work to **GOD** Almighty, the giver of life, wisdom and inspirations. He is the one that supplied inspiration and strength to achieve this great task.

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ABBREVIATIONS

Abbreviations	Meaning
DDT	Dichloro-diphenyl-trichloroethane
ITN	Insecticide treated nets
IRS	Indoor residual spraying
WHO	World Health Organizations
BHC	Benzenehexachloride
GST	Glutathione-S-transferases
OP	Organophosphate
OC	Organochloride
GABA	Gama-(γ)-aminobutyric Acid
Bti	*Bacillus Thuringiensis* Insecticides
A. gambiae	*Anopheles gambiae*
Kdr	Knock down resistance
P450s	Cytochrome-P450-monooxygenases
HCH	Hexacholine
GM	Genetically modified
Na^+	Sodium ion
KEGG	Kyoto Encyclopedia of Genes and Genomes
Pf	*Plasmodium falciparum*
PDB	Protein Data Bank
GUI	Graphic user interface

MAP	Malaria Atlas Project
KOBAS	KEGG Orthology Based Annotation System
GO	Gene Ontology
PPCpred	Predictor of Protein Production, Purification and Crystallization
ParCrys	Parzen Window to estimate a protein's propensity to produce diffraction-quality crystals
LLITNs	Long-Lasting Insecticide Treated Nets
PGDB	Pathway/Genome Database
DKFZ	German Cancer Research Center
GOPET	Gene ontology prediction and evaluation tool
OB - Score	Overton and Barton Score
GRF	Genome Resource Facility
EC	Enzyme Classification
NGS	Next Generation Sequencing

DEFINITION OF TERMS

Choke Point (CP): These are biochemically essential points in the network of an organism. They are reactions that either uniquely produce a specific product or consume a certain specific substrate in a metabolic network.

Reaction without Deviation (RWD): Are also known as **Essential Reactions** if the producing product downstream of it was indicated to hamper.

Load Point (Load Point): Is the identification of nodes with a high ratio of k-shortest paths to the number of nearest neighbor edges or connectivity providing many alternative metabolic pathways.

Damage: Is the procedure that allows access to the enzymes that may serve as drug targets when their inhibition influences a broader amount of downstream reactions and products.

Gene: Is the basic unit of heredity in a living organism. All living things depend on genes. Genes hold the information to build and maintain an organism's cells and pass genetic traits to offspring.

Enzymes: Substance produced by a living organism which acts as a catalyst to bring about or speed up a specific biochemical reaction.

Protein: Proteins are the main building blocks and functional molecules of the cell

Deoxyribonucleic Acid: (DNA) is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms and some viruses.

Ribonucleic Acid: (RNA) is a biologically important type of molecule that consists of a long chain of nucleotide units. Each nucleotide consists of a nitrogenous base, a ribose sugar, and a phosphate.

Insecticidal Target: An enzymatic reactions is considered a potential insecticidal target if such an enzymatic reaction is a choke point (CP) as well as reaction without deviation (RWD)

Resistance Genes: These are the genes that do the flushing, excretion and or refusal of insecticides; such genes are even altered or change their mode of response at some time in the system of the vector in order to neutralize the effects of these insecticides used against them.

ABSTRACT

Insecticide resistance is a genetic characteristic involving changes in one or more insect genes. It is also a major public health challenge combating world efforts on malaria control and strategies. The Malaria vector, *Anopheles gambiae* (*A. gambiae*) has formed resistance to the existing classes of insecticides, especially pyrethroid, the only class approved for Indoor Residual Spray (IRS) and Long-Lasting Insecticide Treated Net (LLITNs). Identification of novel insecticidal targets for the development of more effective insecticides is therefore urgent. However, deciding which gene products are ideal insecticidal targets remains a difficult task in the search. To this end, it has been shown that the dissection and comprehensive studies of biochemical metabolic networks has great potential to effectively and specifically identify and extract essential enzymes as potential insecticidal targets. Using the PathoLogic programme, AnoCyc, a pathway/genome database (PGDB) for *A. gambiae* AgamP3 was constructed, using its annotated genomic sequence and other annotated information from ANOBASE, VECTORBASE, UNIPROT and KEGG databases. Furthermore, additional annotations to proteins annotated as "hypothetical" was gathered using specifically two annotation tools from the DKFZ HUSAR open servers, namely GOPET and DomainSweep and present a more comprehensive annotated PGDB for *A. gambiae* AgamP3. The resulting PGDB for *A. gambiae* AgamP3 has been deployed under the www.bioCyc.org databases. Next, a graph based model that analyzed the topology of the metabolic network of *Anopheles gambiae* was developed to determine the essential enzymatic reactions in the networks. A refined list of 61 new potential insecticidal candidate targets was obtained, which include one clinically validated insecticidal target and host of others with biological evidence in the literature. Finally, the biochemical network of *A. gambiae* was overlaid with two gene expression data obtained from the treatment of *A. gambiae* with pyrethroid (permethrin) to elucidate some tightly linked resistance genes and deduce computationally, for the first time, its resistance mechanism(s) toward this insecticide.