IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 8, Issue 9 Ver. II (Sep. 2015), PP 15-21
www.iosrjournals.org

Nuances of Transcriptomics in Understanding Acaricide Resistance in Ticks

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Abstract: *Ticks are important economic parasites that are involved in the transmission of disease pathogens in cattles around the world. The health and economic burden caused by tick borne infections has warranted better control measures. At present, control of tick and tick borne diseases is mainly achieved by the wide spread use of chemical acaricides like carbamates, pyrethroids, amidines, organophosphates etc. Development of drug resistance in ticks has made the development of these control measures a problematic and challenging task. Understanding the mechanism behind the drug resistance in ticks is an important step towards achieving this goal.Various research groups across the world have reported different genes involved in drug resistance. However the overall pathway involved in the process still remains unclear. Transcriptomics is a new, emerging revolutionary technology that has been increasingly used for solving complex biological problems. Transcriptomics has led to better understanding of the molecular basis of drug resistance in ticks and several genes that are upregulated or downregulated in response to drug exposure has been identified. Hence, transcriptomics can play a major role in understanding drug resistance in ticks and thus in designing novel tick control measures.*

Keywords: *Acaricide resistance, Cytochrome p450, Rhipicephalus (Boophilus) microplus, Ticks, Transciptomics.*

I. Introduction

Ticks are important groups of arthropods and common vectors that are involved in the transfer of disease pathogens in animals. Ticks and tick borne disease (TTBDs) pathogens affect 80 per cent of the world cattle population and are widely distributed throughout the world, particularly in tropical and subtropical countries (De Castro, 1997). They can also act as many viral, bacterial and protozoan disease causing vectors. One of the most common bacterial infections caused by Borrelia type , the Lyme disease is a tick borne disease that has increased steadily over the last two decades increasing the health and economic burden of cattle significantly in the United States of America (Munderloh & Kurtti, 2010).

At present, control of tick and tick borne diseases (TTBDs) is mainly made possible by the extensive use of chemical acaricides like benzoyl phenyl ureas, carbamates, pyrethroids, BHC-cyclodines, amidines, organophosphates, macrocyclic lactones (Ghosh et al., 2007). The incessant and incorrect use of Acaricides have resulted in the development of resistance against Acaricides in these ticks, which is a major setback for the growth of Agriculture sectors and live stock industries . This resistance is an inherited phenomenon (FAO, 2004) and can be described as a reduction in propensity of a parasite to an acaricide when it is used at the recommended concentration. According to a previously published report by FAO (2004), the tick population in India has developed resistance against all commercially available acaricides, which extends to several chemical groups such as pyrethroids, organophosphates, amidines, organochlorines and arsenicals (Angus, 1996; Kemp et al., 1998; Miller et al., 1999). Recent reports on diazinon resistance (Kumar et al., 2011) and synthetic pyrethroids (Sharma et al., 2012) in *R. (B.) microplus* revealed the importance of the problem in Northern India. The tick population which shows the tendancy to develop resistance to acaricide treatment can pass on this trait to the subsequent generations , thereby increasing or amplifying the resistance problem.

II. Types Of Resistance

There are many factors which influence resistance such as genetics, biology/ecology and control operations (Georghiou and Taylor, 1977). Often this resistance is conferred by more than one mechanism and are broadly classified into 3 types (Figure 1) such as reduced sensitivity of the pesticide's target, increase in metabolism of the pesticide and decreased penetration of the pesticide (Scott and Georghiou, 1986).

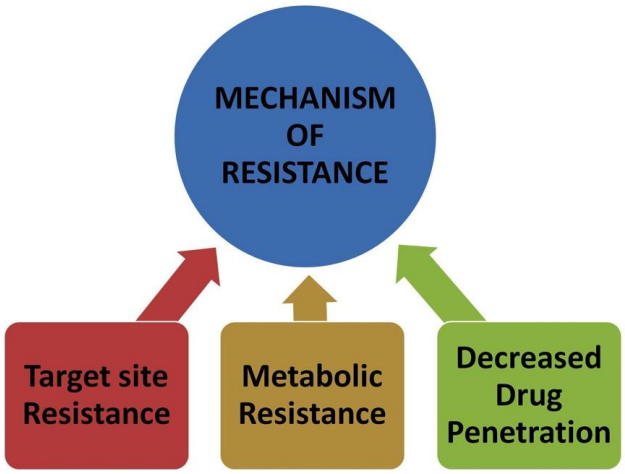

Figure 1 . Mechanism of Resistance

2.1 Target site resistance

Target site resistance mechanism is common and it occurs when an allele of the gene coding for the target molecule striked by the acaricide has an amino acid mutation that confers resistance to the acaricide. This is well-studied in the case of pyrethroid class of acaricides (Guerrero et al., 2012).

2.2 Metabolic resistance

Changes in the ability to sequester or detoxify an acaricide by an individual causes Metabolic resistance. The cytochrome P450s, esterases, and glutathione S-transferases are the enzyme families generally involved in causing metabolic resistance and this type of resistance phenomenon has been studied in *R. (B.) microplus* (Chen A C et al., 2009). Often chemicals known as synergists are used to help discern resistance mechanisms in cattle ticks through bioassays. They are especially helpful in detecting metabolic resistance. Some of the common synergists are triphenyl phosphate (TPP), diethylmaleate (DEM) and piperonyl butoxide (PBO), which are generally assumed to be specific for the carboxylesterases, glutathione S-transferases and Cytochrome P450s, respectively.

2.3 Decreased Drug Penetration

Decreased drug penetration in ticks could arise as a result of the alterations in the ability of an acaricide to penetrate into an individual treated with acaricide. Although this type of resistance mechanism has been detected in *R. (B.) microplus* (Schnitzerling et al., 1983) and in a few arthropods (Noppun et al., 1989), findings related into this mechanism in the cattle tick have not been reported recently.

III. Acaricide Resistance in ticks

Acaricidal resistance in ticks was first reported from Australia in *Rhipicephalus (Boophilus) microplus* against Arsenic (Newton, 1967). Indiscriminate and continuous use of Acaricides could be the major reason behind the development of resistance in Ticks (Mondal et al., 2013). With time, the spectrum of chemicals to which ticks developed resistance has broadened.

Many surveys were conducted by FAO to discover the resistance status of ticks in Indian population. In 2004, FAO conducted a survey based on questionnaire's and concluded that ticks in india have developed resistance against all available acaricides. Based on this, many studies were conducted to detect the resistance status of ticks in india against commonly available acaricides (FAO, 2004). In 2011, Kumar et al reported the presence of large scale diazinon resistance in *Rhipicephalus (Boophilus) microplus* ticks collected from different agro-climatic regions in northern India (Kumar et al., 2011). Similar work was reported by Sharma et al in the year of 2012 where different levels of resistance towards synthetic pyrethroids like deltamethrin and cypermethrin was observed in *Rhipicephalus (Boophilus) microplus* collected from six different agro-climatic regions of Northern India (Sharma et al., 2012). The Deltamethrin resistance status of Ixodid ticks like *Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum* was also carried out in the year 2012 (Vatsya & Yadav 2011; Abdullah et al., 2012, Shyma et al., 2012). Commonly used synthetic pyrethroids (Deltamethrin and Flumethrin) resistance studies were reported in *R.(B) sanguineus* collected from dogs (Mathivathani et al., 2011) using in vitro evaluation techniques. Three in vitro methods; tea bag method, filter paper impregnation method and immersion methods were used to document deltamethrin and cypermethrin resistance populations in Banglore, India (Pradeep et al., 2012).

IV. Understanding Acaricide Resistance : Journey so far

The initial development of these resistance in ticks results from exposure to chemicals, survival and reproduction of ticks that are less affected by the acaricide. In most cases, it is likely that the gene that is responsible for resistance is already present in their population at very low levels before introducing a new acaricide. The rate at which a resistant allele becomes established in a tick population depends upon many factors. These include the frequency of the unusual mutation in the population before treatment, the form of inheritance of the resistant allele (dominant, co-dominant or recessive), the concentration gradient of the acaricide, the frequency of acaricide treatment and the proportion of the total tick population not exposed to the acaricide.

Many reports state that increased Metabolic detoxification and target site modification are the two major mechanisms commonly seen in Arthropods. Increased level of metabolic activity (P450 mediated monooxygenase , glutathione-S-transferses) and reduced sensitivity of sodium ion channels along nerve axons was attributed in pyrethroid resistance insects(Feyereisen, 1999). In 1999, Vulule et al., documented an elevated level of oxidase and esterase in permethrin resistant mosquitoes (Vulule et al.,1999). In addition, there are quite a lot of reports which documents increase in Cytochrome P450 mediated metabolism that contributes to resistance against synthetic pyrethroids (SPs) in the house fly, *Musca domestica* (Wheelock &Scott, 1992; Scott 1996), the cotton bollworm, *Helicoverpa armigera* (Xiao & Hobbs, 1995) and the cockroach, *Blattella germanica* (Scharf et al., 1996; Valles & Yu, 1996).

In the last decade various research groups have identified several target genes that involved in acaricide resistance in *Rhipicephalus (Boophilus) microplus*. According to the global status supported by various research studies, acaricide resistance has been found to be highest in one host hard tick, *Rhipicephalus (Boophilus) microplus* . Despite various research studies available, the exact molecular pathway behind these resistance mechanism still remains elusive. For example a coumaphos resistant Mexican San Roman strain when exposed to coumphas showed an increased expression of Cytochrome P450 which takes part in the detoxification of chemicals (Guerrero et al., 2007). Apart from this protein, other proteins such as Glutathione-S-Transferase, carboxyl esterase (*Est10*) etc have also been identified, which play a role in conferring resistance to ticks. The biochemical nature of acaricide resistant genes involving esterase, glutathione-S-transferase and altered AChE in *R. (B) microplus* was well documented (Baxter et al., 1999; Jamroz et al., 2000; Villarino et.al., 2003). In 2000 Jamroz et al. carried out an esterase mediated hydrolysis of permethrin which elevates *CzEst9* activity in *R. (B) microplus* resistance populations (Jamroz R C et al., 2000). In pyrethroid resistant mexican populations, high levels of mutated sodium channel alleles and enhanced esterase activity was reported (Guerrero et al., 2002). Sodium ion channel mutations leading to these resistant phenotypes in *R.microplus* populations was later reported by Chen et al., 2009. Gene expression and structure of a pyrethroid-metabolizing esterase, *CzEst9*, from a pyrethroid resistant Mexican population of *R. (B.) microplus* (Acari: Ixodidae) has been reported previously (Guerrero & Nene, 2008). Resistance of ticks to other chemicals such as organophosphate (OP) and synthetic pyrethroids (SP) acaricides are widespread and amitraz resistance has been reported but is less common. Resistance to macro cyclic lactones by *Boophilus microplus* has also been reported recently (Martins & Furlong, 2001) . Table 1 shows the list of identified gene targets involved in resistance against different chemicals.

Table 1. List of Gene Targets Identified in various species involved in Resistance to Acaricides

SL NO	GENE TARGET	DRUG	SPECIES	REFERENCES
1	Oxidases and esterases	Pyrethroids	*Aedes ageypti*	Vulule et al.,1999
2	Cytochrome p450	Synthetic pyrethroids	*Musca domestica*	Wheelock & Scott, 1992
3	Cytochrome p450	Synthetic pyrethroids	*Helicoverpa armigera*	Xiao & Hobbs,1995
4	Cytochrome p450	Synthetic pyrethroids	*Blattella germanica*	Scharf et al., 1996
5	Esterase (*CzEst9*)	Pyrethroid	*R. (B.) microplus*	Guerrero & Nene , 2008
6	Esterase(*CzEst9*)	Permethrin	*R. (B.) microplus*	Jamroz et al., 2000
7	Cytochrome P450 (*CYP6G1*)	Organochloride	*Drosophila melanogaster*	Nicole Jouben et al., 2010
8	Cytochrome P450 (*CYP6BQ9*)	Pyrethroid	*Tribolium castaneum*	Zhu et al., 2010
9	Cytochrome P450 (*CYP9Q*)	Pyrethroid	*Apis mellifera*	Wenfu Mao et al., 2011
10	Cytochrome P450 (*CYP6P3*&*CYP6M2*)	Pyrethroid	*Anopheles gambiae*	Djouaka, et al., 2008
11	Cytochrome P450 (*CYP6BG1*)	Pyrethroid	*Plutella xylostella*	Bautista et al., 2007
12	Cytochrome P450 (*CYP6D1*)	Pyrethroid	*Musca domestica*	Zhang & Scott, 1996

The reason for the increased expressed genes, whether due to alteration in the promoter region, mutation in the repressor of this gene or any change in the upstream network is unknown. Additionally there are various chemicals such as Amitraz, Fipronil, Lindane, Malathion etc for which although the targets are known, no detailed studies on resistance mechanism have been done so far.

V. Transcriptomics: A revolutionary Technology

The organism-specific whole genome sequence data availability has revolutionized the study of biological systems, leading to innovative insights and opportunities for solving complex biological problems that affect human health, agriculture and the environment. Due to the high cost of whole genome sequencing projects, alternative techniques such as single pass sequencing of cDNA ends, expressed sequence tag (EST) projects, etc are likely to remain as the major resource of organism-specific DNA sequence data for various eukaryotic species. Such gene discovery projects are likely to benefit the tick and research communities deals with tick-borne diseases as they form the framework for enhancing our understanding of tick biology and molecular mechanisms, which contribute to pathogen transmission, acaricide resistance and parasitism. These insights may be translated into novel and innovative tick and tick-borne disease control options through the identification of biological targets and thus developing novel chemotherapeutic molecules and vaccines for effective tick control (Hill & Gutierrez, 2000). Transcriptome Profiling study is one of the most advanced approach for the finding of new Genes and Gene functions related to any molecular pathway. This is because of its ability to make Genome wide data available within short period of time and at reasonable cost (Lister et al., 2009). When compared with Microarray Technology, Transcriptomic Profiling comes in the category of Open Technology that does not need any sequence or biological information of the organism to be analyzed (Green et al., 2001). The various applications of transcriptome profiling has been illustrated in Figure 2.

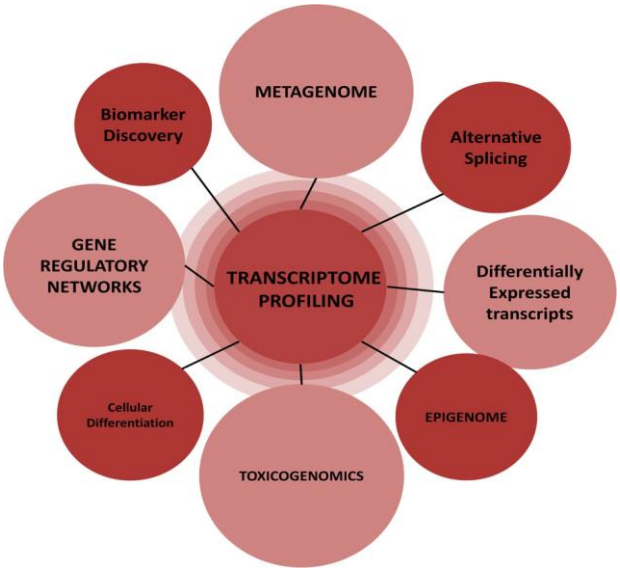

Figure 2. Applications of Transcriptome Profiling

The molecular source of resistance to many insecticides, the associated genes or mutations have been identified in a wide range of insects. The availability of whole genome sequences of the insects has made it possible to better understand the functioning of complex multi-gene systems in metabolic resistance.

VI. Transcriptomics in understanding of resistance mechanisms

Resistance research within the Acaricide has been seriously impeded by the lack of (genome) sequence data. For example, the molecular target sites of a number of chemically diverse acaricidal compounds has neither been elucidated nor characterized at the molecular level. In addition, there is currently very limited molecular information on detoxification genes allied with metabolic resistance, although their importance has been repeatedly highlighted by numerous biochemical studies in arthropods mainly in ticks and mites. The increasing availability of whole genome sequences and EST databases can strongly stimulate mite and tick resistance research and will play an important role in developing novel control tools. Together, this will give a new impetus to toxicology and resistance related researches in the field of acarology, allowing the dissection of environmental adaptation and microevolution as fundamental processes in resistance development.

In 2010, Rodriguez et al reported a comparative microarray analysis and for the first time described the influence of host specificity and host breed on the expression profiles in *Rhipicephalus (Boophilus) microplus*

(Rodriguez et al., 2010). They identified 297 ESTs that are highly expressed in the early stages of *R.microplus* feeding on *B.Indicus* compared to feeding on *B.taurus*.

Transcriptome profiling has been very well used to understand the tick - pathogen interaction while feeding on infected hosts. In a study published in 2013, Heekin et al compared the expression profile in the ovary of *R.microplus* fed on *B.Bovis* infected host with the ovary of *R.microplus* which fed on the unaffected host (Heekin et al., 2013). Several techniques including sequencing of tick ovarian mRNA, microarrays, serial analysis of gene expression (SAGE), and quantitative real-time polymerase chain reaction (qRT-PCR) were used for the study to identify the high priority gene targets affected due to infections. The same research group also reported their whole transciptome sequencing compared the *R.microplus* fed on *B.Bovis* infected and uninfected host using gut samples(Heekin et al., 2013). In the study, they sequenced 4,077 ESTs. The gene expression was measured using microarray designed using the publicly available gene index for *R.microplus* BmiGI Version 2. The study identified 33 ESTs which had high expression level and 43 ESTs which had lower expression levels when fed upon infected host. Figure 3 shows some of the basic steps involved in the transciptome analysis.

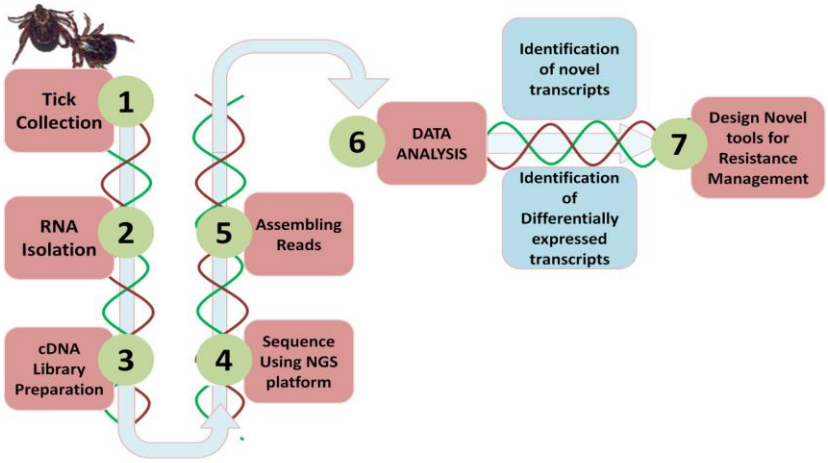

Figure 3. Steps involved in Transcriptome Analysis

Kalajdzic et al 2012, combined genome-wide insertional mutagenesis screen and next generation transcriptomics to identify more genes and transcription factors or microRNAs involved in the regulation of genes involved in resistance (Kalajdzic et al., 2012). They observed an elevated expression of the *Cyp4p2* gene in the resistance strain treated with Imidacloprid and DDT. In another study pubished in 2013, Yang et al reported the gene expression profile of white fly *Bemisia tabaci* which confers thiamethoxam resistance (Yang et al., 2013). Microarray analysis was done to compare the gene expression profile of different life stages of a thiamethoxam-resistant strain with a susceptible strain. Various differentially expressed genes were identified which are involved in the metabolism of xenobiotics, including cytochrome P450 *CYP6CM*. Several ATP-binding cassette transporters which can confer resistance through the active efflux of thiamethoxam were also identified.

Lin et al 2013 did a transcriptomic analysis to better understand the resistance mechanisms of *P. xylostella* to chlorantraniliprole using transcriptome assembly and tag-based digital gene expression (DGE) system. They found a total of 189 up-regulated and 1026 down-regulated genes associated with the resistance mechanism. The genes which are conventionally associated with insecticide resistance such as P450, GST, the ryanodine receptor, and connectin also showed differential expression in response to chlorantraniliprole. These finding were further validated by QT-PCR (Lin et al., 2013).

VII. Conclusion

Ticks are one of the important arthropods that act as vectors for disease pathogens that affect cattle. Currently, different chemicals such as organophosphates, carbamates, pyrethroids, BHC-cyclodines, amidines, macrocyclic lactones, benzoyl phenyl ureas etc has been commonly used for tick control purposes. The increasing cases of ticks developing resistance against these commonly used chemicals has increased the health and economic burden significantly. Several studies have been carried out in tick resistance by different research groups around the world. The studies have resulted in the identification of many genes involved in resistance. Despite of all the available literatures, the exact molecular pathway behind these resistance mechanism mostly remains elusive.

The modern techniques like High throughput deep sequencing and a combination of Next generation sequencing (NGS) technology and other computational techniques have a great impact in the identification and understanding

of fundamental biological mechanisms and cellular pathways. Evolutionary and functional studies of genes may provide insights into various resistance mechanisms in Tick species. This can aid in designing more efficient chemicals or tools for tick control and management. Transcriptomics at a genome scale research has provided relevant insights in to unknown genes and the mechanisms involved in acaricide or insecticide resistance.

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