

RESEARCH ARTICLE

Molecular detection of endosymbiont bacteria *Wolbachia* in bedbug species *Cimex lectularius* from Vidarbha region of India

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Manuscript details:	ABSTRACT
<p>Received: 08.08.2015 Revised : 28.08.2015 Accepted: 02.09.2015 Published : 10.10.2015</p> <p>Editor: Dr. Arvind Chavhan</p> <p>Cite this article as: Siddiqui SS and Raja IA (2015) Molecular detection of endosymbiont bacteria <i>Wolbachia</i> in bedbug species <i>Cimex lectularius</i> from Vidarbha region of India, <i>Int. J. of Life Sciences</i>, 3(3): 200-204.</p> <p>Acknowledgements: The authors thank to Dr. S.G. Bhadange the Principal of Shri Shivaji College of Arts, Commerce and Science Akola for his excellent cooperation.</p> <p>Copyright: © 2015 Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.</p>	<p><i>Wolbachia</i> are maternally inherited bacterial endosymbionts that found in different arthropods. It is now well known that they induce reproductive phenotypic manipulations like feminization, thelytokous parthenogenesis, male killing and cytoplasmic incompatibility in their arthropod hosts. Although diverse insects are infected with <i>Wolbachia</i> bacteria and generally for their insect hosts, it is regarded as facultative/parasitic endosymbiont bacteria. The <i>Wolbachia</i> strain, designated as wCle associated with the bedbug <i>Cimex lectularius</i> was shown to be vital for the growth of host's and reproduction via provisioning of B vitamins. Previously the infection of endosymbiotic <i>Wolbachia</i> bacteria has been shown to the colonies of the human bed bug, <i>Cimex lectularius</i> L. (Heteroptera: Cimicidae) cultured in the laboratory, but no information exists regarding the infection status in natural populations in India. We assayed <i>Cimex lectularius</i> populations from different areas of Vidarbha region (Akola, Amravati, Chandrapur, Nagpur), Maharashtra, India for <i>Wolbachia</i> infection. Detection was done by the polymerase chain reaction (PCR). <i>Wolbachia</i> infections were prevalent in all populations assayed (75–100%). Higher rate of <i>Wolbachia</i> infections were found in bedbugs of Vidarbha region of India, which indicates importance of this association. The characterization of these <i>Wolbachia</i> strains provides a deeper approach into this interaction, which is essential for further studies. The potential utility of <i>Wolbachia</i> for another bed bug control strategies is discussed.</p> <p>Keywords: <i>Wolbachia</i>, <i>Cimex lectularius</i>, PCR</p>

INTRODUCTION

Wolbachia is the maternally inherited and intracellular endosymbiont bacteria found in diverse arthropods and filarial nematodes. This bacteria is known to transfer vertically as well as horizontally across different hosts. The host population can be influenced by *Wolbachia* with different reproductive alterations such as cytoplasmic incompatibility, male killing, parthenogenesis, speciation and feminization (Salunkhe *et al.*, 2014; Werren *et al.*, 2008). Various reports findings said that the infection of *Wolbachia* was observed in 15–25% of insect species (Salunke *et al.*, 2012 and Werren *et al.*, 1995) which was reworked to 40% according to a meta-analysis by Zug and Hammerstein (2012). Members of the genus *Wolbachia* is well known as facultative bacterial endosymbiont bacteria that is associated with different insects, generally conferring negative fitness consequences to their hosts and to enhance their own transmission in a selfish manner it often causes aberrations to the host's reproductive system (Hilgenboecker *et al.* 2008; Werren *et al.*, 2008). However, a *Wolbachia* strain associated with the bedbug *Cimex lectularius*, designated as wCle, was shown to be essential for normal growth and reproduction of the bloodsucking insect host via provisioning of B vitamins (Hosokawa *et al.*, 2010). *Wolbachia* is

estimated to infect up to 70% of all known insect species, making it perhaps the most prevalent symbiotic bacterium on the earth ecosystem (Jeyaprakash and Hoy, 2000).

Hemiptera, an order of class insecta, encompasses bedbug *Cimex lectularius*, insect of the Cimicidae family. Bedbugs are hematophagous arthropods. Medical interest in bedbugs especially *Cimex lectularius* in temperate zones has increased. Adult *C. lectularius* are reddish brown, flat, wingless and ovals (4–7 mm) (Fig.1).

They are hematophagous. They can live for 12 months without feeding and even 1.5–2 years in colder environments. The bedbugs are geographically distributed throughout the world. They may be found in theaters and roots as well as in houses. It affects underprivileged social classes, but also people using collective installations (hotels, trains, dormitories). Cutaneous lesions are the main symptom causes after their bites. In addition, there is no report available on *Wolbachia* infection in *Cimex lectularius* from India so far. Several authors have postulated that these species of *Cimex lectularius* could transmit pathogens to humans (Goddard and deShazo, 2009; Burton, 1963). *Wolbachia*-like inclusions were identified in bed bugs by microscopy almost 90 years ago (Arkwright *et al.*, 1921). More recently, Sakamoto and Rasgon



Fig.1. Photograph of Adult *Cimex lectularius* taken by Carl Zeiss Microscope

investigated the geographic distribution of *Wolbachia* infections in natural *C. lectularius* populations in North America and Africa. *Wolbachia* infections were found at high prevalence (83–100%) in all sampled populations (Sakamoto and Rasgon 2006). Thus goal of this study was to survey the eventual presence of endosymbiont *Wolbachia* bacteria in natural population of bed bugs using PCR. Hence studying *Wolbachia* infection in *Cimex lectularius* may provide deep approach into the evolutionary history of bed bug–*Wolbachia* interaction.

In the present study, we show the presence of *Wolbachia* among a sample of bed bugs collected from Vidarbha region, Maharashtra, India belonging to Cimicidae family. Bed bugs were collected from different areas of Vidarbha region, Maharashtra, (Akola, Amravati, Chandrapur, Nagpur) and 70% ethanol is used for preservation. Taxonomic identification of insects were carried out by using identification keys and then we explored the presence of endosymbiont bacteria *Wolbachia*.

MATERIALS AND METHODS

Collection of Bedbugs

The species of bedbugs *Cimex lectularius* were collected during 2013 to 2014 from their natural habitats from different areas of Vidarbha region, Maharashtra, India (Table 1). Bedbug species were collected from home and theaters. The collected species were transferred to the laboratory carefully in collecting vials, identified and separated with respect to their sex.

Molecular detection of *Wolbachia* infection

The genomic DNA was isolated by the Kit method. The DNA was extracted by the Insect DNA Extraction Kit (Nucleopore Insect DNA Extraction Kit). Molecular diagnosis of *Wolbachia* was done by *Wolbachia*-specific PCR assay using specific primers W-Specf (5'-CATACCTATTCGAAGGGA-

TAG) and W-Specr (5'-AGCTTCGAGTGAAACCAATTC). PCR amplification was carried out with Peltier PCR Processor Model NEO (BioEra) using 50 µl reaction volume consisting of 14 µl of master mix (Thermo scientific) (0.05U/ul Taq DNA polymerase, reaction buffer, 0.4mM of each dNTP (dATP, dCTP, dGTP, dTTP) containing 10X Taq buffer with KCL (100 mM Tris-HCl pH 8.8, 500 mM KCl) 0.2 mM dNTPs, 4mM MgCl₂, 2.5 mM MgCl₂ and 0.5 U Taq DNA polymerase, 100 pm of 3 µl each of forward and reverse primers, 50 ng of 1.5 µl of template DNA, 23 µl of nuclease free water to make up 50 µl. The conditions of PCR reaction consisted of an initial denaturation step at 95°C temperature for 2 min followed by 35 cycles of denaturation at 95°C temperature for 30 sec, annealing at 60°C temperature for 1 min and extension at 72°C temperature for 45 sec and a final extension at 72°C temperature for 5 min. The amplified PCR products were separated through electrophoresis run of 1% agarose gel in 1X TAE (40 mM Tris-HCl, 20 mM acetic acid and 1 mM EDTA)(Puregene genetix brand) buffer for a length of 5–6 cm at a constant 60 V. A standard molecular weight marker DNA ladder (Thermo Scientific GeneRuler 1 kb Plus DNA Ladder) was used in electrophoretic run and the gel documentation system (BioEra's Gel Documentation system, Model Endure) was used to document the gel.

RESULTS AND DISCUSSION

The specific effect of *Wolbachia* on bed bug biology is unknown. We have identified *Wolbachia* infections in *C. lectularius* using PCR. PCR was initially attempted using published primers. Our reports thus suggest that *Wolbachia* infections are most likely common among bed bugs in general.

The major finding of the current study is the presence of endosymbiont bacteria *Wolbachia* that harbour maternally. *Wolbachia* infection is known to cause either feminization or male killing in its host.

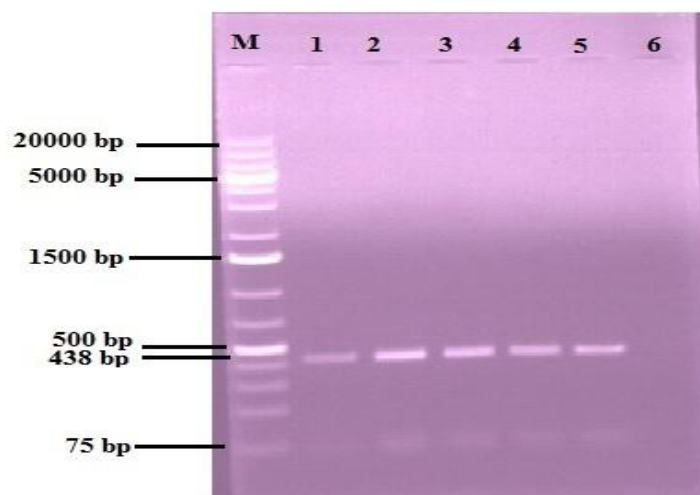


Fig 2. Gel image showing the amplification *Wolbachia* specific *wspec* primer at around ~438 bp; lane M-molecular weight marker (Thermo Scientific Generuler 1 kb Plus DNA Ladder), lanes 1 and 6- positive and negative controls respectively, lanes 2 to 5- *Cimex lectularius* collected from Akola, Amravati, Chandrapur and Nagpur respectively.

Table 1: The PCR results screening the infection status of *Wolbachia* in *Cimex lectularius* species collected from different areas of Vidarbha region, Maharashtra.

Sr. no.	Areas	Bedbug species	Number of detection <i>Cimex lectularius</i>	Body part used for DNA isolation	Positive <i>Wolbachia</i>
1	Akola	<i>Cimex lectularius</i>	18/20	Abdomen	+
2	Amravati	<i>Cimex lectularius</i>	15/15	Abdomen	+
3	Chandrapur	<i>Cimex lectularius</i>	14/18	Abdomen	+
4	Nagpur	<i>Cimex lectularius</i>	15/20	Abdomen	+

Historically, *Wolbachia*-like inclusions were observed in other cimicids besides *C. lectularius* and were identified by PCR (polymerase chain reaction) in the cliff swallow bug *Oeciacus vicarius* Horvath (Rasgon and Scott 2004). A total of seventy three individual of *Cimex lectularius* a bedbug species were screened in the present study for *Wolbachia* infection by PCR amplification (table 1) from different region of Vidarbha. And the results revealed that out of seventy three individuals of *Cimex lectularius* sixty three individuals were found to be infected with *Wolbachia*. This is a primary data on the infection status of *Wolbachia*

in the species of bedbug *Cimex lectularius* from different area of Vidarbha region. In the PCR assays for *Wolbachia*, a PCR product of the expected size (~438 bp) (Figure2) was obtained from all individuals tested. In diagnostic PCR assays, positive and negative control samples were used for checking the impurities or false results as expected in all the diagnostic PCR assays.

The association may be a symbiotic type in which this intracellular proteobacterium may play a key role in the digestive and reproductive functions of

bed bugs (Sakamoto, 2006) . Thus an indirect and alternative strategy of fighting against bed bugs could consist of killing *Wolbachia* species, because they may be necessary for the survival of the bugs.

Our results confirm that *Wolbachia* species is a frequent commensal of bed bugs (62 / 73) in table 1. As yet there has been no information available regarding the *Wolbachia* infection status in *Cimex lectularius* from Vidarbha region of India. This will be the first report from Vidarbha region of India.

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