



Antibiotic alters host's gut microbiota, fertility, and antimicrobial peptide gene expression *vis-à-vis* ampicillin treatment on model organism *Drosophila melanogaster*

Asem Sanjit Singh¹ · Dhruv Pathak¹ · Manoharmayum Shaya Devi² · Abass Toba Anifowoshe¹ · Upendra Nongthomba¹

Received: 11 November 2023 / Revised: 20 February 2024 / Accepted: 10 March 2024
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2024

Abstract

Antibiotics are commonly used to treat infectious diseases; however, persistence is often expressed by the pathogenic bacteria and their long-term relative effect on the host have been neglected. The present study investigated the impact of antibiotics in gut microbiota (GM) and metabolism of host. The effect of ampicillin antibiotics on GM of *Drosophila melanogaster* was analyzed through deep sequencing of 16S rRNA amplicon gene. The dominant phyla consisted of Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, Planctomycetes, Chloroflexi, Euryarchaeota, Acedobacteria, Verrucomicrobia, and Cyanobacteria. It was found that the composition of GM was significantly altered on administration of antibiotics. On antibiotic treatments, there were decline in relative abundance of Proteobacteria and Firmicutes, while there were increase in relative abundance of Chlorophyta and Bacteroidota. High abundance of 14 genera, viz., *Wolbachia*, *Lactobacillus*, *Bacillus*, *Pseudomonas*, *Thiolamproy*, *Pseudoalteromonas*, *Vibrio*, *Romboutsia*, *Staphylococcus*, *Alteromonas*, *Clostridium*, *Lysinibacillus*, *Litoricola*, and *Cellulophaga* were significant ($p \leq 0.05$) upon antibiotic treatment. Particularly, the abundance of *Acetobacter* was significantly ($p \leq 0.05$) declined but increased for *Wolbachia*. Further, a significant ($p \leq 0.05$) increase in *Wolbachia* endosymbiont of *D. melanogaster*, *Wolbachia* endosymbiont of *Curculio okumai*, and *Wolbachia pipientis* and a decrease in the *Acinetobacter* sp. were observed. We observed an increase in functional capacity for biosynthesis of certain nucleotides and the enzyme activities. Further, the decrease in antimicrobial peptide production in the treated group and potential effects on the host's defense mechanisms were observed. This study helps shed light on an often-overlooked dimension, namely the persistence of antibiotics' effects on the host.

Keywords Gut microbiota · Antibiotics · Ampicillin · *Drosophila melanogaster*

Introduction

Globally, 36% surge in antibiotic consumption from 2000 (54 billion) to 2010 (73 billion), with discernible spikes in countries such as Brazil, Russia, India, China,

and South Africa, indicating a burgeoning demand for these pharmaceutical agents (Van Boeckel et al. 2015). Approximately 25 to 75% of administered antibiotics are released into the environment, contributing to the proliferation of antibiotic-resistant bacteria. Penicillin, macrolides, and fluoroquinolones dominate antibiotic use in human medicine, while penicillin, tetracyclines, and sulfonamides find widespread application in animal husbandry, serving roles in treatment, prevention of infectious diseases, and as growth promoters in animal feed (Chopra and Robert 2001). Their toxicity profile varies based on compound composition, administration routes, pathways, and targeted gene/population (Arnold et al. 2013).

Antibiotics also impact the intricate realm of gut microbiota, with broad-spectrum antibiotics eliminating beneficial microbes and reducing diversity in gut microbes

✉ Asem Sanjit Singh
sanjitasem21@gmail.com

✉ Upendra Nongthomba
upendra@iisc.ac.in

¹ Developmental and Biomedical Genetics Laboratory,
Department of Developmental Biology and Genetics, Indian
Institute of Science, Bengaluru, India 560012

² ICAR-Central Inland Fisheries Research Institute,
P.O. Monirampur, Barrackpore, Kolkata, India 700 120

(Blaser 2011; Dubourg et al. 2014). Studies reveal specific effects on gut microbiota composition, such as azithromycin shifting *Lactobacillus johnsonii* abundance and cefactor enriching gut microbiota in a rat model (Khan et al. 2016). Optimal host health relies on rich species diversity and a balanced microbiota composition. Imbalances or reduced diversity in infants' guts has been linked to intestinal illnesses (Volkova et al. 2021; Milani et al. 2017). Maternal antibiotic uptake during pregnancy transforms offspring microbial composition, increasing the risk of allergy and asthma, and reducing levels of bifidobacteria in infants (Coker et al. 2020; Azad et al. 2016; Stokholm et al. 2014; Aloisio et al. 2014). The symbiotic composition of bacteria plays a crucial role in host well-being, with clear links between the gut microbiome and various disorders, such as colorectal cancer, chronic liver diseases, intestinal bowel diseases, pancreatic disorders, irritable bowel syndrome, and coeliac disease (Tilg et al. 2016, 2018; Trebicka et al. 2021; Riquelme et al. 2019; Mars et al. 2020; Leonard et al. 2021). Insects' gut symbionts are vital for core host functions, survival, physiology, and ecological adaptation, serving as a barrier against pathogens and influencing the host's immune system response (Brune 1998; Janson et al. 2008; Hosokawa et al. 2006).

Drosophila melanogaster serves as a well-known model for exploring host-microbiome interactions, host physiology, and pathology due to its simpler bacterial community in the gut (Martino et al. 2017; Broderick and Lemaitre 2012). Bacteria significantly impact the life span, gut homeostasis, interaction with pathogens, and behavior of *Drosophila* (Ryu et al. 2008; Wong et al. 2017). Ampicillin, a member of the penicillin group, stands out as an intrapartum prophylactic drug against Group B Staphylococcus during pregnancy (Aloisio et al. 2014). There are studies regarding impacts of persistent residues of antibiotics, such as hydrophobic ampicillin, to both human and animal hosts. However, there is lack of study on host physiology and gut microbes against Ampicillin exposure to *D. melanogaster*.

With this backdrop, the present study focuses on exploring the impact of ampicillin on the gut microbiota in the *D. melanogaster* model and its effects on the host in terms of antimicrobial peptide production and fertility. The analysis involves assessing the changes in diversity and richness of the *D. melanogaster* gut microbiome through 16S rRNA amplicon sequencing.

Material and methods

Drosophila model and sample collection

Canton-S strain of *D. melanogaster* was grown on standard corn medium, containing yeast, agar, sucrose, and corn flour

in $25 \pm 1^\circ\text{C}$ and 50–60% humidity incubator with 12:12-h light/dark cycle. The 60 flies (30 male and 30 female) in five biological replicates were administered with 100 $\mu\text{g}/\text{ml}$ ampicillin for 8 days and lay eggs in the bottles and the parental flies were discarded. Similarly, for control, standard corn media were used. The eclosion flies were allowed to grow for 15 days both in control and in treatment (Fig. 1A). The treated as well as control adult flies ($n = 60$) were etherized and washed twice with 70% ethanol, sterile phosphate buffer, and dissected with 1-ml sterile syringe (sterilized with 70% ethanol) in 50- μl sterile phosphate buffer to separate the gut (including Malpighian tubules). The samples were transferred to RNA later in sterile tubes and stored at -80°C for total genomic DNA extraction (Fig. 1A).

16S rRNA sequencing and data processing

Gut metagenomic DNA was extracted using QIAamp DNA Mini Kit (Qiagen) as per the manufacturer's guideline. The quality of the extracted total metagenomic DNA was assessed through agarose gel electrophoresis and quantified in Nanodrop (Appendroff, Germany) at A260/280 ratio. The amplicon PCR was set up using the extracted metagenomic DNA with bacterial 16S V3-V4 region-specific primer (16S rRNA F-GCCTACGGGNGGCWGCAG and 16S rRNA R-ACTACHVGGGTATCTAATCC) (Dowd et al. 2008). The quality of the amplicon was checked and purified using Kapa HiFi HotStart Ready Mix and Nextera XT Library Prep Kit (Illumina) as per the manufacturer's protocol. For purification of the PCR enriched library, AMPure XP bead was used to remove un-ligated index primers. Subsequently, the purified library was analyzed on Agilent TapeStation using D100 Screen Tape to cross-check the presence of expected insert size and any adaptor-dimer. Additionally, the library size ranged from 585 to 615 bp and high-quality sequences were dereplicated. The clear sequences were clustered at k D 10 (97% similarity) followed with deletion of chimeras and single ton reads (Eren et al. 2011; Swanson et al. 2011).

The 16S rRNA sequence data sets were processed using the COSMOSID-HUB Microbiomes and One Codex platform. Raw counts and relative abundances of metabolites and enzymes were computed from the platform. The values underwent statistical analysis and were presented as mean $\pm$ standard deviations. One-way ANOVA was employed to compare means among the treatment groups and the control group, with a chosen significance level of $p < 0.05$.

Fertility measurements

The 7-days-old treated and control adult flies were collected and cross them as follows: control $\sigma \times$ control ϕ ; treated $\sigma \times$ treated ϕ ; control $\sigma \times$ treated ϕ ; treated $\sigma \times$ control ϕ .

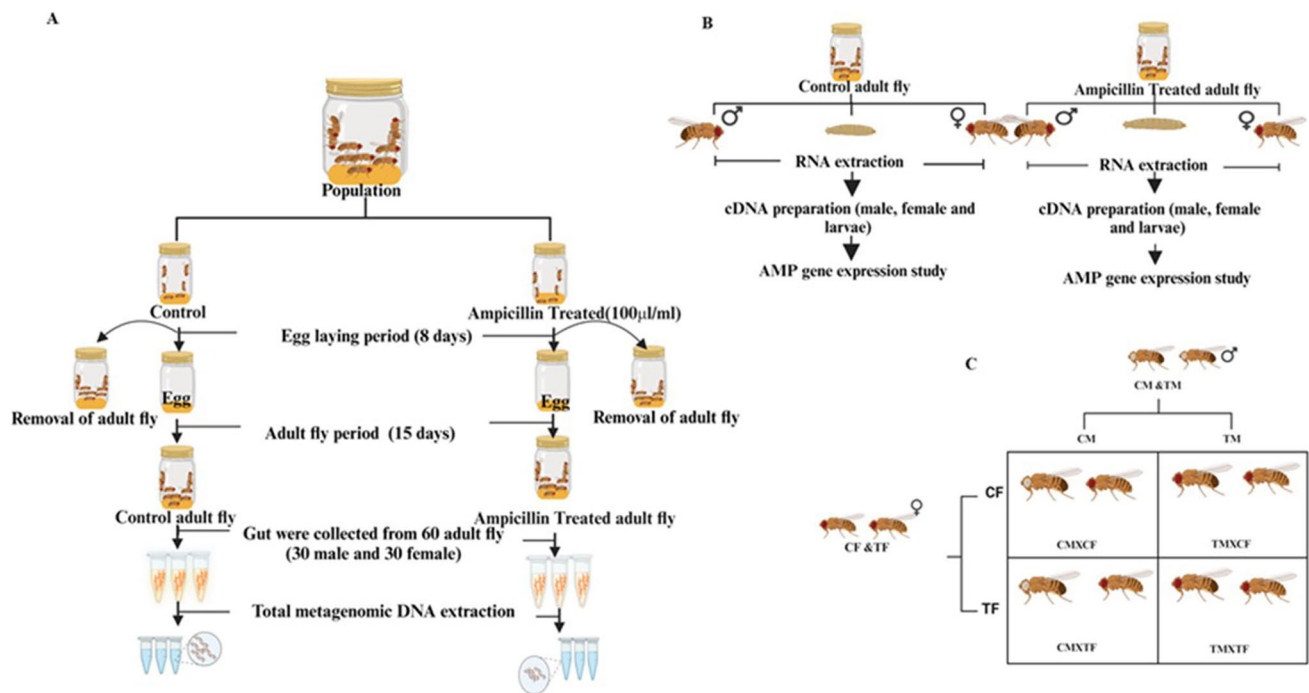


Fig. 1 **A, B, C** The schematic diagram of the experimental setup. **A** Impact of ampicillin exposure on host's gut microbiota, metabolites, and enzymatic pathways. **B** AMP gene regulation. **C** Impact of ampicillin on host's fertility

The flies were allowed to lay eggs in the corn medium vials for 8 days at $25 \pm 1^\circ\text{C}$ and 60–70% humidity. Then the parental flies were discarded on the 8th day and the eclosion was recorded for 15 days in each bottle (Fig. 1C).

RNA extraction and cDNA synthesis

Total RNA was extracted using Trizol (Sigma) from 5 whole *D. melanogaster* of both control and treated. The extracted total RNA was qualitatively checked in 1% agarose gel and quantitatively measured by nanodrop spectrophotometer (Appendroff, Germany). DNase Treatment (Ambion™, DNA free™, AM1907; Thermo Fisher Scientific) was done for removal of DNA content from the extracted RNA sample. Afterwards, cDNA was synthesized from total RNA using Revert Aid H minus First Strand cDNA Synthesis Kit (K1632; Thermo Fisher Scientific) and stored at -20°C for quantitative PCR (qPCR) (Fig. 1B).

Quantitative PCR

cDNA prepared from *D. melanogaster* tissue was subjected to quantitative PCR using SYBR green mastermix in Light cycler 480 Real-Time Instrument (Roche, Germany). Initially, *rp49* was used as reference gene for the expression study. Seven antibiotic response genes *attacin*, *cecropin*, *defensin*, *metchnikowin*, *drosomycin*, *drosocin*, and *dip-tericin* were assessed for gene expression study. The qPCR

primers employed were sourced from Chatterjee et al. (2016). Each reaction of 20 µl containing 10 µl of $2 \times \text{SYBR}$ green mastermix (Thermo Scientific), 1 µl of 5 pmol of forward and reverse primer, 2 µl of diluted cDNA, and 7 µl autoclaved MilliQ water was run in triplicates. Light cycler 480 (Roche, Germany) was programmed as pre-incubation at 95°C for 5 min, followed by amplification of 35 cycles (95°C for 10 s, annealing for 20 s for respective genes and 72°C for 10 s), melting (95°C for 5 s, 65°C for 2 min and 97°C for continuous), and cooling at 40°C for 20 s. Negative control should also have been mentioned here. The specificity of the reaction was verified through melt curve analysis and threshold cycle values were obtained at the end of the program. Gene expressing value was expressed in terms of fold change values and estimated using $2^{-\Delta\Delta\text{CT}}$ method (Livak and Schmittgen, 2001) considering *rp49* as a reference gene. $\Delta\Delta\text{CT}$ was obtained as.

Statistical analysis

The microbiome community identification and profiling based on next-generation sequencing were calculated using a specific tool called "Cosmos ID." The results of the statistical tests are presented as means $\pm$ standard deviations (SD). One-way ANOVA was used to compare the means between the treatment groups and the control group. A p -value < 0.05 is chosen as the level of significance. The alpha diversity, the Shannon, and Simpson indexes are calculated. In AMP

gene expression analysis, the fold change values were plotted and statistically analyzed for significant difference of gene expression between treated and control on Graph pad PRISM 5.

Result

Dominant bacterial community

A total of 28 numbers of phyla were identified in the gut system of *D. melanogaster* encompassing both control and treated samples. Ten numbers of phylum contributed $\geq 1\%$ concentration with varying in abundances both control and treated samples. Four phyla, viz., Proteobacteria, Firmicutes, Bacteroidota, and Actinobacteria with $\geq 1\%$ concentration but varied in number of read counts were common in control and treated. A total of 149 families were detected; among them, 135 were common in the system and Acetobacteraceae, Flavobacteriaceae, Lachnospiraceae, Rhodobacteraceae, and Planctomycetaceae were dominant. Two hundred five genera were detected in control samples while 169 were detected in the treated group. The *Acetobacter* and *Lactobacillus* were dominant in control. A total of 229 species in control and 182 in treated sample were observed. There were significant ($p=0.0001$) reduction in number of genera and lower species diversity ($p=0.0055$) in the ampicillin-treated groups.

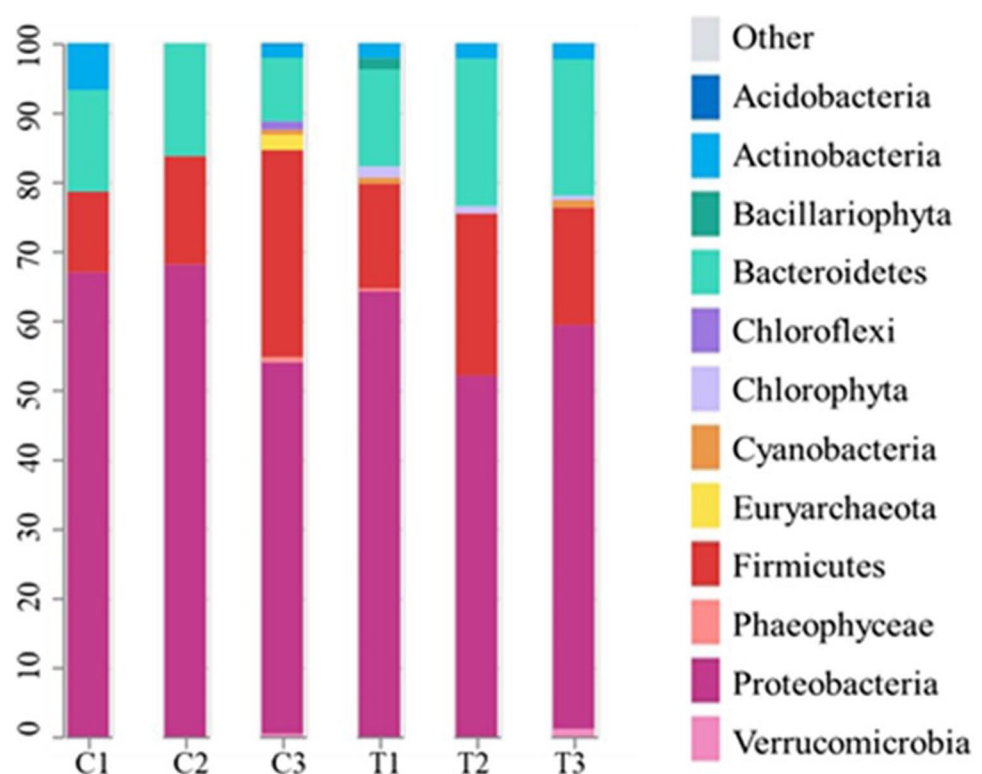
Most of the sequences read in control phyla were Proteobacteria ($62.85 \pm 8.05\%$), Firmicutes ($19.06 \pm 9.57\%$), Bacteroidota ($13.38 \pm 3.72\%$), and Actinobacteria ($2.9 \pm 3.49\%$). Similarly, in treated groups, Proteobacteria ($58.16 \pm 6.1\%$), Firmicutes ($18.49 \pm 4.4\%$), Bacteroidota ($18.34 \pm 3.8\%$), Actinobacteria ($2.25 \pm 0.1\%$), and Chlorophyta ($1.10 \pm 0.5\%$) (Fig. 2).

The higher number of genera (205) was detected in the control group while lower (169) in the case of ampicillin-treated group. The *Acetobacter* ($87.86 \pm 11.2\%$), *Lactobacillus* ($16.9 \pm 1.72\%$), *Wolbachia* ($1.26 \pm 1.2\%$), *Leuconostoc* ($1.5 \pm 1.6\%$), and *Prevotella* ($0.66 \pm 0.79\%$) dominate in control while *Wolbachia* ($16.42 \pm 2.3\%$), *Lactobacillus* ($14.06 \pm 12.84\%$), *Vibrio* ($4.72 \pm 1.85\%$), *Pseudomonas* ($4.32 \pm 2.67\%$), and *Bacillus* ($4.24 \pm 0.4\%$) were the dominant genera associated with treated group (Fig. 4).

Number of species observed was 229 and 182 in the control and treated samples respectively. *Acetobacter pasteurianus* ($29.65 \pm 18.60\%$), *Wolbachia* endosymbiont of *D. melanogaster* ($12.74 \pm 10.47\%$), and *Commensalibacter intestine* ($8.9 \pm 9.6\%$) were dominant in the control group and *Wolbachia* endosymbiont of *D. melanogaster* ($35.12 \pm 5.7\%$) followed by *Prevotella copri* ($6.01 \pm 5.7\%$) and *Wolbachia pipientis* ($4.03 \pm 0.6\%$) in treatment samples (Fig. 5).

In the study, *Wolbachia* sp. was abundant in the treated and *Acetobacter* sp. in the control group. Significant

Fig. 2 Average relative density of the dominant bacteria phyla in gut of control and treated *D. melanogaster*. The x-axis shows the taxon groups and the y-axis displays the average percentages on the sequences reads. The cutoff point for selecting the dominant phyla was set to $\geq 1\%$. “Other” indicates minor phyla. C1, C2, C3—control; T1, T2, T3—treatment exposed to ampicillin



decrease in species ($p=0.0055$) and genera ($p=0.0001$) was observed in treated sample (Fig. 3).

Gut microbiota differences between control and treated

The ampicillin-modulated changes in gut microbiota in terms of phyla were statistically insignificant in the control and treated samples. The phylum Proteobacteria, Firmicutes, Actinobacteria, and Euryarchaeota were higher in the control group while Bacteroidetes, Pasteurellales, and Chloroflexi were increased in the treated groups (Fig. 2).

The gut microbiota of the control samples was predominantly composed of the families Acetobacteraceae ($61.41 \pm 22.36\%$), Anaplasmataceae ($8.08 \pm 6.81\%$), and Prevotellaceae ($2.1 \pm 2.8\%$), whereas, in treated group, the density of the Acetobacteraceae ($0.48 \pm 28.72\%$) was drastically declined and Anaplasmataceae ($34.52 \pm 4.73\%$), Prevotellaceae ($4.9 \pm 5.19\%$), Bacillaceae (3.21 ± 0.95), and Flavobacteriaceae ($2.19 \pm 0.42\%$) were increased (Fig. 3).

Alteration of gut microbiota in response to ampicillin

Ampicillin led to decrease in Acetobacteraceae ($p=0.004$) significantly, whereas Anaplasmataceae ($p=0.004$), Bacillaceae ($p=0.01$), and Flavobacteriaceae ($p=0.004$) were increased in the treated sample. The significant increase in

the Rhodobacteriaceae ($p=0.002$) was in the treated group (Fig. 3). Similarly, the genera *Acetobacter* ($p=0.004$) was decreased and *Wolbachia* ($p=0.002$), *Bacillus* ($p=0.02$), *Pseudomonas* ($p=0.03$), *Vibrio* ($p=0.01$), *Staphylococcus* ($p=0.04$), *Alteromonas* ($p=0.05$), *Clostridium* ($p=0.001$), *Lysinibacillus* ($p=0.004$), and *Janthinobacterium* ($p=0.02$) were increased in the treated group (Fig. 4). *Wolbachia* endosymbiont of *D. melanogaster* ($p=0.01$), *Wolbachia* endosymbiont of *Curculio okumai* ($p=0.02$), and *Wolbachia pipiens* ($p=0.01$) were increased significantly in the ampicillin-treated group while *Acetobacter cerevisiae* ($p=0.05$) and *Acetobacter pasteurians* ($p=0.02$) were decreased (Fig. 5).

Biodiversity and microbial richness

The diversity of the species were estimated through the Simpson index and Shannon index. The Simpson index was calculated and observed that the control (0.218033) group was more diverse than treatment (0.926155). Evenness was more in control (Shannon index = 1.0966623) group than treated (Shannon index = 5.266927) (Fig. 6). Principal component analysis (PCA) of species was done and found that treated samples were closer to each other compared to control samples (Fig. 7).

The study found that ampicillin induces higher microbiome functional capacity for the biosynthesis of guanosine deoxyribonucleotides (de novo biosynthesis II)

Fig. 3 Comparative analysis of the dominant and significantly different families in the gut microbiota of control versus ampicillin-treated *Drosophila melanogaster*. The x-axis shows the taxon groups and the y-axis displays the average percentages on the sequences reads. Abbreviations are as described in Fig. 1

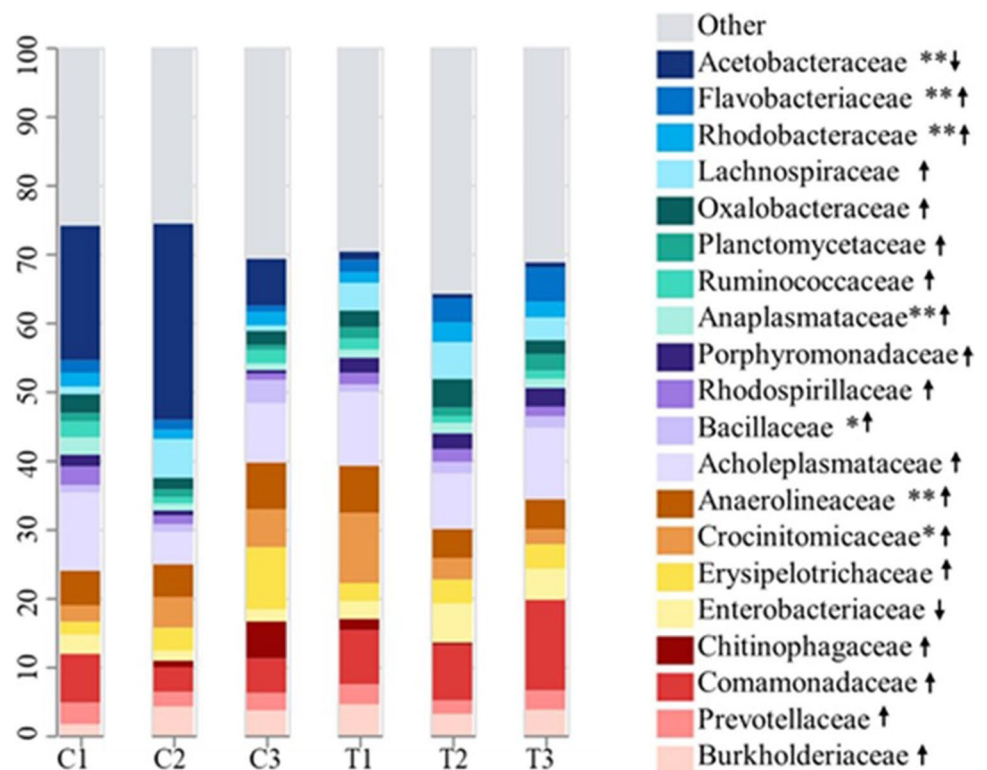


Fig. 4 Comparative analysis of the dominant and significant different genera in the gut microbiota of control versus ampicillin-treated *D. melanogaster*. The direction of arrow is indicating increase or decrease in the density of each genus in compared to control. *Indicating significant at $p < 0.05$. ** indicates high significant at $p < 0.01$. Abbreviations are as described in Fig. 1

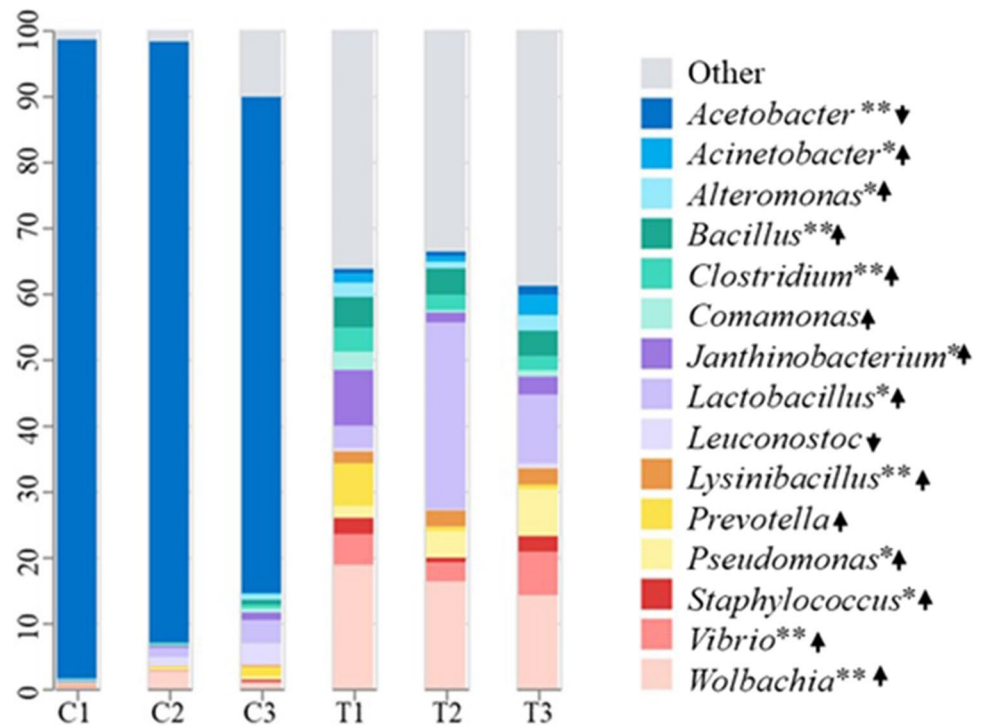
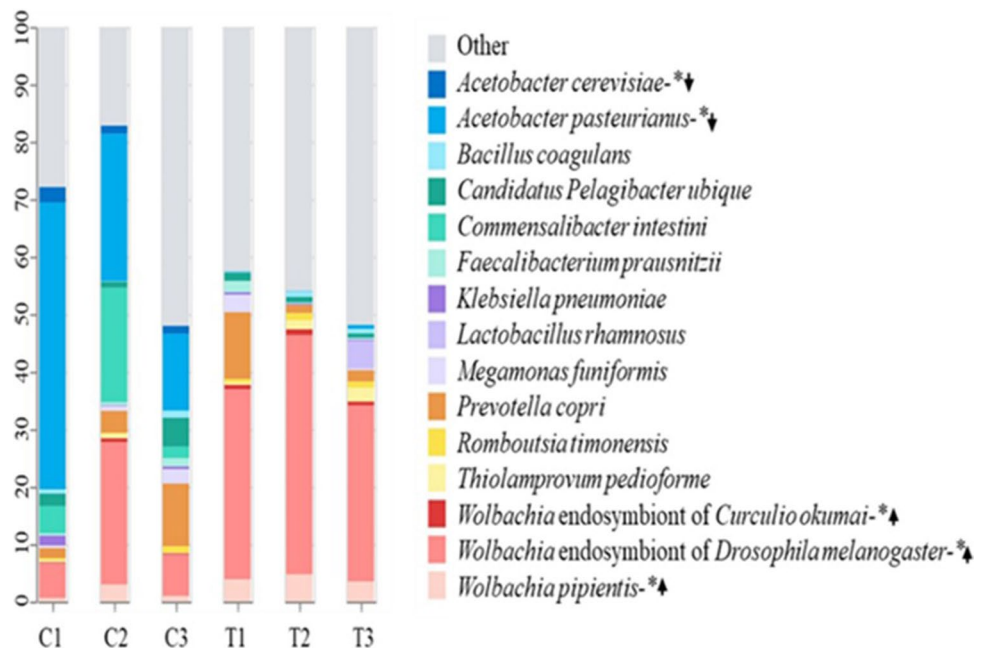


Fig. 5 Dominant and significantly different species in the gut microbiota of control versus antibiotic-treated *D. melanogaster*. The direction of the arrow is indicating increase or decrease in the density of each species in compared to control. *Indicating significant at $p < 0.05$. Abbreviations are as described in Fig. 1



and adenosine deoxyribonucleotides (de novo biosynthesis II). Additionally, the study identified increase in activity of aerobic respiration I (cytochrome c), superpathways of adenosine nucleotides de novo biosynthesis-I, and TCA (tricarboxylic acid cycle) cycle I (prokaryotic) (Fig. 8). The study also revealed that the cytochrome-C-oxidase, RNA-directed DNA polymerase, ubiquinone reductase (H⁺-translocating), H⁺ transporting two sectors ATPase,

and L-threonine aldolase enzyme activities were increased in the treated (Fig. 9).

Effect on fertility

Ampicillin treatment of *Drosophila* led to significant ($p < 0.05$) decrease in the eclosion number of offspring. Control ♂ × control ♀ has the highest eclosion followed by

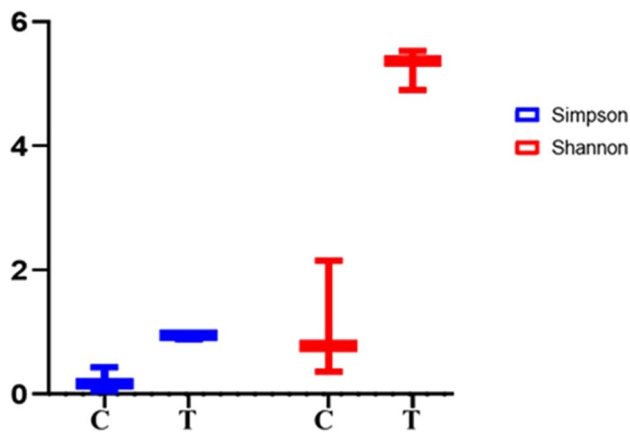


Fig. 6 The difference of the Shannon diversity index and Simpson diversity index. Simpson index showed a significant ($p=0.001$) increase in the treated sample and Shannon index $p=0.0009$ significantly increased in the treated group

treated ♂ × control ♀. While the control ♂ and treated ♀ have higher eclosion compared with treated ♂ and treated ♀ (Fig. 10). The fertility mainly affects the female *Drosophila* when compared to male flies.

Effect on AMP gene regulation

The AMP gene regulation and antimicrobial peptides production is an important aspect of host defense in multicellular organisms. The present study revealed that

the AMP genes were significantly downregulated in treated compared to that of control flies (Fig. 11A, B, C). AMP gene expression was significantly downregulated in the treated males, females, and larvae. In treated males, expressions of attacin ($p=0.0129$), cecropin ($p=0.0006$), defensin ($p=0.0040$), metchnikowin ($p=0.0123$), drosomycin ($p=0.0030$), drosocin ($p=0.0060$), and dipteracin ($p=0.0108$) were significantly decreased (Fig. 11A) whereas in treated female, expressions of attacin ($p=0.0007$), cecropin ($p=0.0001$), metchnikowin ($p=0.0008$), drosocin ($p=0.0009$), and dipteracin ($p=0.0003$) were significantly decreased except defensin ($p=0.0647$) and drosomycin ($p=0.0726$) (Fig. 11B). In larval treated sample, significant decrease in the AMP genes, i.e., attacin ($p=0.0012$), cecropin ($p=0.0007$), defensin ($p=0.0008$), metchnikowin ($p=0.0002$), drosomycin ($p=0.0128$), drosocin ($p=0.0002$), and dipteracin ($p=0.011$), was detected (Fig. 11C).

Discussion

Impact of antibiotic exposure on host's GM

Broad-spectrum antibiotics, such as ampicillin, can disrupt microbial communities, leading to reduced species diversity in the gut microbiota (Heinsen et al. 2015). This imbalance may impact both harmful and beneficial microbes, potentially causing dysbiosis and health issues. Our study found

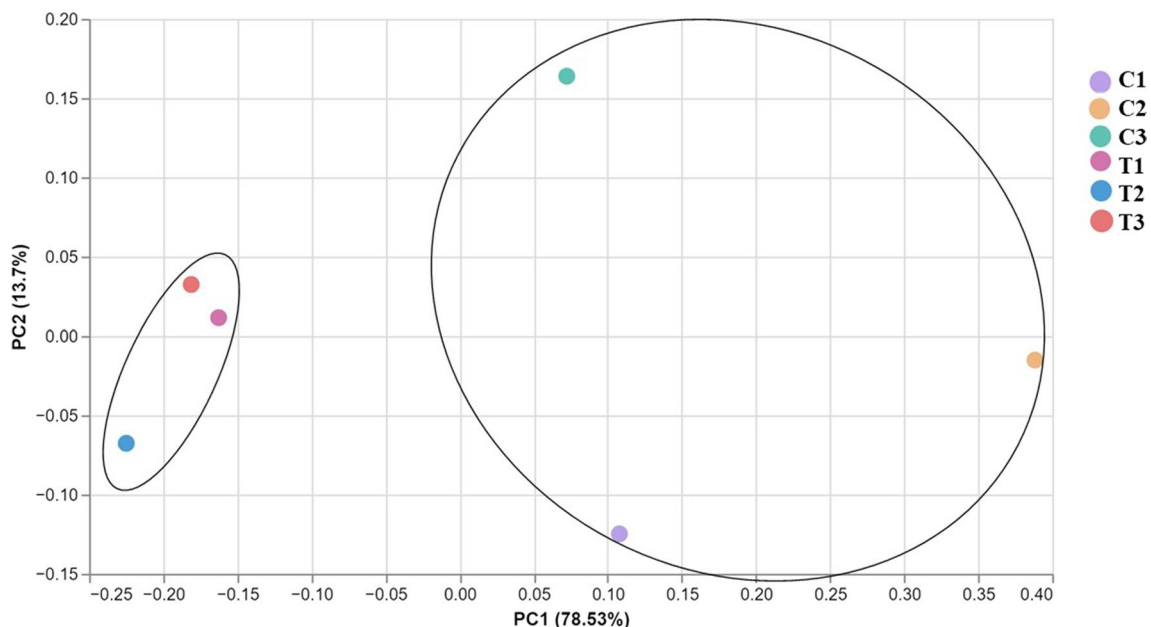


Fig. 7 Principal component analysis (PCA) ordination of the microbial diversity data in the control and treated. The first axis explains 78.53% of the variation in the data, and the second axis explains

13.7% of the variation in the data. Principal coordinate analysis of the Weighted Unifrac distance matrix revealed a clear clustering of the RD. Abbreviation are as described in Fig. 1

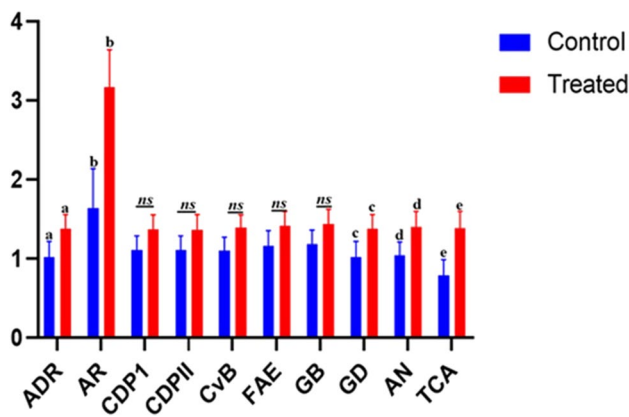


Fig. 8 Metabolite production in gut of control versus ampicillin-treated *D. melanogaster*. The x-axis shows the metabolites and the y-axis displays the fold change. ADR, adenosine deoxyribonucleotides de novo biosynthesis II; AR, aerobic respiration I (cytochrome c); CDP1-CDP, diacylglycerol biosynthesis I; CDP2-CDP, diacylglycerol biosynthesis II; CvB-Cis, vaccenate biosynthesis; FAE, fatty acid elongation-saturated; GB, gondoate biosynthesis (anaerobic); GD, guanosine deoxyribonucleotides de novo biosynthesis II; AN, superpathway of adenosine nucleotides de novo biosynthesis I; TCA, TCA cycle I (prokaryotic). The bar with same letters indicates significant difference between each other, $p \leq 0.05$

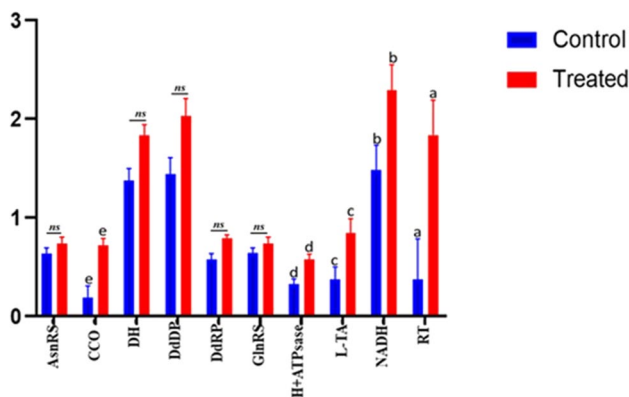


Fig. 9 Enzyme production in gut of control and treated *D. melanogaster*. The x-axis shows the enzymes and the y-axis displays the fold change. (AsnRS, asparaginyl-tRNA synthase (glutamine-hydrolyzing); CCO, cytochrome C oxidase; DH, DNA helicase; DdDP-DNA, directed DNA polymerase; DdRP, DNA-directed RNA polymerase; GlnRS, glutaminyl tRNA synthase; H⁺-ATPase, H⁺-transporting two sector ATPase; L-TA, L-threonine aldolase; NADH, ubiquinone reductase (H⁺)-translocating; RT-RNA-directed DNA polymerase). The bar with same letters indicates significant difference between each other, $p \leq 0.05$

a decrease in Proteobacteria and Firmicutes in treated flies, similar to observations in rat gut microbiota (Dethlefsen and Relman 2011), indicating a potential shift in microbiota composition due to treatment or environmental factors. The rise in opportunistic pathogens like *Wolbachia*, *Vibrio*, *Bacillus*, and others suggests competition among

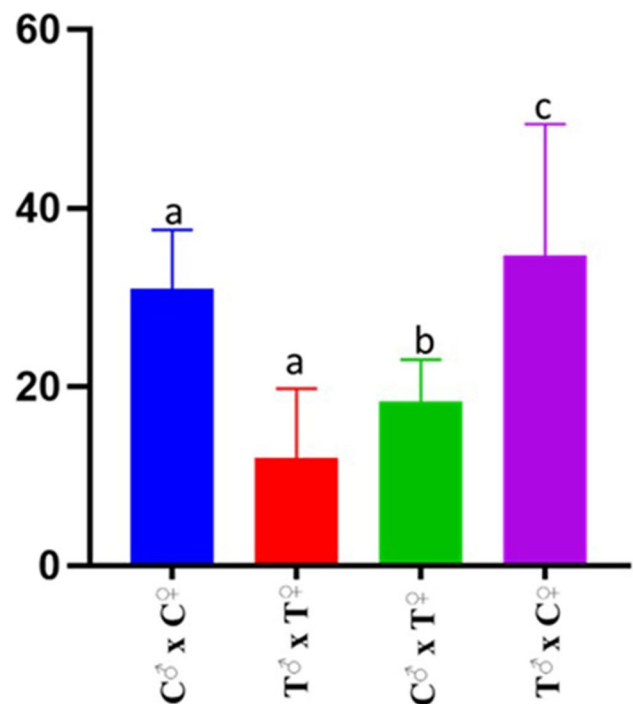


Fig. 10 Effect of ampicillin on fertility of *D. melanogaster*. The bar with same letters indicates significant difference between each other; $N=3$, $n=30$, $p \leq 0.05$. C♂, control male; C♀, control female; T♂, treated male; T♀, treated female

bacterial communities, contributing to intestinal microbiota dysbiosis. Antibiotics selectively promote the growth of certain bacteria, including potential pathogens, leading to a significant reduction in species and increased instability in treated groups. This shift in richness and evenness of intestinal microbiota, as seen in honeybees exposed to antibiotics (Raymann et al. 2017), highlights concern about the impact of antibiotic exposure on gut microbiota and overall health.

Impact of antibiotic on microbial functional capacity

The study revealed an increased microbiome functional capacity in treated samples for the biosynthesis of guanosine deoxyribonucleotides (de novo biosynthesis II) and adenosine deoxyribonucleotides (de novo biosynthesis II). Additionally, heightened activity in adenosine nucleotides de novo biosynthesis-II and superpathways of adenosine nucleotides de novo biosynthesis-I was noted. Adenosine and guanosine are essential components of purine nucleotide metabolism which is crucial for cellular processes (Antonioli et al. 2013). Elevated levels may have physiological implications, influencing the body's response to conditions (Jimmerson et al. 2017; Antonioli et al. 2013). Guanosine derivatives, like 8-hydroxydeoxyguanosine, act as endogenous ligands for Toll-like receptor 7 (TLR7), suggesting potential immunomodulatory effects

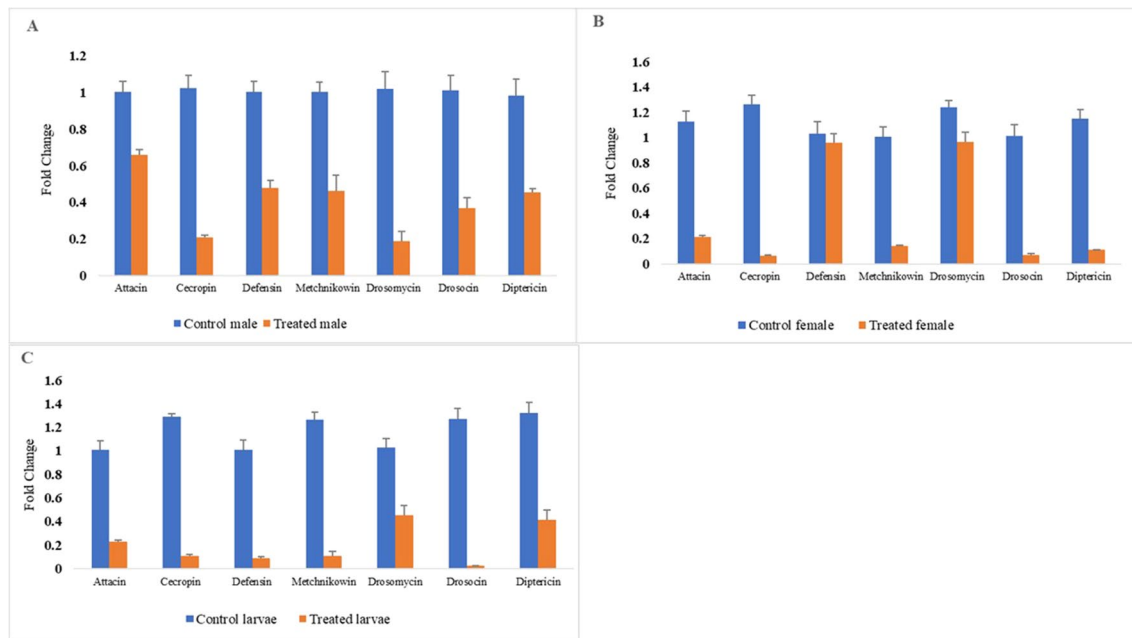


Fig. 11 A, B, C AMP gene expression in *D. melanogaster* was analyzed using quantitative RT-PCR in both control and ampicillin-treated samples. The induction of seven anti-microbial peptides was

estimated by normalizing AMP mRNA levels to the coamplified housekeeping gene *rp49*

and immune response triggering (Shibata et al. 2016). Moreover, 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage, associated with strong cytokine production, indicates the microbiome's production of guanosine derivatives, including 8-OHdG, could impact DNA integrity and immune responses (Shibata et al. 2016).

Impact of antibiotic on enzymatic activities

The treated samples exhibited higher cytochrome-C-oxidase (CCO) activities, a crucial regulator of the intrinsic apoptotic pathway and a defense mechanism against pathogens (Schultz-Cherry et al. 2001). Ampicillin treatment increased L-threonine and RNA-directed DNA polymerase activity, similar to observations with antibiotic treatments (Li et al. 1977). L-Threonine, a precursor for carbohydrate synthesis, increased in treated samples, impacting infection strategies for Enteric pathogens (Kato and Ishiwa 2015). Dysbiosis of gut microbiomes affects gut transcriptome, immunity, and metabolism, increasing infection risk (Elya et al. 2016). Enhanced production of microbial metabolites from dietary nutrients benefits host cells, impacting host disease and health, and maintaining gastrointestinal homeostasis. These microbial metabolites also signal to various organs, including the brain (Elya et al. 2016).

Impact of antibiotic on host's fertility

The broad antimicrobial activity of ampicillin may contribute to a significant reduction in host fertility, evident in the diminished population in the treated group and the prevalence of *Wolbachia* sp. This association suggests a potential link between the two phenomena. *Wolbachia*, commonly found in insects and arthropods, plays roles in parthenogenesis, feminization, and cytoplasmic incompatibility (Stouthamer et al. 1993; Rousset et al. 1992; Hoffmann et al. 1990). Infections by various *Wolbachia* strains can lead to reproductive failure, where uninfected individuals are unable to successfully reproduce with infected males or females (Werren et al. 2008; Serbus et al. 2008). *Wolbachia*-induced cytoplasmic incompatibility disrupts sperm function during and after egg penetration, potentially decreasing overall population fertility (Snook et al. 2000). Additionally, *Wolbachia* infection induces oxidative stress, creating an imbalance between host antioxidant defenses and reactive oxygen species production, which can negatively impact cellular function and fertility (Serbus et al. 2008).

The presence of *Wolbachia* may also influence dopamine production, a neurotransmitter involved in various biological functions, including the regulation of juvenile hormone in female flies (Hanna et al. 2015; Ueno et al. 2012). Alterations in dopamine production induced by *Wolbachia* may disrupt hormonal balance, potentially affecting

the reproductive biology of female flies (Gruntenko and Rauschenbach 2008; Argue et al. 2013). In summary, the presence of *Wolbachia* in treated samples appears to have multifaceted effects on the host population, including potential negative impacts on sperm and male fertility, induction of oxidative stress, and alterations in dopamine production influencing juvenile hormone levels in female flies (Serbus et al. 2008; Werren et al. 2008).

Impact of antibiotic on host's antimicrobial peptide gene regulation

The present study reveals a reduction in the regulation of antimicrobial peptide genes across male, female, and larval *Drosophila*, with the exception of defensin and drosomycin in female individuals. This decline in antimicrobial peptides (AMPs) coupled with a decrease in gut microbial diversity establishes a correlation between these two phenomena. The gut dysbiosis can compromise the host's defense mechanism, rendering it more susceptible to infections or altering the dynamics of pathogenic interactions. Insect gut studies emphasize the pivotal role of microbes in mediating interactions between the host and potential pathogens (Broderick et al. 2006). While Kaufman and Klug (1991) suggested that the presence of gut microbiota may not be imperative for the basic survival of insects, in *Drosophila*, these microbes play a significant role in the production of AMPs and microbial reactive oxygen species (ROS), thereby activating the host's antimicrobial defense (Ha et al. 2005; Ryu et al. 2006). In our study, the disruption of gut microbiota seems to influence host immune systems and may be through diverse mechanisms, including the modulation of inflammatory signaling via microbial metabolites, alteration of Toll-like receptor signaling, and degradation of secretory IgA (sIgA) (Levy et al. 2017). While the anomaly in females may be due to the robust production of antimicrobial peptides, i.e., the expression of defensin and drosomycin remains unaffected even under certain conditions. This phenomenon aligns with the parallel outcome observed when the production of defensin and drosomycin did not significantly decrease upon infection with specific pathogenic bacteria in female *Drosophila* (Buchon et al. 2009). These findings underscore the critical role played by antimicrobial peptides in the innate immune response of female *Drosophila*, enabling them to effectively combat microbial invaders.

Conclusion

The present study presented a detailed findings on the impact of ampicillin on *Drosophila* GM, host fertility, and AMP gene regulation. Ampicillin caused a longitudinal decrease in the overall gut microbial diversity in the *D. melanogaster*

model and enrichment of specific taxa. The antibiotic used in the present study resulted into microbial imbalance, disturbance in bacterial community networking, reduction in host fertility, and downregulation of AMP genes. The complex host-microbiome interaction and the current usage of antibiotics need careful stewardship, with an emphasis on the application of antibiotic alternatives while limiting collateral damage.

Conflict of interest

The authors declare no competing interests.

Author contribution Asem Sanjit Singh: Conceptualisation, designed the experiment, carried out the experiments, analyzed the experiment results, and writing original draft. Dhruv Pathak: Carried out the experiment; Upendra Nongthomba: Conceptualisation, designed the experiments, commented on the manuscript; Manaharmayum Shaya Devi: Data analysis, assisted in manuscript preparation; Abass Toba Anifowoshe: Commented on the manuscript. All authors reviewed the manuscript.

Funding The authors express their gratitude to the Indian Institute of Science (IISc) for offering essential facilities to conduct this research. The first author acknowledges the financial support received through the DBT-RA post-doctoral program in Biotechnology and Life Science, including fellowship and contingency, under Grant ID-DBT-RA/2022/January NE/994.

Data availability The data supporting the conclusions of this study can be accessed through the NIH Sequence Read Archive under submission ID SUB148280, associated with BioProject ID PRJNA1078087, titled "*Drosophila melanogaster* Gut Microbiomes."

Declarations

Competing interests The authors declare no competing interests.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication All authors have provided their consent for the publication of this manuscript.

References

- Aloisio I, Mazzola G, Corvaglia LT, Tonti G, Faldella G, Biavati B, Di Gioia D (2014) Influence of intrapartum antibiotic prophylaxis against group B Streptococcus on the early newborn gut composition and evaluation of the anti-Streptococcus activity of Bifidobacterium strains. Appl Microbiol Biotechnol 98:6051–6060
- Antonioli L, Blandizzi C, Pacher P, Haskó G (2013) Immunity, inflammation and cancer: a leading role for adenosine. Nat Rev Cancer 13(12):842–857
- Argue KJ, Yun AJ, Neckameyer WS (2013) Early manipulation of juvenile hormone has sexually dimorphic effects on mature adult behavior in *Drosophila melanogaster*. Horm Behav 64(4):589–597

- Arnold KE, Boxall AB, Brown AR, Cuthbert RJ, Gaw S, Hutchinson TH, Jobling S, Madden JC, Metcalfe CD, Naidoo V, Shore RF (2013) Assessing the exposure risk and impacts of pharmaceuticals in the environment on individuals and ecosystems. *Biol Lett* 9(4):20130493
- Azad MB, Konya T, Persaud RR, Guttman DS, Chari RS, Field CJ, Sears MR, Mandhane PJ, Turvey SE, Subbarao P, Becker AB (2016) CHILd Study Investigators Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. *BJOG* 123(6):983–993
- Blaser M (2011) Stop the killing of beneficial bacteria. *Nature* 476(7361):393–394. <https://doi.org/10.1038/476393a>
- Broderick NA, Lemaitre B (2012) Gut-associated microbes of *Drosophila melanogaster*. *Gut Microbes* 3:307–321. <https://doi.org/10.4161/gmic.19896>
- Broderick NA, Raffa KF, Handelsman J (2006) Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. *Proc Natl Acad Sci* 103(41):15196–15199
- Brune A (1998) Termite guts: the world's smallest bioreactors. *Trends Biotechnol* 16(1):16–21
- Buchon N, Broderick NA, Chakrabarti S, Lemaitre B (2009) Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in *Drosophila*. *Genes Dev* 23(19):2333–2344. <https://doi.org/10.1101/gad.1827009>
- Chatterjee A, Roy D, Patnaik E, Nongthomba U (2016) Muscles provide protection during microbial infection by activating innate immune response pathways in *Drosophila* and zebrafish. *Dis Model Mech* 9(6):697–705
- Chopra I, Roberts M (2001) Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev* 65(2):232–260
- Coker MO, Hoen AG, Dade E, Lundgren S, Li Z, Wong AD, Zens MS, Palys TJ, Morrison HG, Sogin ML, Baker ER (2020) Specific class of intrapartum antibiotics relates to maturation of the infant gut microbiota: a prospective cohort study. *BJOG: An Int J Obstetrics & Gynaecology* 127(2):217–227
- Dethlefsen L, Relman DA (2011) Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc Natl Acad Sci USA* 108(supplement_1):4554–4561
- Dowd SE, Sun Y, Wolcott RD, Domingo A, Carroll JA (2008) Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) for microbiome studies: bacterial diversity in the ileum of newly weaned Salmonella-infected pigs. *Foodborne Pathog Dis* 5(4):459–472
- Dubourg C, Lagier JC, Robert C, Armougom F, Hugon P, Metidji S, Dione N, Dangui NPM, Pfeleiderer A, Abraham J, Musso D, Papazian L, Brouqui P, Bibi F, Yasir M, Vialettes B, Raoult D (2014) Culturomics and pyrosequencing evidence of the reduction in gut microbiota diversity in patients with broad-spectrum antibiotics. *Int J Antimicrob Agents* 44(2):117–124. <https://doi.org/10.1016/j.ijantimicag.2014.04.020>
- Elya C, Zhang V, Ludington WB, Eisen MB (2016) Stable host gene expression in the gut of adult *Drosophila melanogaster* with different bacterial mono-associations. *PLoS ONE* 11(11):e0167357
- Eren AM, Zozaya M, Taylor CM, Dowd SE, Martin DH, Ferris MJ (2011) Exploring the diversity of *Gardnerella vaginalis* in the genitourinary tract microbiota of monogamous couples through subtle nucleotide variation. *PLoS ONE* 6(10):e26732
- Gruntenko NE, Rauschenbach IY (2008) Interplay of JH, 20E and biogenic amines under normal and stress conditions and its effect on reproduction. *J Insect Physiol* 54(6):902–908
- Ha EM, Oh CT, Bae YS, Lee WJ (2005) A direct role for dual oxidase in *Drosophila* gut immunity. *Science* 310(5749):847–850
- Hanna ME, Bednářová A, Rakshit K, Chaudhuri A, O'Donnell JM, Krishnan N (2015) Perturbations in dopamine synthesis lead to discrete physiological effects and impact oxidative stress response in *Drosophila*. *J Insect Physiol* 73:11–19
- Heinsen FA, Knecht H, Neulinger SC, Schmitz RA, Knecht C, Kühbacher T, Rosenstiel PC, Schreiber S, Friedrichs AK, Ott SJ (2015) Dynamic changes of the luminal and mucosa-associated gut microbiota during and after antibiotic therapy with paromomycin. *Gut Microbes* 6(4):243–254
- Hoffmann AA, Turelli M, Harshman LG (1990) Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. *Genetics* 126(4):933–948
- Hosokawa T, Kikuchi Y, Nikoh N, Shimada M, Fukatsu T (2006) Strict host-symbiont cospeciation and reductive genome evolution in insect gut bacteria. *PLoS Biol* 4(10):e337
- Janson EM III, Stireman JO, Singer MS, Abbot P (2008) Phytophagous insect-microbe mutualisms and adaptive evolutionary diversification. *Evolution* 62(5):997–1012
- Jimmerson LC, Bushman LR, Ray ML, Anderson PL, Kiser JJ (2017) A LC-MS/MS method for quantifying adenosine, guanosine and inosine nucleotides in human cells. *Pharm Res* 34:73–83
- Kato K, Ishiwa A (2015) The role of carbohydrates in infection strategies of enteric pathogens. *Trop Med Health* 43(1):41–52
- Kaufman MG, Klug MJ (1991) The contribution of hindgut bacteria to dietary carbohydrate utilization by crickets (Orthoptera, Gryllidae). *Comp Biochem Physiol A-Physiol* 98:117–123
- Khan I, Azhar EI, Abbas AT, Kumosani T, Barbour EK, Raoult D, Yasir M (2016) Metagenomic analysis of antibiotic-induced changes in gut microbiota in a pregnant rat model. *Front Pharmacol* 7:104
- Leonard MM, Valitutti F, Karathia H, Pujolassos M, Kenyon V, Fanelli B, Troisi J, Subramanian P, Camhi S, Colucci A, Serena G (2021) Microbiome signatures of progression toward celiac disease onset in at-risk children in a longitudinal prospective cohort study. *Proc Natl Acad Sci* 118(29):e2020322118
- Levy M, Kolodziejczyk AA, Thaïs CA, Elinav E (2017) Dysbiosis and the immune system. *Nat Rev Immunol* 17(4):219–232
- Li LH, Cowie CH, Gray LG, Moran DM, Clark TD, Rinehart KL Jr (1977) Effects of streptovaricins and their degradation products on RNA-directed DNA polymerase of Rauscher leukemia virus. *J Natl Cancer Inst* 58(2):239–243
- Mars RA, Yang Y, Ward T, Houtti M, Priya S, Lekatz HR, Tang X, Sun Z, Kalari KR, Korem T, Bhattarai Y (2020) Longitudinal multi-omics reveals subset-specific mechanisms underlying irritable bowel syndrome. *Cell* 182(6):1460–1473
- Martino ME, Ma D, Leulier F (2017) Microbial influence on *Drosophila* biology. *Curr Opin Microbiol* 38:165–170. <https://doi.org/10.1016/j.mib.2017.06.004>
- Milani C, Duranti S, Bottacini F, Casey E, Turrone F, Mahony J, Belzer C, Palacio SD, Montes SA, Mancabelli L, Lugli GA, Rodriguez JM, Bode L, de Vos W, Gueimonde M, Margolles A, van Sinderen D, Ventura M (2017) The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. *Microbiol Mol Biol Rev* 81(4):1–67
- Raymann K, Shaffer Z, Moran NA (2017) Antibiotic exposure perturbs the gut microbiota and elevates mortality in honeybees. *PLoS Biol* 15(3):e2001861
- Riquelme E, Zhang Y, Zhang L et al (2019) Tumor microbiome diversity and composition influence pancreatic cancer outcomes. *Cell* 178:795–806
- Rousset F, Bouchon D, Pintureau B, Juchault P, Solignac M (1992) Wolbachia endosymbionts responsible for various alterations of sexuality in arthropods. *Proc Roy Soc London, Ser B, Biol Sci* 250(1328):91–98
- Ryu JH, Ha EM, Oh CT, Seol JH, Brey PT, Jin I, Lee DG, Kim J, Lee D, Lee WJ (2006) An essential complementary role of NF- κ B pathway to microbicidal oxidants in *Drosophila* gut immunity. *EMBO J* 25(15):3693–3701
- Ryu JH, Kim SH, Lee HY, Bai JY, Nam YD, Bae JW, Lee DG, Shin SC, Ha EM, Lee WJ (2008) Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in *Drosophila*. *Science* 319:777–782. <https://doi.org/10.1126/science.1149357>

- Schultz-Cherry S, Dybdahl-Sissoko N, Neumann G, Kawaoka Y, Hinshaw VS (2001) Influenza virus ns1 protein induces apoptosis in cultured cells. *J Virol* 75(17):7875–7881
- Serbus LR, Casper-Lindley C, Landmann F, Sullivan W (2008) The genetics and cell biology of Wolbachia-host interactions. *Annu Rev Genet* 42:683–707
- Shibata T, Ohto U, Nomura S, Kibata K, Motoi Y, Zhang Y, Murakami Y, Fukui R, Ishimoto T, Sano S, Ito T (2016) Guanosine and its modified derivatives are endogenous ligands for TLR7. *Int Immunol* 28(5):211–222
- Snook RR, Cleland SY, Wolfner MF, Karr TL (2000) Offsetting effects of Wolbachia infection and heat shock on sperm production in *Drosophila simulans*: analyses of fecundity, fertility and accessory gland proteins. *Genetics* 155(1):167–178
- Stokholm J, Sevelsted A, Bønnelykke K, Bisgaard H (2014) Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study. *Lancet Respir Med* 2(8):631–637
- Stouthamer R, Breeuwer JAJ, Luck RF, Werren JH (1993) Molecular identification of microorganisms associated with parthenogenesis. *Nature* 361(6407):66–68
- Swanson KS, Dowd SE, Suchodolski JS, Middelbos IS, Vester BM, Barry KA, Nelson KE, Torralba M, Henrissat B, Coutinho PM, Cann IK (2011) Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals similarities with humans and mice. *ISME J* 5(4):639–649
- Tilg H, Cani PD, Mayer EA (2016) Gut Microbiome and Liver Diseases *Gut* 65:2035–2044
- Tilg H, Adolph TE, Gerner RR et al (2018) The intestinal microbiota in colorectal cancer. *Cancer Cell* 33:954–964
- Trebicka J, Macnaughtan J, Schnabl B et al (2021) The microbiota in cirrhosis and its role in hepatic decompensation. *J Hepatol* 75(Suppl 1):S67–81
- Ueno T, Tomita J, Kume S, Kume K (2012) Dopamine modulates metabolic rate and temperature sensitivity in *Drosophila melanogaster*. *PLoS ONE* 7(2):e31513
- Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Teillant A, Laxminarayan R (2015) Global trends in antimicrobial use in food animals. *Proc Natl Acad Sci* 112(18):5649–5654
- Volkova A, Ruggles K, Schulfer A, Gao Z, Ginsberg SD, Blaser MJ (2021) Effects of early-life penicillin exposure on the gut microbiome and frontal cortex and amygdala gene expression. *iScience* 24(7):102797. <https://doi.org/10.1016/j.isci.2021.102797>
- Werren JH, Baldo L, Clark ME (2008) Wolbachia: master manipulators of invertebrate biology. *Nat Rev Microbiol* 6(10):741–751
- Wong AC-N, Wang Q-P, Morimoto J, Senior AM, Lihoreau M, Neely GG, et al (2017) Gut microbiota modifies olfactory-guided microbial preferences and foraging decisions in *Drosophila*. *Curr Biol* 27:2397–2404.e4 <https://doi.org/10.1016/j.cub.2017.07.022>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.