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Gut Microbiome Characterisation of *Chrysomya megacephala*: Isolation, Identification, Antibiotic Profiling, and Initial Documentation of *Leclercia adecarboxylata* from the Fly

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Abstract

***Chrysomya megacephala*, known for its vector potential, harbors a diverse microbiota crucial in understanding disease transmission dynamics. Herein, we report the first documentation of *Leclercia adecarboxylata* isolated from *C. megacephala*. *L. adecarboxylata* is an *Enterobacteriaceae*, gram-negative bacillus that cause infections in human and animals. Additionally, we have reported the presence of *Pseudomonas aeruginosa* and *Enterococcus faecalis* from *C. megacepahala.* The study carried out the antibiotic profiling and hemolytic assays, which revealed distinct resistance patterns and virulence characteristics, shedding light on potential public health implications. *L. adecarboxylata, Pseudomonas aeruginosa* and *Enterococcus faecalis* showed positive result for hemolysis and in terms of antibiotic resistance *P. aeruginosa* strains showed resistance to Amoxicillin, Ampicillin and Tetracycline while, *E. faecalis* showed resistance towards Streptomycin and Tetracycline. However, *L. adecarboxylata* showed sensitivity to all antibiotics. This study was conducted from Kozhikode, Kerala, India, and this is the first of its kind of study from the region to analyse the vector potential of *C. megacephala*. These findings underscore the significance of comprehensive microbiological investigations in vector-borne disease surveillance and management strategies.**

Keywords: *Leclercia adecarboxylata*, *Chrysomya megacephala*, Calliphoridae, Hemolysis, Antibiotic Resistance

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INTRODUCTION

Calliphoridae flies (blow flies) are non-biting dipterans which play an important role in the fields of medical, veterinary and forensic sciences. They forage and breed mostly on garbage and unhygienic areas and thus have a constant association with the pathogens making them an important vector in pathogenic diseases.[1] Several kinds of pathogenic and non-pathogenic bacteria, fungus and parasites have been found to be associated with different species of blow flies; for example, *Lucilia* sps has been found to be a carrier of *Pseudomonas* and Corynebacterium,[2-4] *Chrysomya albiceps* carries *Erysipelothrix rhusiopathiae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecium*, and *Staphylococcus haemolyticus*.[5] *Chrysomya megacephala* (Fabricius, 1794), commonly known as Oriental laterine fly, is one such species under *Calliphoridae* that act as a vector for pathogenic microorganisms thus putting them in the realm of medical entomology.[6] Studies show that *C. megacephala* has a higher vector potential than that of common flies such as *Musca domestica*.[3] They were found to be causing myasis in humans and were able to harbor enteric pathogenic bacteria which will eventually get disseminated by them. *C. megacephala* is known to carry pathogens such as *Escherichia coli*, *Salmonella* spp, *Staphylococcus* spp, *Entrococcus* spp, *Shigella* spp, *Bacillus* spp, *Klebsiella pneumoniae*, *Viridans streptococci*, *Morganella morganii*, *Providencia* spp, *Citroabacter* spp.[3,7,8] Besides their role as a vector, the global distribution of *C. megacephala* has favored it to be part of several vital roles in our ecosystem.[9] *C. megacephala* acts as a pollinator for wild flowering plants,[10] and also come under the list of primary colonizers of carrions which in turn makes them useful in determining Post Mortem Interval (PMI).[11]

The synanthropic nature of blow flies makes them an important agent in spreading bacteria among human living environment. Through regurgitation and defecation, flies transfer the internal microbiome to the environment which indirectly contaminate human environment.[12] Being necrophagous species, blow flies can spread pathogens from affected animal to the surrounding environment as well as to naive animals thereby increasing the severity of epizootic diseases like *Bacillus anthracis.*[13] Based on the reports from various studies, one of the main problems faced by humans is from foodborne pathogens like, *Campylobacter* spp*, Clostridium perfringens, Escherichia coli* 0157:H7, *Listeria monocytogenes, norovirus, Salmonella enterica, Toxoplasma gondii*. These pathogens mainly cause contamination in food categories such as poultry, pork, deli meats, dairy, beef, eggs. This has led to a loss of more than $8 billion, and has been the reason 1,28,000 cases of hospitalization of individuals and 3000 deaths annually.[14] One such example involves the pathogen *Salmonella* where the infection caused the maximum number of deaths in USA, which costs around $2.71 billion in terms of hospitalization and treatment of individuals. In Europe, more than 91,000 cases have been reported which has created a loss of around €3 billion a year. These pathogens are persistent in the gut of humans and animals and are eliminated by feces which are transported by insects to other environments thus spreading the diseases.[15] Since blow flies breed and feed in the fecal matter and decaying organic matter,[16] the study regarding the microbiome of insect could lead a better understanding of the role of blow flies as vectors of pathogenic bacteria. In addition to this, an outbreak of pathogenic avian influenza virus H5N1 that occurred in 2004, in Japan resulted in the death of numerous poultry animals and the blow flies samples collected from the premises indicated that blow flies possible could act as a mechanical vector of these outbreak as they were able to detect the virus in its system.[17]

Leclercia adecarboxylata belonging to the family *Enterobacteriaceae* is a gram-negative, rod shaped, opportunistic pathogen which is often associated with the gut of humans and animals.[18-20] *L. adecarbocylata* is isolated from insects like *Coleoptera*, *Culicidae*, *Muscidae* and *Calliphoridae*.[21-24] There have been several case reports saying that *L. adecarboxylata* has been isolated from the blood of humans. This bacterium can cause bacteremia, wound infection, cholecystitis, and endocarditis, especially in patients who are immuno-compromised with cancer, leukemia, renal failure, and cirrhosis.[25,26] This pathogen has been isolated from different bodily fluids of humans such as blood, wound

secretions, synovial fluids, cerebrospinal and peritoneal fluids.[27,28] However, with respect to previously available literature, no data regarding the presence of *Leclercia* in *C. megacephala* is available. With regard to these data, our current study is the first report of the presence of *Leclercia* sps from the gut of *C. megacephala*. The results from the current study provide a new insight on *Leclercia* carried by *C. megacephala* and can be studied on basis of infections.

The boundless use of antibiotics has caused the emergence of antibiotic-resistant pathogenic and non-pathogenic bacteria related to humans and animals. The rapid spread of resistant bacteria is a global health concern that needs to be carefully monitored.[29] India is one of the largest consumers of antibiotics in the world and also ranked top in the world in terms of carrying antibiotic-resistant pathogens, like multidrug-resistant tuberculosis. According to various regional reports, the common antibiotic resistant pathogens that are prevalent in India include *Salmonella typhi*, *Shigella*, *Pseudomonas* and *Acinetobacter*.[30] In India by 2050, an estimated death of 2 million is projected to occur due to antibiotic resistance. Annually more than 50,000 newborn mortality have been reported due to antibiotic resistant sepsis pathogens because of their resistance towards first-line antibiotics.[30] Insects like houseflies, ants, mosquitoes were confirmed to harbor antibiotic resistant bacteria which act as a vector for disseminating pathogens to humans and animals. Extensive use of antibiotics in animal husbandry, poultry, ranching, and swine farms had led to the harboring of antibiotic resistant bacteria, which can be transmitted directly to humans through their egg, meat and milk.[31] The proper monitoring of these vectors can help in cautious use of antibiotics and also help in policy making when passing laws related to use of antibiotics.[32] Hence the current study checks for the antibiotic susceptibility of bacterial strains isolated from the gut of *C. megacephala.*

Considering all these factors, identification of the bacterial pathogens in blow flies to estimate their vector potential is crucial in order to monitor their transfer in both invertebrates and human habitation. Along with that, knowledge of antibiotic resistant strains carried by *C. megacephala* can help in implementing policies related to antibiotic use in both farms and by humans.

MATERIALS AND METHODS

Collection and identification of flies

C. megacephala sample were collected from Kozhikode, Kerala, India (11°13’34.5"N 75°48’04.0"E). For collection, decaying chicken carcasses were placed in the sample site and were collected using a sweeping net. A total of 20 adult flies were collected in glass jars. Collected flies were then anaesthetized using ethyl acetate and identified using taxonomic keys.[33] Then the flies were subjected to external body sterilization using 70% ethanol and sodium hypochlorite for 1 min. Thereafter, the samples were rinsed in 10 mm phosphate buffered saline (PBS). The adult fly sample was dissected in PBS.[34,35]

Microbial assay

The individual dissected gut contents were then pooled together and then subjected to serial dilution. The diluted sample were then plated on Nutrient agar media. The sample plates were then incubated overnight. Bacterial colonies formed were then selected randomly from the plates based on the distinction in the colony morphology. The selected colonies were then re-streaked into further sub cultures to get pure colonies. A total of 18 colonies were formed and from this the bacterial colony were then subjected for Hemolytic assay.

Hemolytic assay and Biochemical tests

The selected colonies were plated onto the blood agar for overnight incubation. After incubation, the blood agar plate showing Hemolytic assay were selected for molecular sequencing. Blood agar media is a type of enriched medium for the culturing of fastidious organisms which can be used to differentiate between pathogenic and non-pathogenic bacteria. Pathogenic bacteria often undergo hemolysis in blood agar medium which is of different kind like alpha, beta and gamma hemolysis.[36] In addition to this, the bacterial strains were subjected for biochemical tests to differentiate between their phenotypic characters

which include tests like IMVIC, Catalase, Urease, Gelatinase, Acid fermentation test.[37-39]

Molecular sequencing and phylogenetic analysis

Four samples (8,9,14,18) which showed positive result for hemolytic assay were subjected for 16S RNA sequencing. Genomic DNA was isolated from the tissue in leg using NucleoSpin® Tissue Kit and the quality of the isolated DNA was checked using agarose gel electrophoresis. Furthermore, forward primer 16S-RS-F (CAGGCCTAACACATGCAAGTC) and reverse primer

Table 1. Biochemical tests

Bacterial strains	BIOCHEMICAL TESTS											
	Indole	Methyl Red	Voges-Proskauer	Citrate test	Catalase	Urease	Gelatin	Acid Fermentation test (Sorbitol)	Acid Fermentation test (Inositol)	Acid Fermentation test (Glucose)	Acid Fermentation test (Sucrose)	References
Leclercia adecarboxylata BMNKOZ	+	+	-	+	+	+	-	+	-	+	+	37
Enterococcus faecalis BMNJMC	-	-	+	-	-	-	-	+	-	+	+	38
Pseudomonas Aeruginosa BMNCM	-	-	-	-	+	-	+	-	-	-	-	39
Pseudomonas aeruginosa BMNCKJM	-	-	-	-	+	-	+	-	-	-	-	39

+ indicate positive result, - indicate negative result

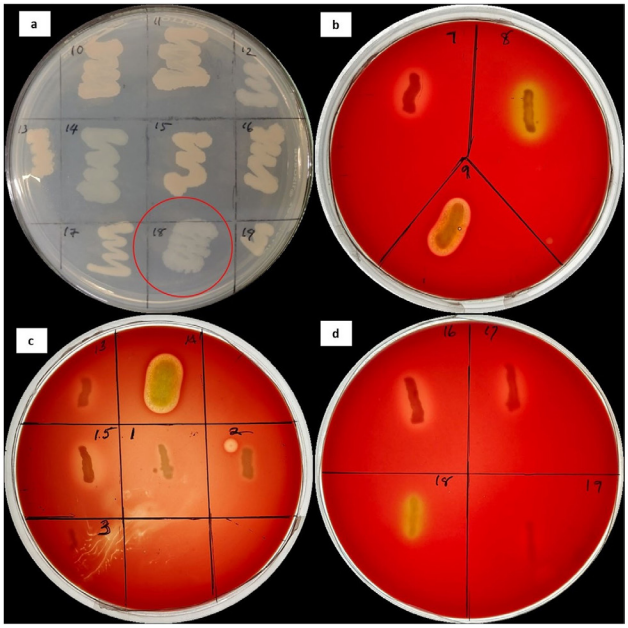

Figure 1. (a) Showing *L. adecarboxylata* (marked) along with bacterial colonies isolated from the gut of *C. megacephala.* (b), (c) and (d) Showing hemolytic activity of strains

16S-RS-(RGGGCGGWGTGTACAAGGC) were used for PCR amplification. The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). Post PCR, BigDYe Terminator v3.1 were used for the sequencing. The sequence alignment was carried out using Geneious Pro v5.1.[40,41] The sequences obtained from molecular sequencing were submitted to NCBI GenBank and compared with other sequences to get sequences that are having maximum identity score. Clustal W, multiple alignment software programme was used for aligning the sequences which are then used for constructing the phylogentic tree using MEGA X software.[42]

Antibacterial susceptibility assay

Kirby-Bauer disc diffusion method was used for the susceptibility test. The antibiotic discs were placed on Mueller-Hinton Agar (MHA) plates inoculated with our selected bacterial strains and kept for overnight incubation for 24 hrs. at 37°C.[43] In our current research, we used Amoxicillin (10 mcg), Ampicillin (30 mcg), Tetracycline (10 mcg), Ciprofloxacin (1 mcg), Streptomycin (300 mcg), Neomycin (10 mcg) for the antibiotic susceptibility test.

RESULTS

Hemolytic assay and biochemical tests

The colonies plated in the blood agar plates showed alpha, beta and gamma hemolysis. The colonies were marked numerically starting from 1-18 (Figure 1). The colonies marked as 8,9,14 and 18 showed alpha hemolysis. These samples were then selected for molecular identification using 16S rRNA. Biochemical assay revealed positive test for the strains especially for *L. adecarboxylata* which can be used to differentiate between *E. coli*. The further results obtained for the strains were given in Table 1 and the confirmatory images for *L. adecarboxylata* were given in Figure 2.

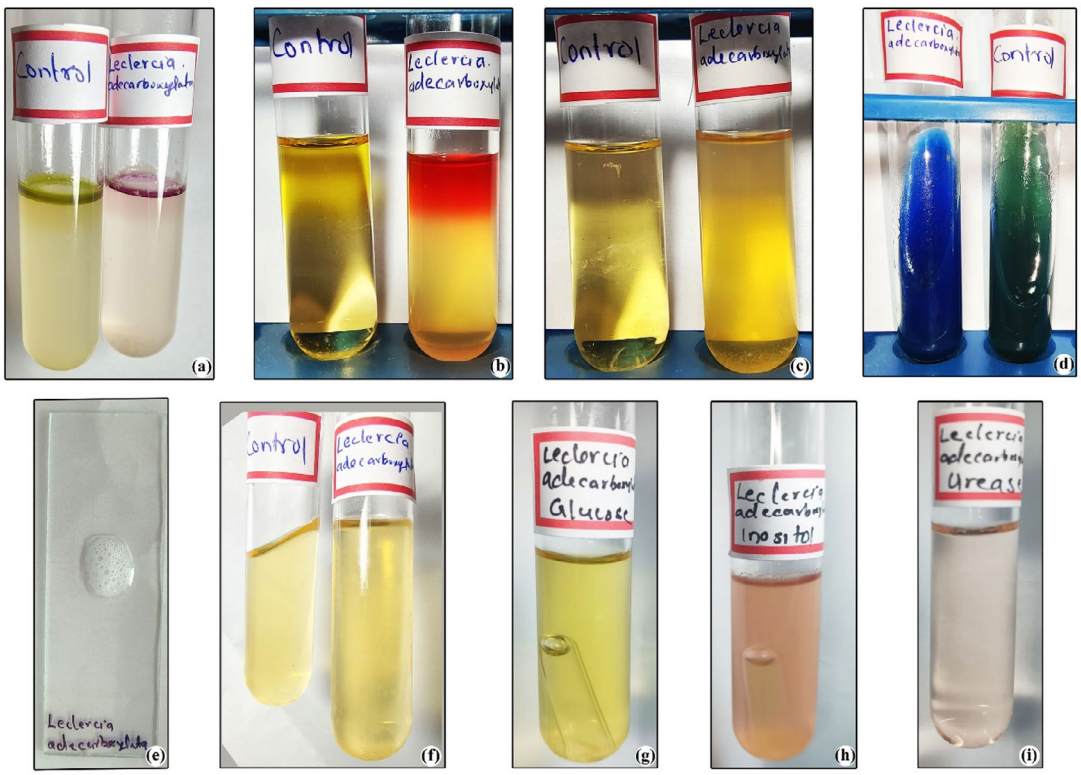

Figure 2. Biochemical Tests; (a) Indole Test, (b) MR Test, (c) Voges-Proskauer Test, (d) Citrate Test, (e) Catalase Test, (f) Gelatinase Test, (g) Glucose Acid Fermentation Test, (h) Inositol Test, (i) Urease Test

Molecular sequencing

Based on the 16S rRNA sequencing, the bacterial samples were identified as *Pseudomonas aeruginosa, Enterococcus faecalis* and *Leclercia adecarboxylata*. Bacterial sequences generated from the molecular sequencing were analyzed to check the quality. Sequence editing and assembling were done using BioEdit sequence alignment tool version.[44] Aligned samples were then used to check the species similarity with the help of BLAST function of NCBI. The generated sequences were submitted to NCBI and accession

Table 2. Zone of inhibition values (in mm) shown by antibiotics against bacterial strains

	Pseudomonas aeruginosa (9)(mm)	*Pseudomonas aeruginosa* (14)(mm)	*Enterococcus faecalis* (8)(mm)	*Leclercia adecarboxylata* (18)(mm)	S[a]	MS[b]	R[c]
Antibiotics							
Amoxicillin (30)	NE	NE	27	22	≥18	14-17	≤13
Ampicillin (10)	NE	NE	25	21	≥17	14-16	≤13
Ciprofloxacin (1)	35	32	20	30	≥21	16-20	≤15
Neomycin (10)	14	17	NE	16	≥20	15-20	≤14
Streptomycin (300)	26	25	14	18	≥21	15-20	≤14
Tetracycline (10)	8	8	2	19	≥19	15-18	≤14

S[a] - Sensitive, MS[b] - Moderate sensitive, R[c]- Resistant[98] , NE- No Effect

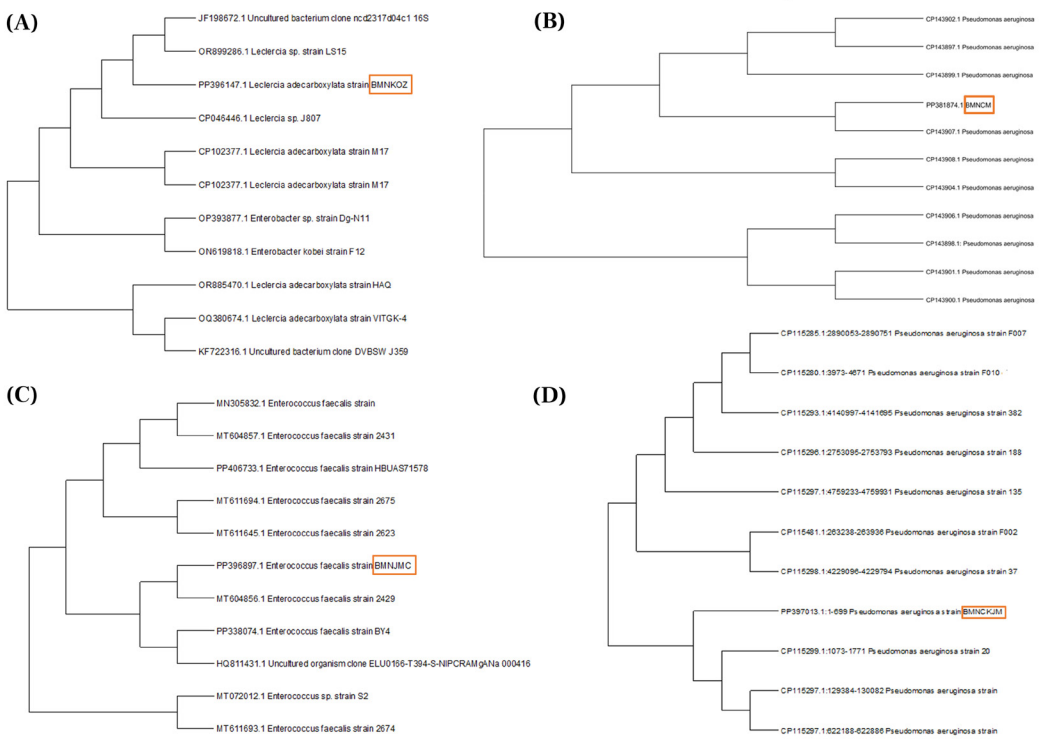

Figure 3. Phylogentic tree (a) *L. adecarboxylata* BMNKOZ (b) *P. aeruginosa* BMNCM, (c) *E. faecalis* BMNJMC (d) *P. aeruginosa* BMNCKJM

numbers were generated as follows: PP381874, PP396147, PP396897, PP397013. The percentage of query coverage values ranged from 80-99%. The evolutionary history was constructed using Neighbor Joining method (NJ).[45] Along with that, the evolutionary distances were calculated using Maximum Composite Likelihood method.[46] In addition to molecular sequencing, phylogenetic relationship between species based on 16S rRNA gene were carried out to identify the sample species and the phylogenetic tree is represented in Figure 3.

Antibacterial susceptibility

The zone of inhibition values is presented in Table 2. Ciprofloxacin was found to be the most effective antibiotics against all four strains of bacteria, followed by Streptomycin (Figure 4). However, Tetracycline showed the least effect against all four bacterial strains. While Amoxicillin and ampicillin had no effect on *P. aeruginosa,* Neomycin also had no effect on *E. faecalis* and also the strain showed some resistance to Streptomycin. In the case of *L. adecarboxylata*, all antibiotics showed considerable sensitivity making the strain not resistant towards the common antibiotics in use.

DISCUSSION

Flies are considered to be an important vector of pathogens due to their abundance and close association with people, animals and their waste.[47] Poor hygiene in animal rearing areas attracts flies to breed and feed on waste, acting as vectors and increase pathogen transmission near human settlements.[48] *Calliphoridae* are abundant in and around the farms and can travel up to 2-3 km making it to transfer the pathogens from one area to another.[49] A study by Urban and Broce, suggested that flies can disseminate loads of bacteria directly to the raw meat which are given as food for dogs that results in intestinal infections and mortality.[49] Basson *et al*. showed

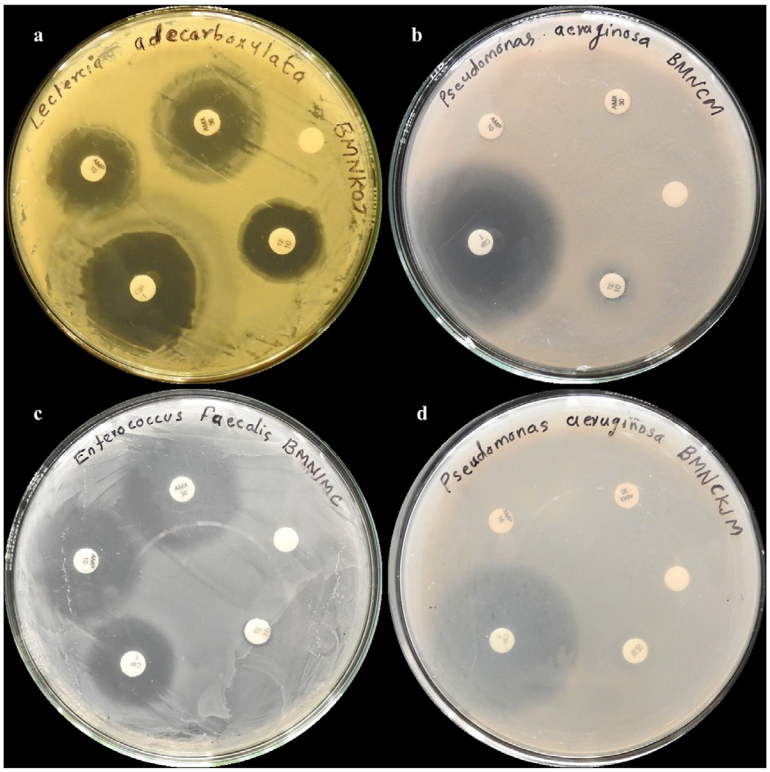

Figure 4. Antibiotic assay showing zone of inhibition by (a) *L. adecarboxylata* BMNKOZ, (b) *P. aeruginosa* BMNCM (c) *E. faecalis* BMNJMC (d) *P. aeruginosa* BMNCKJM

Table 3. Antibiotic resistant strains isolated from various Calliphoridae flies from different studies

Calliphoridae sps	Strains isolated	Antibiotic Resistances	References
Green bottle flies	*Pseudomonas putida*	AMP, CEF, NAL	99
	Pseudomonas spp.	CEF, NAL, AMPP	
	Enterococcus faecalis	KAN, CIP, NAL	
Blow flies	*E. coli*	Amp, AML, CIP, IMI, E, P-G, TE	100
	Klebsiella spp.	P-G, CHL	
	Salmonella spp.	AMP, IMI, P-G, TE	
	Shigella spp.	AMP, AML, IMI, TE,	
	Enterobacter spp.	P-G, TE	
	Staphylococcus spp.	AMP, AML, C, E, P-G, TE	
	Bacillus spp.	P-G	
Lucilia sericata	*E. coli*	AMP, STR, TET, CHL	101
	Staphylococcus spp.	AMP, TET,	
Lucilia sericata	*S. aureus*	TET, AMP,	102
	E. coli	TET AMP CEP, COT	
Chrysomya megacephala	*Lemef17 K. ascorbate*	AMP	103
Lucilia cuprina	*Lemef105 E. coli* (MDR)	AMP, ASB, CPM, CAZ CRO, CIP, ETP, PPT	

Ampicillin (AMP), Amoxicillin (AML), Ampicillin-Sulbactam (ASB), Cefepime (CPM), Ceftazidime (CAZ), Cephalexin (CEP), Chloramphenicol (CHL), Ciprofloxacin (CIP), Cotrimoxazole (COT), Ceftriaxone (CRO), Ertapenem (ETP), Erythromycin (E), Imipenem (IMI), Penicillin-G (P), Piperacicllin-Tazobactam (PPT), Streptomycin (STR), Tetracycline (TE)

that species like *C. albiceps* and *Chrysomya marginalis* can disseminate *Bacillus anthracis* and the high abundance of blow flies feeding on disease affected carcass can lead to the continuation of anthrax infection to other animals due to frequent visit of blow flies with animals. Due to its capability of transfer, it can also take up antibiotic strains of bacteria from one region to other.[50] To support this, studies have shown that flies like *Lucilia sericata* can harbor both antibiotic-resistant and sensitive strains of bacteria, such as *Proteus mirabilis*, within their gut for extended periods, potentially serving as reservoirs and vectors for transmission.[51] Similarly, isolation of antibiotic resistant strains from *Calliphoridae* flies were done by several researchers which is represented in Table 3. Research by Deel suggests that new bacteria introduced into the environment could potentially integrate into the microbiome of flies and also there is a chance that while these flies colonize the human cadaver having pathogenic bacteria, it will get incorporated into fly microbiome and disseminate them thus acting as a carrier.[52] The early and prominent colonization of carcasses by *C. megacephala*,[6] necessitates studies on their vector potential due to inevitable contact with pathogenic environments. With respect to these findings, from our study we were able to isolate and identify *P. aeruginosa* , *E. faecalis* and *L. adecarboxylata* from the gut of *C. megacephala*. *L. adecarboxylata* and is reported for the first time from *C. megacepahala.*

The first report of *Vibrio parahaemolyticus* in *C. megacephala* from Thailand has been reported highlighting the potential of *C. megacephala* as a significant mechanical vector for *V. parahaemolyticus*, a bacterium known for causing gastroenteritis from Calliphoridae family.[53] The first report of *L. adecarboxylata* was found from *Calliphora vicina* collected from hospital premises along with strains of *E. coli* serotype E1525. The authors concluded that, bacterial strain *E. coli* serotype E1525 obtained from *C. vicina* pointed that these flies should be carefully monitored considering it as a mechanism of dispersing bacteria strains. The recommendation is that fly-proof measure should be employed in order to prevent the ingress of fly, thereby limit the introduction of bacteria from a pathogenic environment to non-pathogenic environment.[23] However, till present no literature survey showed the presence *of L. adecarboxylata*

from *C. megacephala*. They mostly cause human infections that are polymicrobial and usually occur in immunocompromised humans.[20] The data and studies focusing on *L. adecarboxylata* were scanty which often results in the misdiagnosis of the pathogen as they share similar biochemical features with *E. coli* and causes diseases like sepsis, septic arthritis, diarrhoea, peritonitis, gallbladder infections which are common diseases of *Enterobacteriaceae*.[19,54] However to study the phenotypic characters, biochemical aspects of the bacterial strains were studied and considerable results were obtained. The *L. adecarboxylata* strain was found to be positive for tests like catalase, indole, MR, citrate, urease, and acid fermentation tests which come in concordance with the previous study. By considering these difficulties in identification we considered the molecular sequencing of *L. adecarboxylata* to confirm the species. Owing to the importance, recently several case reports have confirmed the problems associated with *L. adecarboxylata*. A medical study report showed the death of a 24-week-old premature neonate due to the nosocomial sepsis infection from *L. adecarboxylata* [55] The researcher further suggested that a thorough knowledge of *L. adecarboxylata* is necessary for the neonatologist to understand the nature of neonatal sepsis. In addition to this, a case report showed that a 48-year-old female was diagnosed with Peritonitis from *L. adecarboxylata* and the possible route of *Leclercia* infection was through water source or from farm animals.[56] *L. adecarboxylata* also found to cause Necrotizing Soft Tissue Infection (NSTI) which can lead to serious health problems like damage to skin, muscle, soft tissues and even septic shock and consequent multiorgan failures.[57,58] Also, a multidrug resistant *L. adecarboxylata* which causes respiratory distress in cow was reported from India. The authors suggested that the epidemiology and antimicrobial resistance of *L. adecarboxylata* should be studied with respect to different geographic locations around the world and more studies will bring the zoonotic significance of the species.[59] While keeping the suggestion of Choudhary to conduct studies in different geographical locations, we selected the district of Kozhikode, Kerala, India. The district of Kozhikode in currently a hotspot of disease outbreaks like NIPAH 2018 and Shigella.[60,61] Not only this particular district, every place should by carefully monitored to mitigate the chance of a new disease outbreak. To mitigate the disease, outbreak a better understanding of new strains along with their transmission pattern through vectors should be monitored. Since *Calliphoridae* flies are commonly associated with farm animals, the chance of *Leclercia* spp to reach these animals and to humans cannot be neglected.[55]

Several bacteria secrete virulence factors (toxins), that allow them to disrupt the host defensive mechanisms and impair host functions. Bacterial hemolysins are exotoxins which attack blood cell membrane thereby disrupting its cell wall. This action could increase the severity of infections.[62] Therefore, the hemolysis is one virulence factor that can determine the pathogenicity of bacteria strain. In our study *L. adecarboxylata* showed hemolysis which contradicts the studies done by Muratoglu and Anuradha where the specimen showed negative blood hemolysis.[25,37] A possible explanation for this contrasting result was suggested by Snak, where the author pointed that this change might be due to the presence of hemolysin gene in the strain.[63] Our strain showed alpha hemolysis which can interpreted in such a manner that they can act as pathogens and cause infection in humans and animals. Also, in the era of emerging antimicrobial resistance, a worldwide focus was given in terms to reduce the susceptibility of public to various resistant pathogens. *Leclercia* is beginning to exhibit antibiotic resistance, much like the majority of antibiotic-resistant bacteria. This indicates that they are a potentially harmful organism and should be treated with caution.[64] Even though *L. adecarboxylata* is susceptible to many antibiotics, resistant strains been reported recently. The resistance of *L. adecarboxylata* isolated from pig farms to aminoglycosides, quinolones and trimethoprim-sulfamethoxazole was been reported by Yao *et al*.,[65] The transferable drug resistance observed in *L. adecarboxylata* via R plasmids raises concerns, as these plasmids have the potential to be transferred to human pathogenic bacteria, thereby heightening the risk of drug-resistant bacterial development. This underscores the emergence of antibiotic resistance in *L. adecarboxylata* as an issue linked to environmental antibiotic exposure in food

production.[66] From our study *L. adecarboxylata* strain showed susceptibility to all antibiotics namely Amoxicillin, Ampicillin, Ciprofloxacin, Neomycon and Streptomycin. In our study, Ciprofloxacin showed the best result whereas neomycin showed the least effect. Regular monitoring of *L. adecarboxylata* for antibiotic resistance changes is crucial, warranting attention in future research endeavors. Furthermore, educating individuals involved in farming is imperative. Promoting educational initiatives that underscore the prevention of antibiotic misuse and the avoidance of multi-drug resistant bacteria emergence should also be prioritized.

Investigating the vector harboring capacities of flies is crucial for various biotechnological applications including biomedicine (treatment of diseases), agrobiotechnology (consume organic waste rapidly thereby help in disposal of meat and fish as well as manure) and insect-borne disease prevention.[67-70] Moreover, Zaher *et al*., isolated bacteria species like *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, and *Staphylococcus hominis* from *Calliphoridae* and other forensically important necrophagous insects.[71] Our research isolated two strains of *P. aeruginosa* from the gut of *C. megacephala*, consistent with prior findings of Gaszv *et al*., where the author suggested *P. aeruginosa* prevalence in *Lucilia* spp., where both are important carrion feeders under *Calliphoridae*.[4] *P. aeruginosa* is found to be a pathogen for causing infections such as nosocomial pneumonia, urinary tract infections, malignant external otitis, endophthalmitis, endocarditis, meningitis and septicemia.[72] In our study, *P. aeruginosa* strains exhibited hemolytic activity, similar to findings from (Al-Saffar and Jarallah) and Macin *et al*., where the isolated *P. aeruginosa* from hospital environments showed hemolysis.[73,74] Furthermore, our hemolytic strain showed antibiotic resistance to few antibiotics which was similar to the antibiotic resistant hemolytic strain isolated by Macin *et al*.,[74] Hemolysis is a crucial pathogenicity deciding factor in *P. aeruginosa*, observed consistently across different hosts and environments, as demonstrated by Hossain *et al*., in cattle samples.[75] While taking the case of antibiotic resistance in *P. aeruginosa*, Sh AL-Salihi and Hameed, show resistance of *P. aeruginosa* to the antibiotic in the following order as ampicillin (100%), amoxicillin (97.3), neomycin (91.4%), ciprofloxacin (84%).[76] In our study, *P. aeruginosa* strains show high resistant to amoxicillin, ampicillin and moderate resistant to neomycin. However, it shows contradicting result in the case of ciprofloxacin where we got high zone of inhibition values. The authors suggested that the reason for the development of resistance is due to the beta-lactamase production, the presence of strong barrier to diffusion at the outer membrane of bacteria and bacterial efflux of *P. aeruginosa.* Furthermore, our *P. aeruginosa* strain has no effect from amoxicillin and ampicillin which come in accordance with the study by Ahmed *et al*., where the researcher pointed that amoxicillin and ampicillin were ineffective against *P. aeruginosa.* These insights will help while administrating antibiotics against *P. aeruginosa* infection in humans and animals.[77]

In our current study, we also isolated *E. faecalis* from the gut of *C. megacephala*. *Enterococcus* species are gram-positive cocci which causes enterococcal bacteremia, urinary tract infections, meningitis, endocarditis which subsequently results in hospitalization and mortality. Out of this, *E. faecalis* is one common species that causes bacteremia.[78-80] Our *E. faecalis* strain showed hemolytic activity which is consistent with the previous findings of Izumi *et al*.,[81] Wang *et al*., detected the *cylA* gene in majority of *E. faecalis* strain (71%) potentially contributing to γ-hemolytic activity. These studies aimed to evaluate the probiotic and safety aspects of *E. faecalis*, suggesting that strains lacking hemolytic activity and other virulence genes could be potential probiotic candidates.[82] This was supported by Hashem *et al*., who investigated the virulence factors contributing to *E. faecalis* pathogenicity including hemolytic assay and found a significant correlation between hemolysis and the presence of *Cyl* gene which produce the Cytolysin toxin that increases the severity of infection.[83] Although our study did not investigate gene-level correlations, the observed hemolysis activity in our samples may contribute to increased infection levels of *E. faecalis*, warranting further investigation. In our study, *E. faecalis* showed no resistance towards Ciprofloxacin and rather show sensitivity towards

amoxicillin, ampicillin and streptomycin. However, bacteria strain has no effect by neomycin and tetracycline. This resistance towards tetracycline result was come in accordance with the study by Kim *et al*., where the isolated *E. faecalis* strain showed resistance towards tetracycline. The authors further supported the resistant phenomenon with gene level studies and showed that common genes responsible for the resistance were tet (M), tet (L).[84] In addition to this, research by Xuan *et al*. showed that *E. faecalis* is resistant towards erythromycin (91.1%), tetracycline (100%), ciprofloxacin (66.1%), bacitracin (87.8%) and chloromycetin (41.1%). The resistance to ciprofloxacin contradicts with our strains result which showed moderate sensitivity. Xuan *et al*., pointed out that resistance of bacteria towards few antibiotics and absence of resistance to few antibiotics like nosipheptide and enramycin results can be used in policy making while suggesting the use of antibiotics in pig farms.[85]

Another important aspect to look into the vector potential of fly is the phenomenon of horizontal gene transfer among bacteria. Horizontal transmission of antibiotic resistance was found to occur in the crop of insects.[86] Petridis *et al*. reported that the gastrointestinal tract of houseflies provides a suitable environment for the horizontal transfer of resistant genes and virulence genes.[87] Additionally, the plasmid mediated horizontal resistant gene transfer were illustrated by Akhtar *et al*. in houseflies.[88] Zurek and Ghosh reported that in the gut of housefly there is a frequent transfer of tetracycline resistance gene tet (M) between *E. faecalis* strains. So, inside the gut of insects, bacteria can actively share toxins and antibiotic genes along with proliferation of bacterial species.[89] With respects to these studies, the same phenomenon in blow flies cannot be neglected as the feed and breed in pathogenic environments. Also, according to our knowledge, no studies has been reported to find out the horizontal gene transfer in *C. megacephala* and limited studies being reported about the pathogens associated with this fly. Additional importance of understanding the blow fly microbiome is that the information regarding blowfly associated microorganisms is found to be crucial in the development of PMI prediction models. Previous researches showed that soil and insects are two main factors associated with carrions. Hence, microbial communities associated with soil and insects also have significant role in the development of PMI models.[90,91] Therefore, the current study of microbiome association with blowflies along with future metagenomic studies will help in increasing the accuracy of PMI by forensic scientists.[92,93]

Furthermore, while looking into the relevance, our current study also plays an influence in economic aspects. Due to the infestation of blow flies, into the sea food industry a large economic loss was found and it was shown by several research studies. Fish samples were taken from the Puthiyappa fishing harbor along the Kozhikode district's coastline to check for the presence of *Campylobacter* spp. The results showed the presence of *Campylobacter coli* and *Campylobacter jejuni*, which can be a source of food-borne campylobacteriosis transmission.[94] Similar to this, it was confirmed that there is antibiotic-resistant *Listeria* spp. present in fishes in Kerala's fish harbors. This is concerning because Kerala is a major seafood exporter and the presence of these bacteria can cause regulatory alerts from importing nations thus causing huge economic loss.[95] *C. megacephala* is found to be abundant in and around harbors where sea food processing takes place and they can spread the pathogens to nearby areas.[96] Aak *et al*. reported an estimated loss of half a million euros from stock fish industry in Norway due to the infestation by *Calliphoridae*.[97]

CONCLUSION

In our current study, *L. adecarboxylata* is the bacteria was reported for the first time from *C. megacephala.* The strain showed hemolysis which contribute to their pathogenicity whereas the strain is susceptible to antibiotics. Since there is a potential that it will eventually develop antibiotic resistance, a thorough investigation into antibiotic pathogens pertaining to blow flies have to be conducted which will help in policy making and use of antibiotics in various animal and human related fields. Also, the results from the current study indicates that *C. megacephalas* has a vector potential in carrying pathogenic bacteria. To get a better understanding future studies focusing on in

vivo experimental studies to determine vectoral capacity of flies, epidemiological aspects, and transmission high resolution sequence-based studies are to be carried out.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

BMN and MT conceptualized the study. BMN performed investigation. MT supervised the study. BMN wrote the manuscript. MT reviewed and edited the manuscript. Both authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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