ORIGINAL ARTICLE

# Isolation and Characterization of Human Health-Concerned Bacteria from the Musca Domestica in Urban Environments of Districts Rawalpindi and Chakwal, Pakistan

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**ABSTRACT**

**Objective:** To explore and characterize the microbial fauna associated with the houseflies under different environments.

**Study Design:** Cross-sectional study.

**Place and Duration of Study:** The study was carried out at the Department of Health Sciences, Capital University of Science and Technology, Islamabad, Pakistan from June 2022 to October 2022.

**Materials and Methods**: The 50 samples per location were taken from the urban environment of Rawalpindi and Chakwal districts. Then the species of bacteria were isolated for Biochemical and Molecular characters for the most prevalent bacterial species. Antibiotic sensitivity testing was also done for identified species.

**Results**: The biochemical analysis gave significant results regarding the *Proteus* and *Staphylococcus* species. Then the phylogenetic analysis of isolated strains showed their close association with *Proteus mirabilis* in the case of R1_785 and with *Proteus Vulgaris* and *Staphylococcus Xylosus*. The identified strains, after the antibiotic sensitivity testing identified to be the most resistant ones moreover, their phylogenetic history showed that they diverged independently as per their evolutionary analysis.

**Conclusion**: The *Staphylococcus Xylosus* susceptibility was highly resistant against Gentamycin and least resistant against Imipenem and Tazobactam. These findings suggest houseflies' potential role in transmitting pathogenic bacteria with antibiotic resistance in households.

**Keywords**: *Bacteria, Houseflies, Musca Domestica, Parasites, Phylogenetic Analysis, Proteus Mirabilis, Proteus Vulgaris.*

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## Introduction

*Musca domestica,* commonly called a housefly, is mostly mentioned as filth flies. From the start of human life houseflies also learn how to live in association with humans. The housefly [*Musca domestica]* belongs to the order Diptera and the family Muscidae. They are cosmopolitan in nature and present worldwide. Their habitat includes decaying organic matter like animal manure, litter and animal bedding, where they undergo reproduction and development. *M. domestica* belongs to species that are endophilic and synanthropic in nature, which means that it completes its lifecycle in human habitats. [1] The environment is contaminated by these flies with antimicrobial-resistant bacteria. The fly adapts to any environment with that of humans, animals, and maggots. These all are enriched with organic matter and possess microbial flora.[2]

The adult house flies are highly mobile and carry

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bacteria from the septic environment by coming in contact with these surfaces through wings, feet, or bodies. Interactions that are present between the microorganism and the host are categorized as the source of nature for modulating animal physiology, fitness, and social behavior of the host.[2] Flies adapt four ways to transmit diseases at the surface of their body, vomitus regurgitation, the hair, and alimentary canal passages. The structure of the fly is excellently adapted to carry pathogens and collect them. It has a profusion of hairs that can collect environmental detritus. Bacteria can be isolated from external surfaces, internal surfaces, vomitus, and feces of the house fly samples.[3] The maggots of flies are rich in microbial flora. They can carry pathogens physically as they have an exoskeleton made up of a cuticle and that of the double layer Type 11 peritrophic matrix [PM], which helps to provide the site of attachment.[4]
According to the U.S. Food and Drug Administration, houseflies act as a tributary factor for the dispersion of various infectious and other foodborne diseases. Houseflies have the ability to carry 100 agents of different etiological diseases like viral, bacterial, and protozoan diseases.[5] The diseases caused by the houseflies as vectors include diphtheria, dysentery, intestinal parasites, typhoid, leprosy, and fowl cholera. Molecular analysis depicted that house flies are dispersing groups of microorganisms.[6] The evidence suggests *M. domestica* plays an important role in the transmission of diseases.
It is found that the risk of diarrhea is more in the area where the number of flies increases suggesting the link between its transmission by houseflies.[7] Life-threatening diseases in humans and animals are caused by pathogens house flies carry.[8] Allergy cases that occur due to houseflies are rare but various respiratory allergies due to occupational exposure have been reported.[9]
As antibiotic resistance develops, many bacterial isolates play a significant role in clinical terms. It is estimated that flies carry specific pathogenic bacteria and different nonpathogenic bacteria that are carriers of different antibiotic-resistant genes. Pathogens vary in their characteristics, depending on the area of collection. The samples of houseflies that are collected from the hospitals have higher numbers of bacteria as compared to other locations and are highly resistant to some antibiotics like Cephalothin and Gentamycin. Antimicrobial resistance of bacteria and fungi present in houseflies is higher, especially in samples of those taken from hospital environments or animal farms.[10] Transmission of many infections is associated with the hospital environment.[11] It has affirmed its relationship with the foodborne pathogens *Escherichia coli, Salmonella,* and *Shigella* spp. Antimicrobial resistance (AMR) is a global risk to human health.[12,13]
This study aims to compare the species richness and relative abundance of bacteria carried by houseflies in houses of different locations.

## Materials and Methods

### Ethical Permission

This analytical study was conducted in the Department of Health Sciences, Capital University of Science and Technology, Islamabad Pakistan from July 2022 to December 2022 after getting approval from the Institutional review board (BI&BS/ERC/22-3).

### Sampling Locations and Collection of Samples

Different locations were selected from Rawalpindi and Chakwal districts. The sampling areas were the kitchens of domestic houses. Altogether 300 samples were collected. The collection was done during the month of June to September. Adult houseflies were collected by the use of a nylon insect net.
Flies were transferred to the glass bottles, immediately transported to the laboratory and kept in refrigerator at -2◦C. Houseflies from each location were transferred to autoclaved centrifuge tubes containing 10ml Phosphate Buffer Saline solution [PBS] and 100ml of distilled water. All centrifuge tubes were vortex for 3-5 minutes. The centrifuged tubes were labeled according to the location from where the sample was collected.[15]

### Enumeration of Microbial Load

About 0.1 ml of the sample homogenized in Butterfield's phosphate-buffered water and then inoculated into Plate Count Agar, Nutrient Agar, Baird Parker agar, Bacillus cereus agar, and Violet Red Bile Agar using the surface spread method.[16] The plates were incubated at 35◦C for 24 to 48 h, and the


**Fig 1: Cities of Chakwal and Rawalpindi in the Potohar Region of Punjab, Pakistan.**[14]

colonies were counted, and results were expressed as colony-forming units per gram (cfu/g). The presence or absence of *Staphylococcus, Enterococcus, Bacillus, Salmonella, E. Coli* and *Coliform* was assessed according to the recommended standard methods.[17,18]

**Phenotypic and Biochemical Characterization**

The isolated bacteria samples were characterized on the basis of colony morphology, Gram's reaction, sporulation test, motility tests, enzymatic reactions, and biochemical tests.[17,19-21]

**Validation of Biochemical Tests using API 20E**

The bacteria were also characterized biochemically using the API 20E kit. The standard procedure was undertaken for the biochemical characterization of bacteria includes 20 miniaturized tests for the identification and characterization of bacteria.[22]

**Genetic Characterization**

**16S rRNA Sequencing:** The high throughput and the earliest technique to study microbial ecology is the use of 'the 16SrRNA sequence which seems to be the most conserved one. It is cost-effective approach in a community for the survey of bacteria.[2] To determine the microbiota associated with the houseflies, the preserved strains were sent for 16S sequencing, and the samples were sequenced from Microgen Korea. , The sequences were submitted to the National Centre for Biotechnology Information (NCBI).[23] (Accession Numbers: 190605-012_A01_R1_785F.ab1, 190605-012_C01_R1_907R.ab1 and MN252579.1)

**Antibiotic Sensitivity Test:** Kirby Bauer test was performed to check the resistance of isolated and sequenced strains that are either resistant to antibiotics or susceptible. The strains, with less zone of inhibition showed resistance to that specific antibiotic, and the strains with more area of inhibition were susceptible.[24]

**Phylogenetic Analysis**

MEGA 11[25] was used for Phylogenetic Analysis.

## Results

The growth obtained on the differential media was streaked further to obtain the bacteria isolates. Different types of bacteria were obtained, having

different morphology, color characteristics, colony characteristics, and pigmentations. All these colonies predicted which species they belonged and their character. The bacterial plates that seemed to be more prevalent were further purified by streaking and culturing them repeatedly; hence, the purified strains were obtained. These were further stored in the glycerol stock and put in the refrigerator for future use. These pure strains also contained duplicates, which means one strain had two copies.

**Biochemical Analysis**

**Staining of Pure Cultures**

The Gram staining method performed the staining of pure cultures. The results were significant and concluded that the bacterial species obtained on MaConkey were stained purple. The duplicate pink sample was also stained purple, which concluded that the species grown on MaConkey were Gram-Purple. Moreover, their microscopic examination exhibited circles. The strains obtained on the Mannitol Salt agar were stained purple in the case of the first strain but pink in the case of the other strain, which indicated that the first was Gram -Positive and the other was Gram- Negative, respectively. The strains obtained on the EMB were stained pink, meaning they were gram-negative.

The urease test that was coined for the analysis that the strains either use the urea or acquire urea after the two days examination was positive for just two strains while the other showed a negative result. The result was considered positive if the yellow color of the media turned into pink after the utilization by the strain culturing in that plate.

In the Citrate Utilization Test, Simmons Citrate agar was a defined medium containing sodium citrate as the sole carbon source. The pH indicator, bromothymol blue, turns from green at neutral pH (6.9) to blue when a pH higher than 7.6 is reached alkaline[26] The results showed that just one strain gave positive results in the media and turned blue after 4 days. This indicates that a specific strain was utilizing citrate for metabolic activities.

The results for catalase tests were predicted by observing the bubbles that spontaneously produced after adding 3% hydrogen peroxide. The bubbles appeared suddenly and disappeared after some time. The results are summarized in Table 1.

**Validation of API 20E kit results by Using Media**

**Table 1: The summary of biochemical characterization and microscopic analysis of pure bacterial strains obtained from *Musca domestica* after the incubation period of 96 hours at 37 °C**

| S. No | Strain ID | Microscopic Analysis | Gram Staining | Citrate Test | Catalase Test | Urease Test |
|---|---|---|---|---|---|---|
| 1 | MSA(R)ISL9/4DU P1(P) Res | Circle | Purple violet | Negative | Positive | Negative |
| 2 | MSA(R)ISL9/4DU P2.2(P) Res | Circle | Pink | Negative | Negative | Positive Slightly Pink |
| 3 | EMB (R)ISL9/4DUP2(P) RES | Rod | Pink | Negative | Positive | Negative |
| 4 | EMB(R)ISL9/4DU P1(P)RES | Rod | Pink | Negative | Positive | Positive Slightly Pink |
| 5 | MACC(R)(G.K)9/ 4PURE 1(RES) | Circle | Purple | Positive | Positive | Negative |
| 6 | MACC(R)(G.K)9/ 4 PURE2 (RES) | Circle | Purple | Negative | Positive | Negative |
| 7 | MACC(R)(G.K)29 /5 Pure | Circle | Purple | Positive | Negative | Positive |
| 8 | MACC(R)(G.K)30 /5 Pure | Circle | Purple | Positive | Positive | Positive |

The results of biochemical tests were validated by using API20E, which provided evidence that the results obtained after the biochemical tests were true with reference to the API 20E strip results. Using the API 20E, the change in color was predicted using the reference guide (Table 2).

**Table 2: API20E kit results that are interpreted by using the reference guide**

| S. No | Test Name | Result | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | ONPG | Negative | Negative | Negative | Positive | Positive | Positive |
| 2 | ADH | Negative | Negative | Positive | Positive | Positive | Positive |
| 3 | LDC | Negative | Negative | Negative | Negative | Negative | Negative |
| 4 | ODC | Negative | Negative | Negative | Negative | Positive | Positive |
| 5 | CIT | Negative | Negative | Positive | Positive | Positive | Positive |
| 6 | H2S | Negative | Negative | Positive | Positive | Positive | Positive |
| 7 | URE | Positive | Positive | Positive | Positive | Positive | Positive |
| 8 | TDA | Negative | Negative | Positive | Positive | Positive | Positive |
| 9 | VP | Negative | Negative | Negative | Negative | Positive | Positive |
| 10 | GEL | Negative | Negative | Positive | Positive | Negative | Positive |
| 11 | GLU | Negative | Negative | Positive | Positive | Positive | Positive |
| 12 | MAN | Negative | Negative | Positive | Positive | Positive | Positive |
| 13 | INO | Negative | Negative | Positive | Negative | Negative | Negative |
| 14 | SOR | Negative | Negative | Negative | Negative | Negative | Negative |
| 15 | RHA | Negative | Negative | Negative | Negative | Positive | Positive |
| 16 | SAC | Negative | Negative | Positive | Positive | Positive | Positive |
| 17 | MEL | Negative | Negative | Negative | Negative | Positive | Positive |
| 18 | AMY | Negative | Negative | Positive | Positive | Positive | Positive |
| 19 | ARA | Negative | Negative | Negative | Negative | Positive | Positive |

The results showed that strain 1 is the non-sugar fermenting, non-citrate utilizing, urease-producing strain; moreover, it also has negative effects on hydrogen peroxide production. The strain 2 is thought to be the same as the strain. Strain 3 that is grown on the MacConkey gave positive sugar results which means they are sugar fermenting; on the other hand, they also provide positive urease results that illustrate that they utilize the urea and metabolize it by producing urease enzyme. Strain 3 is also predicted to produce hydrogen peroxide and use citrate as well. The biochemical test results for strain 4 are more or less than the same as that of strain 3; the difference lies in the Inositol test, which seems negative in contrast to strain 3. Strain 5 is thought to give positive results for sugars. It gives negative results for Inositol, Sorbitol, and gelatin. Strain 6 is thought to predict almost the same results as strain 5, the differences lie in inositol and sorbitol tests, but it gives positive results in the case of gelatin.

**Molecular Characterization**

The strains that are prevalent are sent for sequencing. The 16S rRNA sequences of three strains were obtained. Their BLAST result predicted that the strain that is labeled as R1_785 sequence Id and obtained from MACC(R) (G.K)29/5 Pure shows 95.98% similarity to that of other closely reported Proteus mirabilis strain S3 16S ribosomal RNA gene, the partial sequence with the query coverage of 66%. On the other hand, MACC(R)(G.K)30/5 Pure, whose sequence Id is R1_907 shows a sequence similarity of 97.27% with 76% query coverage with *E* value 0.0. The *Staphylococcus* sp. Strain RS 4 785 16S with accession code MN252579 closely resembles *Staphylococcus Xylosus* (KY992565) and is grouped with only a 0.10% difference. There is a 0.28% difference between MN252579 with KJ6341142, and a difference of 0.39 % between MN252579 and MH144255; MN252579 has a maximum difference of 1.06% with FN646069.

After the sequencing (190605-012_A01_R1_785F.ab1, 190605-012_C01_R1_907R.ab1 and MN252579.1) and

BLAST results for strain R1_785 that gave a 95.98% similarity index with 66% query coverage and 0.0 E, value, further procedure was carried out towards the phylogenetic history of specie. The sequences closest to the strain R1_785 were taken; these are total of 21 sequences that give the closest similarity to that of R1_785.

The results of the phylogenetic history of strain R1_785 (Figure 2) by using the maximum likelihood tree were interpreted and illustrated by the branching pattern of the tree that the *Proteus Vulgaris* strain PW108 was thought to come top in the evolutionary history, which further diverged into the *Proteus Vulgaris* strain MAR after the period of time. In the time-lapse, the *Proteus Vulgaris* strain CIP103181T diverged from both strains by evolution which further led toward the uncultured Proteus spp. clone W60 divergence. This strain further evolved into *Proteus Vulgaris* U133. The *Proteus Vulgaris* U133 has changed its 16S rRNA sequence and diverged into DSM 13387T; it has given the divergence to *Proteus Vulgaris* strain ATCC29905. The strain *Proteus Vulgaris* strain PCS2 converged to *Proteus Vulgaris* strain ATCC 29905. On the other hand, *Proteus Vulgaris* strain PCS2 further diverged the *Proteus Vulgaris* strain NBRC 3045. Three strains further diverged from the *Proteus Vulgaris* strain NBRC 3045and these were *Proteus Vulgaris* strain SP13, which split into two more *Proteus Vulgaris* strain AIII and side by side another strain as well, diverged from it that is *Proteus* spp. strain PMCPr.

Moreover, another strain that is actually diverged from *Proteus Vulgaris* strain NBRC 3045 is *Proteus* spp. strain KL14 that further diverged into another strain i.e. *Proteus vulgaris* strain BN 1954. A strain diverged from all these strains named *Proteus Vulgaris* strain knp3.

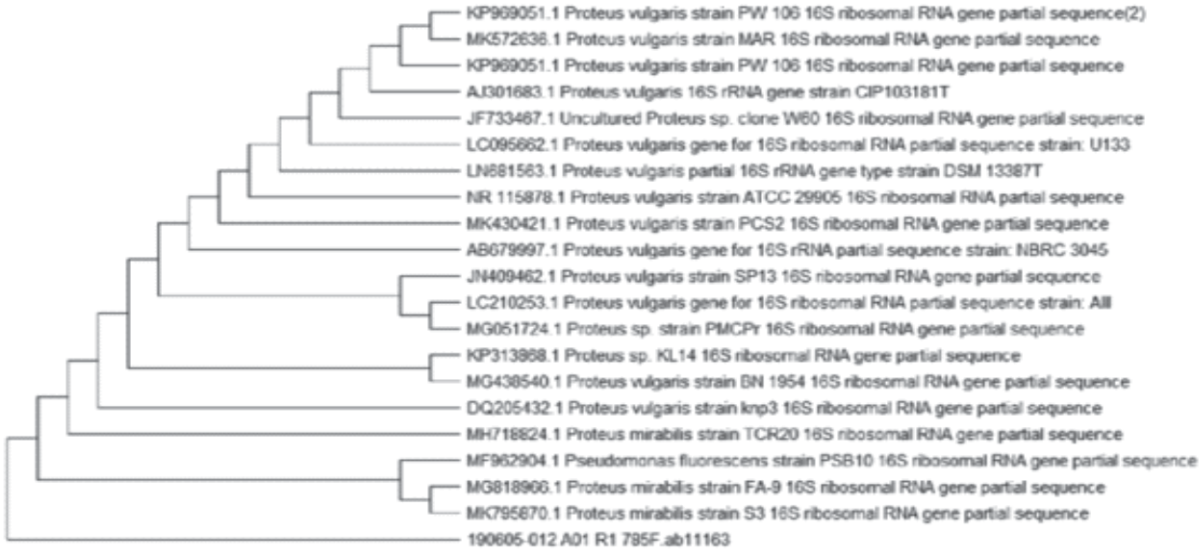


**Fig 2: Phylogenetic Tree for strain RI_785 and among 21 closely related strains. Cladogram was generated on the basis of the similarity of genome sequence among the strains of different bacterial species and the isolated strain. Our strain of interest is at the bottom end of the tree.**

**Antibiotic Sensitivity Testing**

The antibiotic sensitivity testing was performed for all the preserved strains. The drug resistance pattern and the zone of inhibition of all of the strains are mentioned in Table 3.

Bactria on Musca DomesticaStrain 1 seems to have less resistance against all these antibiotics, which means it is susceptible to these all, so these antibiotics may harm the bacteria by either ceasing its growth or disrupting the cell wall. On the other hand, strain 2 seems to be resistant to Ampicillin, Fluconazole, cephalexin, and Nalidixic acid. However, it seems to be susceptible to other antibiotics. This strain is more resistant as compared to strain 1. Strain 3 seems to be resistant to Imipenem, Kanamycin, Ampicillin, Fluconazole, and cephalexin. It is the more resistant strain among all strains. Besides this, strain 4 is resistant to Fusidic


**Fig 3: Phylogenetic Tree for strain RI_907 and among 21 closely related strains. Cladogram was generated on the basis of the similarity of genome sequence among the strains of different bacterial species and the isolated strain. Our strain of interest is at the bottom end of the tree.**

**Table 3: Antibiotic sensitivity pattern of bacterial strains**

| Sr. No. | Antibiotic Name | Symbol | Strain 1 | | | Strain 2 | | | Strain 3 | | | Strain 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Chloramphenicol | C | 2.0 | 2.1 | 2.1 | 1.8 | 1.8 | 1.9 | 1.2 | 1.1 | 1.1 | 2.2 | 2.1 | 2.5 |
| 2. | Tetracycline | TE | 1.5 | 1.7 | 1.6 | 1.3 | 1.2 | 1.3 | 1.1 | 1.1 | 1.2 | 2 | 2 | 2 |
| 3. | Piperacillin Tazobactum | TZP | 2.3 | 3.1 | 2.4 | 2 | 2 | 2 | 2.3 | 2.2 | 2.2 | 1.9 | 1.8 | 1.8 |
| 4. | Imipenem | IMP | 2.5 | 2.7 | 2.5 | 2 | 2.1 | 2.1 | 0 | 0 | 0 | 2.1 | 2.2 | 2.3 |
| 5. | Ciprofloxacin | CIP | 2.3 | 2.5 | 2.3 | 1.6 | 1.6 | 1.6 | 2 | 2 | 2.1 | 2.2 | 2.2 | 2.3 |
| 6. | Norfloxacin | NOR | 2.6 | 2.1 | 2.4 | 1.5 | 1.4 | 1.5 | 1.9 | 1.6 | 1.9 | 1.6 | 1.5 | 1.6 |
| 7. | Fusidic Acid | FD | 1.1 | 1.3 | 1.3 | 1.8 | 1.6 | 1.8 | 0.7 | 0.6 | 0.7 | 0 | 0 | 0 |
| 8. | Gentamycin | GEN | 2.4 | 2.2 | 2.4 | 1.6 | 1.6 | 1.7 | 1.9 | 1.8 | 1.7 | 1.4 | 1.2 | 1.1 |
| 9. | Cephalexin | CL | 1.5 | 1.6 | 1.9 | 1.3 | 1.4 | 1.2 | 1 | 1 | 1 | 0 | 0 | 0 |
| 10. | Kanamycin | K | 1.6 | 1.7 | 1.8 | 1.5 | 1.4 | 1.5 | 0 | 0 | 0 | 1.8 | 1.8 | 1.8 |
| 11. | Nelidixic Acid | NA | 1.4 | 1.3 | 1.3 | 0.9 | 0.6 | 0.8 | 0.7 | 0.6 | 0.7 | 2.2 | 2.1 | 2 |
| 12. | Cefotaxime | CXM | 1.5 | 1.6 | 1.6 | 2.5 | 2.1 | 2.4 | 1.3 | 1.3 | 1.4 | 1.4 | 1.3 | 1.4 |
| 13. | Ceftriaxone | CRO | 1.4 | 1.5 | 1.5 | 2.5 | 2.7 | 2.6 | 0.9 | 0.8 | 0.8 | 1 | 1 | 1 |
| 14. | Ceftazidime | CAZ | 1.4 | 1.4 | 1.5 | 1.5 | 1 | 1 | 1 | 1 | 1 | 1.8 | 1.7 | 1.8 |
| 15. | Ampicillin | AMP | 1.5 | 1 | 1.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16. | Fluconazole | FCA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17. | Cephalexin | CTX | 2 | 1.5 | 1.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 1.5 | 1.3 |

acid and Cephalexin. However, it is susceptible to other antibiotics.

## Discussion

During the time-lapse *Proteus mirabilis* TCR20 diverged from *Proteus Vulgaris* strains according to the tree, which further divided the branch into three more strains and showed their divergence as *Pseudomonas* fluorescence strain PSB10 that led towards the divergence of another branch of strain *Proteus mirabilis* strain FA-9 that further diverged into *Proteus mirabilis* strain S3 so after a certain time it gave rise to R1_785 strain which diverged independently. This tree demonstrated that the R1_785 diverged previously from *Proteus vulgaris* strains, and in the near evolutionary history, the *Proteus mirabilis* is the descendent of R1_785 strain.

The phylogenetic tree for the R1_907 (Figure 3) illustrates that the *Proteus Vulgaris* strain FM39 comes at the top of evolutionary history, which after a certain time, has diverged into *Proteus Vulgaris* strain MAR. During evolutionary time-lapse, this strain was, diverged into *Proteus Vulgaris*

CIP103181T. The strain *Proteus vulgaris* CIP103181T further diverged into *Proteus Vulgaris* strain NCIM 2813. After time passed, this strain diverged into *Proteus* spp. JCM 2148. *Proteus* spp. JCM 2148 further evolved into *Proteus* sp. strain J4. However, during this evolutionary time, firstly, the *Proteus* spp. Strain PMCPr independently evolved into a different strain, and the other two were undertaken the evolution with each other and diverged into two separate strains that are named *Proteus Vulgaris* strain NBRC 3045 and *Proteus Vulgaris* strain HSC 51S18.

On the other hand, the strain *Proteus* spp. Strain J4 converged to *Proteus* spp. JCM 2148 in the evolutionary era and further diverged into *Proteus Vulgaris* strain A111 that gave rise to the other strain in evolutionary history that was *Proteus Vulgaris* strain LSRC 158. This strain has evolved into *Proteus Vulgaris* strain DSM 13387T, which is further responsible for the evolution of *Proteus* sp. L2 strain. *Proteus* sp. SBP 10 was diverged further and hence responsible for the evolution of another strain that was ZMd44. A new branch originates due to the evolution of *Proteus Vulgaris* strain PCS2 that further developed one more branch dwelling downward that is the representative of *Proteus Vulgaris* strain FC2953. So, at that spot, two sub-branches are formed: the strain Swine manure bacterium RT-2C and *Proteus* sp. W15Dec34 were diverged with each other and in the end, responsible for the evolution of strain R1_907 by forming another branch.

The *Staphylococcus* sp. Strain RS 4 785 16S with accession code MN252579 closely resembles *Staphylococcus Xylosus* (KY992565) and is grouped with only a 0.10% difference. There is a 0.28% difference of MN252579 with KJ6341142, and the contrast of 0.39 % exists between MN252579 and MH144255, MN252579 has a maximum difference of 1.06% with FN646069, as shown in the above figure.

The NCBI has submitted the sequences and allotted the accession numbers as SUB5880396 190605-012_A01_R1_785F.ab1 was given the accession number MN173863, and that of SUB5880500 190605-012_C01_R1_907R.ab1 has the accession number MN173859 as per NCBI publishing.

In different clinical laboratories and microbiology laboratories, the most common and frequent microorganism isolated is from the genus *Staphylococcus*, the Coagulase-negative *Staphylococcus* (CoNS).[27] From as early as the 1970s, this bacteria CoNS has been known as the cause of different infections and is of great importance as a pathogen.[28] The infection caused by CoNS occurs mostly in patients suffering from neutropenia, in neonates, and in patients with indwelling foreign devices. They cause infections at different metastatic sites, such as the central nervous system, heart, bones, and joints, and such infections in these vulnerable populations are difficult to treat.[29]

For the nine antibiotics, the percentage of resistance is mentioned in Table 3. The highest percentage of resistance i.e, 93.30% was recorded in Gentamycin, with the least resistance for Tazobactam. Whereas in terms of sensitivity, Tazobactam was found to be highly sensitive at 86.6%, and no intermediate value was recorded. Cefotaxime and Nalidixic acid showed 0% sensitivity for *S. Xylosus,* whereas 0% intermediate values were recorded for Chloramphenicol, Ciprofloxacin, Fusidic acid, Gentamycin with resistance values of 53.3%, 86.60%, 40% and 93.3%.

*Staphylococcus Xylosus* proved to be resistant to Cefotaxime, Chloramphenicol, Ciprofloxacin, Fusidic acid, and Gentamycin. Staphylococcus aureus, involved in the Micrococcaceae family, is a Gram-positive bacteria. *Staphylococcus* species are the most common bacteria and are present in all environments. *Staphylococcus* aureus strain is gram-positive and coagulase-negative. These are commonly present and have developed resistance against the environment and many aseptic chemicals. These are vectors to many diseases causing skin diseases and severe infections, so they should be removed from sites.[30] The *Staphylococcus* genus contains different species which are disease-causing and live in commensals to skin of animals. Strains including *S. aureus* is a pathogen resistant to methicillin, mostly called as methicillin-resistant *Staphylococcus* aureus, and to vancomycin, mostly called as vanomycin resistant *Staphylococcus aureus* and this antibiotic is also termed as “drug of last resort”.[31] Moreover, in the last decade, the methicillin-resistant *Staphylococcus aureus* has

changed their location from hospitals to now being commonly present in living societies and restaurant places.[32]

Community-acquired strains have been isolated from areas such as daycare centers, fire stations, and educational institutes. These resistant bacteria cause diseases in humans and animals, mostly in horses, with high treatment expenses, morbidity, and mortality. Both groups of *Staphylococcus*, Coagulase-positive *Staphylococcus* (CoPS), and coagulase-negative *Staphylococcus* (CoNS) are pathogens causing many serious infections. All the species of this CoPS are coagulase positive and can develop resistance against many antibiotics used for different treatments of animals and humans. CoNS isolated from animals have developed resistance against gentamycin, macrolides, tetracycline, streptomycin, trimethoprim, sulfamethoxazole, and fluoroquinolones. The high levels of antimicrobial resistance observed in this study is consistent with the observations in a study conducted in South Africa, reporting that up to 95.1% of the samples were MDR, and only 3.7% were susceptible to all antibiotics tested in the study.[33] It has been reported that a variety of bacteria have developed resistance against antibiotics. It is a very serious issue worldwide. The extensive use of antibiotics in the field of medicine produces resistance in different Gram-positive bacteria against the antibiotic. It has been reported in different studies that *Staphylococcus,* which has developed resistance against many antibiotic drugs, is found in vegetables, poultry, egg, milk, and raw meat. In another research, it was reported that the *Staphylococcus* with the highest percentage of resistance was from chicken (23.3%), vegetable salad (20%), raw meat (13.3%), raw egg-surface (10%) and unpasteurized milk (6.7%). *Staphylococcus Xylosus* is the Gram-positive bacteria and the most common pathogen in humans. So now a day's, antibiotic resistance in *Staphylococcus xylosus* is the main concern because it is responsible for a number of infectious diseases it is the main cause of nasal infection, a common cause of hospital-acquired infections.

The resistant *Staphylococcus Xylosus* bacterial strains transmit the antibiotic resistance determinants to other strains of *Staphylococcus*, and it is reported in different studies that the resistant *Staphylococcus* have the ability to transmit the antibiotic-resistant causing bovine intramammary infection. It has been observed that fruits and meat contain a large number of *Staphylococcus* spp. These bacterial strains were extracted from the patients who consumed contaminated fruits and vegetables. The bacteria which pass alive through the digestive tract to the colon are often transient. The resident flora has a protective effect against intruders. The bacteria which are responsible for the transmission of antibiotic drug resistance are still possible, so if our consumed food contains resistant bacteria, it could be an important source of creating resistance in the gastrointestinal tract. The bacterial populations spread the resistance from one ecosystem to the other.

The spreading of antimicrobial resistance among different bacterial species is a major problem worldwide, which is increasing daily. Antibiotic drugs are mostly used to treat infected persons against different infections. The number of findings recommends that poor selection of antibiotics may create resistance in various bacteria, resulting in the treatment against bacterial infections becoming more difficult.[34] The resistance against antibiotics in *Staphylococcus Xylosus* is reported worldwide. In present, infections Staphylococcus Xylosus caused have been increasingly problematic due to the production of resistance in bacteria. Hence the aim of this research was to find the antimicrobial sensitivity pattern of *Staphylococcus Xylosus* that was isolated from the M. domestica, which were collected from rural areas of Rawalpindi and Chakwal, Pakistan.

## Conclusion

The most frequent genus of bacteria that were isolated from domestic kitchen samples of houseflies collected from three different locations was *Proteus* and *Staphylococcus*, which was further confirmed by biochemical and molecular characterization. The phylogenetic analysis showed its close association with *Proteus mirabilis* and *Staphylococcus Xylosus* with a similarity of 99.9%. The antibiotic sensitivity tests were also performed. The *Staphylococcus Xylosus* susceptibility was highly resistant against Gentamycin and least resistant against Imipenem

and Tazobactam. These findings suggest houseflies' potential role in transmitting pathogenic bacteria with antibiotic resistance in households. Exposure of houseflies to animal farming and human habitats has led to the greater prevalence of antibiotic resistant bacteria.

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