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Prevalence and Characterization of Disease-Causing Bacteria Isolated from Pigeons (*Columba livia)* in India

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Pigeons harbour and spread numerous zoonotic diseases, including bacterial diseases caused by pathogenic bacterial communities. Pigeons are the hosts for various bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. In the present study, pigeon (*Columba livia*) from farms and houses were subjected to determine the major causative bacterial pathogens. The samples were collected from the oral cavity, cloaca and ocular regions of pigeons. The distribution of bacteria was tested using the plate count agar method, and virulence bacteria were further isolated using a selective agar base.

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Among the three different sources, cloaca harbours a higher (54) bacteria ($p<0.05$) than other sources. The prevalence of common bacterial pathogens among pigeon samples was analyzed. Among the characterized bacterial species (*E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, and *S. pyogenes*), *E. coli* was predominant in almost all selected samples, especially cloaca samples. The isolates were characterized based on their growth in selective media, biochemical properties and 16S rDNA gene sequencing.

Keywords: Pigeon; Bacteria; drug-resistance; pathogenic; zoonotic disease.

1. INTRODUCTION

Pigeons are widely distributed almost all over the world, and *Columba livia* is one of the major pigeon species in India. They pose serious public health risks as they carry >100 zoonotic bacterial, fungal, and viral pathogens [1]. It has been reported that humans generally come into close contact with pigeons and their fecal materials in public places, roosting sites, and other regular activities. Pathogenic bacterial populations such as *Escherichia coli*, *Salmonella typhimurium*, *Chlamydophila psittaci*, *Streptococcus* spp., *Corynebacterium* spp. *Enterococcus* spp., were isolated and characterized from pigeons [2-4]. In addition, the increased prevalence of bacterium such as *Salmonella* spp. was recorded in healthy pigeon populations. In live-bird markets and yards, the feces of birds greatly contribute to the wide spread of highly infectious bacterial agents into the surrounding environment. It has been previously reported that healthy pigeons may generally carry pathogenic bacterial strains, including skin-associated *Salmonella* spp., which contribute to zoonotic disease [5]. Moreover, in the unhealthy states in the slaughterhouses, the flesh of pigeons might be highly contaminated with bacteria such as *Salmonella* spp. [6]. *Escherichia coli* are one of the major bacterial pathogen widespread in animals and environments and a high zoonotic risk was reported. In humans, these pathogenic bacteria caused urinary tract and ocular infections [7]. The important factor of bacterial resistance is the presence of drug resistance genes. Through plasmid interchange molecular mechanism at the gene level, non-pathogenic bacteria receive resistant genes from the pathogenic bacteria. In bacteria, transposons, integrons, and plasmids harbouring antibiotic resistance genes are accountable for horizontal gene transfer between identical bacterial or other species [8]. The unrestricted application of antibiotics induced resistance among bacterial communities. Despite the potential significance of pigeons as sources of several pathogenic bacteria, reports on drug-susceptibility patterns of pathogenic bacteria in pigeons are highly limited. The main objective of the study is to detect drug-resistant bacterial pathogensin the infected pigeon population from India.

2. MATERIALS AND METHODS

2.1 Samples

In this study, a total of 12 samples (n=12) including oral, ocular, and cloacal swabs, were collected from the infected pigeon (*Columba livia*) raised in farms and households in Tuticorin, Tamilnadu state, India between January 2023 and May 2023. Pigeons were screened, and the infection state was visually observed. A wound or haemorrhage in the oral cavity, cloaca, or eye was considered a diseased bird and was selected for this study. A steriled swab was used for sampling. The swabs were stored in steriled vials containing phosphate saline (pH 7.2, 0.1 M) and transported to the laboratory. All pigeons were handled with care during sampling, and consent was given by the owner. After sampling, pigeons were freed from the cages, and ethical approval is not required for this kind of sampling protocol.

2.2 Analysis of the Total Bacterial Population

The samples (steriled swab samples) were diluted serially with sterile double-distilled water and plated on total plate count agar (M091S-500G) (Himedia, Mumbai, India). The plates were incubated for 24 h at 37 °C,and the bacterial colonies were counted using an automatic colony counter [9].

2.3 Determination of Pathogenic Bacteria

The samples were enriched in Nutrient broth medium overnight at 37 °C. The samples were further diluted serially and plated on nutrient agar plates using steriled double-distilled water. The samples were inoculated on MacConkey agar

plates. The plates are incubated at 37 ℃ for 24 h. Pink to red coloured colonies were counted and considered as *E. coli*. Cetrimide agar was used for the determination of *Pseudomonas aeruginosa* from the samples. Blood agar and MacConkey were used for the determination of *Klebsiella pneumoniae*, *S. aureus*, and *S. pyogenes* [2,6,9].

2.4 Morphological and Biochemical Characteristics of Pathogenic Bacteria

The isolated bacterial strains were characterized based on colony morphology and the Gram-staining method. Biochemical tests, including the triple sugar iron test, carbohydrate fermentation, methyl red test, indole test, Voges–Proskauer test, and catalase test were carried out according to the previous methods [10].

2.5 Identification of Multidrug-Resistant *S. aureus* PG1

The selected multidrug-resistant bacterial strain was subjected to 16S rDNA gene sequencing. The selected strain was inoculated in Luria Bertani (LB) broth medium and incubated for 24 h at 37℃. Then, it was centrifuged at 10,000 rpm for 10 min,and the cell pellet was collected. Genomic DNA was isolated using a DNA purification kit as described by the manufacturer's instructions (Merck, Germany). The 16S rDNA was amplified using forward (5′AGAGTTTGATCMTGGCTCAG3′) and reverse (ACGGCTACCTTGTTACGA, 5′ to 3) primers [11]. The amplified 16S rDNA gene was sequenced using Applied Biosystems, and the GenBank accession number was assigned.

2.6 Statistical Analysis

A one-way analysis of variance (ANOVA) was performed, and the variation of the bacterial population among the sampling sources was analyzed. The values were the means of three different experiments, and a p-value <0.05 was considered significant.

3. RESULTS

3.1 Analysis of the Bacterial Load in the Pigeon Samples

In our study, the pathogenic bacteria were determined from a total of 30 swab samples (10 oral, 10 ocular, and 10 cloaca). The bacterial population ranged from 4.8×10^6 to 5.7×10^9 CFU/mL. The bacteria population was 4.8×10^6 in sampling site 1 and it was maximum at site 4 (5.7×10^9 CFU/mL). The sampling site 1, was associated with household farming, and the sampling site 4 was farm-rearing pigeon. The total bacteria populations were 4.8×10^6, and 4.8×10^6, respectively, for sites 2 and 3. The sample sites 2 and 3 were associated with household farming.

3.2 Determination of Pathogenic Bacteria from the Pigeon Samples

The prevalence of bacteria among the oral, ocular and cloacal samples of pigeons was determined. A total of 10 morphologically different bacteria were isolated from the oral swabs, and ocular and cloaca samples showed 12, 20 morphologically different bacterial colonies. Oral swab showed 44 bacteria, whereas ocular and cloaca samples presented 45, and 54 morphologically different bacteria, respectively ($p<0.05$).

3.3 Prevalence of Pathogens in the Pigeon Samples

The prevalence of bacterial pathogens among pigeon samples was analyzed. Among the characterized species (*E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, and *S. pyogenes*), *E. coli* was predominant in almost all selected samples, especially cloaca samples. The isolates were characterized based on their growth in selective media and biochemical properties.

3.4 Distribution of Bacterial Pathogens Among the Samples

The distribution of pathogens varied significantly among the samples. In oral samples, *P. aeruginosa* is a dominant species (n=8) and one *S.pyogenes* was characterized. In the ocular samples, the distribution of these pathogens is limited. *E. coli* and *S.pyogenes* were not detected in the oral samples, and seven *S. aureus* strains were isolated/*E. coli* was the dominant bacteria isolated from the cloaca sample, followed by *K. pneumoniae*, and *P. aeruginosa*.

4. DISCUSSION

In this study, pathogenic bacteria were found in the oral, ocular, and cloacal samples.

Escherichia coli strains were the most abundant microbes in our study, and the present finding was consistent with previous findings [12,13]. In addition, several pathogenic bacteria were characterized from the pigeon samples.Close contact with pigeon dropplings can cause health hazards and lead to the transmission of highly virulent bacterial species. In earlier studies, *Campylobacter* spp. was determined from the pigeon samples and it caused foodborne diseases in human populations [14]. In this study, many opportunistic pathogens such as *Pseudomonas aeruginosa*. *Klebsiella pneumoniae*, *S. aureus*, and *S. pyogenes* were determined from the cloacal, ocular and oral samples. The opportunistic human pathogens such as *Staphylococcus*, *Enterococcus*, *Pseudomonas*, *Streptococcus*, *Streptococcus*, *Brevibacterium*, *Curtobacterium*, and *Corynebacterium* were reported earlier from the fecal samples [15,16], and the present finding was corroborated with previous reports. The presence of pathogenic bacterial strains isolated in our study among the pigeon population shows a serious risk to public health caused by opportunistic bacterial pathogens and adequate monitoring strategies are required to manage public health issues. The microbial composition varied between pigeons and this result was consistent with previous reports [17]. The variation in bacterial composition could be assumed to be a result of the differences in environmental conditions, especially poor sanitation, and contaminated food uptake and contamination in the sampling sites [18]. The bacterial population among pigeons may vary based on food intake and types of food. The diet of the pigeon could have varied widely, and the occasional diet provided by farmers with an increased interest in wild birds. These variations in the habitual food sources cause significant population changes in the bacterial composition of pigeons. Moreover, exposure to pesticides or antibiotics could significantly cause variation in the microbial composition that would affect bacterial species in pigeons [19].

Many investigations revealed the interactions of microbial flora between humans and birds and also reported the microbial shift between humans and animals. Microbial transfer between animals and humans was reported through very close habitual interactions [20]. It has been reported that people who have very close contact with dogs have much more skin-associated pathogenic bacteria than other people. Likewise, a microbial shift was reported between nearby livestock and humans [21]. In the present study, potential differences were observed between the pigeon population and variation in the communities depending on the sampling sites. The sample collected near the contaminated site exhibited the maximum bacterial population. The fecal microbial population of pigeons varied based on the availability of homeless people and environmental contamination [22,23]. One of the important human characteristics is to feed birds and this behaviour involves the gentle exchange of bacteria between humans and birds [24]. The repeated interactions between pigeons and humans could greatly contribute to an exchange of bacteria, resulting in population variations.

5. CONCLUSIONS

The present study revealed the isolation and characterization of various pathogenic bacteria from pigeon body parts (oral, ocular, and cloaca). The number of pathogenic bacterial populations varied widely. Cloaca region is a source of various pathogenic bacterial pathogens. The microbial population varied based on human interactions with pigeons. A pathogenic *S. aureus* strain PG1 was isolated from the cloaca sample. The availability of pathogenic bacteria in pigeons poses a serious threat to humans.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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