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Isolation and Molecular Detection of Antibiotic Resistant *Staphylococcus aureus* from Pet Birds of Mymensigh City Corporation Areas, Bangladesh

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Abstract **Background:** Pet bird (ornamental bird) rearing is gradually increasing in Bangladesh. Pet serves as a best friend and confidant for elderly and young people. For unemployment, pet bird farming acts as an income source. Many people are directly or indirectly involved in this business through pet rearing, breeding, and selling. Antibiotic resistance is a global public health concern. Pet birds are considered one of the reservoirs or carriers of AMR bacteria in humans. As a result, it is necessary to know the risks associated with antibiotic-resistant bacteria for humans who are directly involved in pet bird rearing. This research was performed for the isolation and molecular detection of antibiotic-resistant *Staphylococcus aureus* (MRSA) from pet birds. **Methodology:** A total of 169 feces samples, including pigeon (n = 57), budgerigar (n = 56), and cockatiel (n = 56), were collected from different pet shops in Mymensingh city corporation. The bacterial isolates were identified using staining and biochemical assays, followed by molecular identification using PCR. Isolated organisms were then tested for antibiotic sensitivity using disk diffusion methods with ten frequently used antibiotics. **Results:** Among the 169 samples, 35 (20.71%) were positive for *Staphylococcus* spp. by conventional and molecular tests. The prevalence of the *nuc* gene was 33.34% in pigeons, 22.23% in budgerigar, and 7.14% in cockatiel. The prevalence of the mecA gene in *S. aureus* was 28.57% in pigeons. But no *mec*A gene was found in budgerigar or cockatiel. *S. aureus* isolates were resistant to methicillin (100%), vancomycin (71.43%), cotrimoxazole (85.71%), and tetracycline (71.43%). **Conclusion:** It can be concluded that pet birds harbor enteric bacteria that are resistant to most antibiotics used in our study, and the presence of such antibiotic resistant bacteria in pet birds might pose a potential threat to humans and animals' health.

Keywords: *pet birds, antibiotic resistance, Staphylococcus aureus*

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1. Introduction

A bird that is kept and raised solely for decorative purposes is referred to as a "pet bird." This group mostly consists of Passeriformes (commonly known as songbirds, biz canaries, finches, and sparrows) and Psittaciformes (parrots, parakeets, budgerigars, and love birds). According to statistical research conducted by the American Veterinary Medicine Association (AVMA), 11 to 16 million companion and exotic birds were found in the United States in 2007 [1]. In Bangladesh, pet ownership has turned into a self-sustaining industry, with animals being bred for a number of reasons, including their worth as breeding stock [2].

Zoonotic pathogens are infectious agents that may infect humans via direct contact, food, drink, the environment, or uncommon agents such as viruses, bacteria, parasites, or other unknown substances. Due to our intimate relationship with pets, they pose a potential public health hazard around the world. For example, veterinary hospitals for pet animals and birds can spread zoonotic infections, including *Escherichia coli*, *Staphylococcus aureus*, and others [3]. A vast number of individuals in the United States are hospitalized on account of staphylococcal food poisoning every year [4]. Methicillin-resistant *S. aureus* (MRSA), for example, has

emerged as a serious veterinary disease because pets can serve as a reservoir for human MRSA infections. Over the past 10 years, human MRSA cases have been drastically raised in hospitals [5]. A bunch of studies revealed that circulating MRSA clones in dogs and cats are similar to those in humans, specifically the hospital-acquired clones [5,6]. MRSA can be transferred between pets and their owners [7,8,9,10,11,12].

Antimicrobial resistance is a warning burning globally, and it gets worse in developing countries, where the spread of antimicrobial-resistant organisms is often caused by complicated social, cultural, and behavioral factors. [13]. Adding antibiotics to the feed of commercial birds is a common practice in Bangladesh. Some common feed antibiotics are penicillin, oxytetracycline, doxycycline, azithromycin, amoxicillin, cephalexin, etc. The popular reason for using feed antibiotics is to increase efficiency and as a growth promoter to prevent cases of infectious diseases [14]. Pet bird sales are rising in Bangladesh. So, dealers utilize various strategies to make their pets more enticing. This mentality leads them to combine medications with bird feed, unknowingly completing the antibiotic resistance cycle. Multiple research investigations have been carried out on animals, revealing a clear connection between the widespread application of antibiotics and the emergence of antibiotic-resistant bacterial species. However, there is a lack of knowledge and research on antibiotic resistant bacteria from pet animals and birds in Bangladesh, despite the historical recognition of these creatures as a significant source of human health concerns. Thus, this research might have a significant influence in assessing the antibiotic resistance of bacteria found in pet birds.

2. Materials and Methods

2.1. Collection of Samples

A total of 169 samples (feces) were collected from pet shops in Mymensingh city, comprising 57 pigeons, 56 budgerigars, and 56 cockatiels, respectively. All the samples for the present study were collected aseptically using sterile instruments and transferred carefully into an ice box containing ice packs. All the samples were brought to the bacteriology laboratory of the Department of Microbiology and Hygiene, BAU, Mymensingh, for the isolation and identification of bacteria.

2.2. Isolation and Identification

Nutrient broth was used for primary enrichment, and then selective media were inoculated and incubated for the whole night at 37°C. After primary culture of the organism, a 10-fold dilution was made to prevent overgrowth of organisms. After that, 100 µl was inoculated onto nutrient agar, MSA, and blood agar. The colonies showed typical cultural characteristics of *S. aureus* and were selected for subculture on selective and differential media to confirm the isolation [15]. Morphological characteristics were identified by Gram's staining according to the method described by Merchand and Packer [16].

2.3. Molecular Identification

2.3.1. DNA Extraction

The boiling method was used for the extraction of genomic DNA from each *S. aureus* isolate [17, 18].

2.3.2. Molecular Detection of Staphylococcus Spp. and *Staphylococcus aureus* by PCR

Detection of Staphylococcus spp. and *Staphylococcus aureus*, was done by targeting *tuf* and *nuc* genes according to the methods described by Martineau et al. (2001) [19] and Kalorey et al. (2007) [20].

2.4. Antimicrobial Susceptibility Testing

The antimicrobial sensitivity/resistance for the mentioned eight isolates was evaluated using eight antimicrobials that represent three groups: carbapenems (Imipenem, Ertapenem, and Meropenem), cephalosporins (Ceftazidime, Cefotaxime, Ceftriaxone, and Cefpodoxime), and macrolides (Azithromycin).

Cefotaxime/Clavulanic acid (30/10 mcg) and Ceftazidime/Clavulanic acid (30/10 mcg) discs showed an increase of ≥5 mm in the zone of inhibition as compared to cefotaxime or ceftazidime alone, indicating the production of ESBLs. By following the Clinical and Laboratory Standards Institute (CLSI) guidelines, antibiogram result was interpreted [21].

2.5. Determination of Methicillin Resistant Gene

2.5.1. Amplification of *mec*A Gene by PCR

Primers designed from *mec*A genes were used to detect methicillin resistance. The total volume of the PCR mixture was 25 µl, consisting of 12.5 µl of the PCR master mixture, 1 µl of each primer, 1 µl of template DNA, and 9.5 µl of nuclease-free water. The thermal profile of the PCR of the *mec*A gene was 95 °C for 5 minutes for initial denaturation, followed by 30 cycles of 95 °C for 1 minute for denaturation, 55 °C for 45 seconds for annealing, 72 °C for 1 minute for elongation, and 72 °C for 10 minutes for final extension. The holding temperature was 4 °C [22].

3. Results

This study aimed to determine the prevalence and molecular identification of pathogenic *Staphylococcus aureus* and their antimicrobial resistance patterns. Considering this, a total of 169 feces samples were collected from 57 pigeons, 56 budgerigars, and 56 cockatiels.

3.1. Molecular Detection of *Staphylococcus* Spp. and *S. aureus* by PCR

PCR was performed to confirm the *Staphylococcus* spp. by using specific primers designed for the *tuf* gene (Figure 1). Out of 35 *tuf* gene-positive *Staphylococcus* spp., only 7 were found positive for the *nuc* gene (Table 1, Figure 2,

and Figure 3). A 279-bp band appeared after PCR and electrophoresis for *nuc* gene.

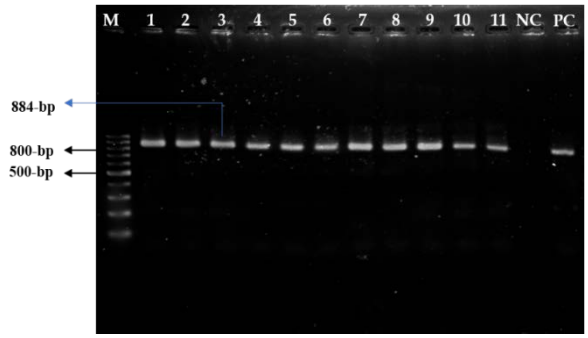

Figure 1. Amplification of *tuf* gene (884-bp) for *Staphylococcus* genus. Lane M: 100 kb DNA ladder (Thermofisher), Lane 1-11: Positive for tuf gene of *Staphylococcus*, Lane NC: Negative control, Lane PC: Positive control. PCR products were electrophoresed in 1.5% LE agarose at 100 volt for 30 minutes at TAE buffer.

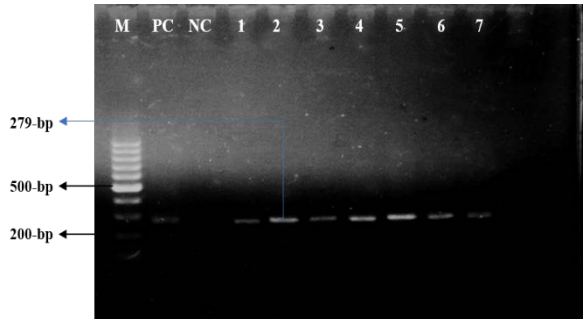

Figure 2. Amplification of *nuc* gene (279-bp) for *S. aureus*. Lane M: 100 kb DNA ladder (Thermofisher), Lane PC: Positive control, Lane NC: Negative control, Lane 1-7: Positive for *nuc* gene of *S. aureus*. PCR products were electrophoresed in 1.5% LE agarose at 100 volt for 30 minutes at TAE buffer.

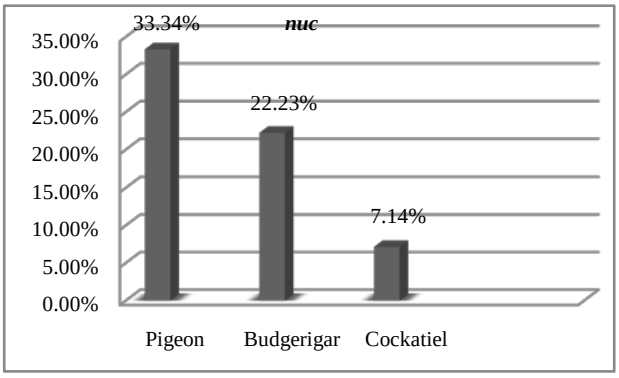

Figure 3. Prevalence of *nuc* gene in Pigeon, Budgerigar and Cockatiel.

Table 1. Prevalence of *S. aureus* in Pigeon, Budgerigar and Cockatiel

Group	No. of sample	No. of *tuf* gene positive sample	*nuc* gene	
			No. of positive sample	Prevalence (%)
Pigeon	57	12	4	33.34%
Budgerigar	56	9	2	22.23%
Cockatiel	56	14	1	7.14%
Total	169	35	7	62.71%

3.2. Molecular Detection of *mec*A for by PCR

Out of 7 samples, only 2 were positive for the *mec*A gene (Table 2, Figure 4 and Figure 5). A 533-bp band appeared after PCR and electrophoresis for the *mec*A gene.

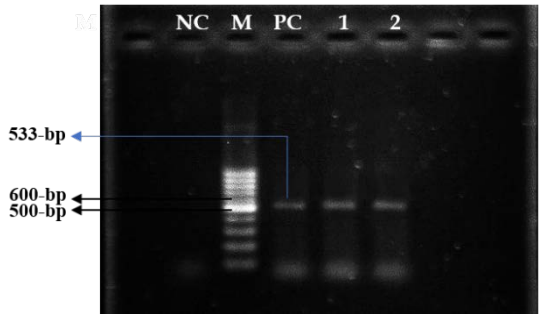

Figure 4. Amplification of *mec*A gene (533-bp) for methicillin resistance. Lane NC: Negative control, Lane M: 100 kb DNA ladder (Thermofisher), Lane PC: Positive control, Lane 1-2: Positive for *mec*A gene of *S. aureus*. PCR products were electrophoresed in 1.5% LE agarose at 100 volt for 30 minutes at TAE buffer.

Table 2. Distribution of *mec*A gene of *S. aureus* (total=7) isolates with their prevalence

Group	No. of sample	No. of *S. aureus* positive sample	*mec*A	
			No. of positive sample	Prevalence (%)
Pigeon	57	4	2	28.57%
Budgerigar	56	2	0	0%
Cockatiel	56	1	0	0%
Total	169	7	2	28.57%

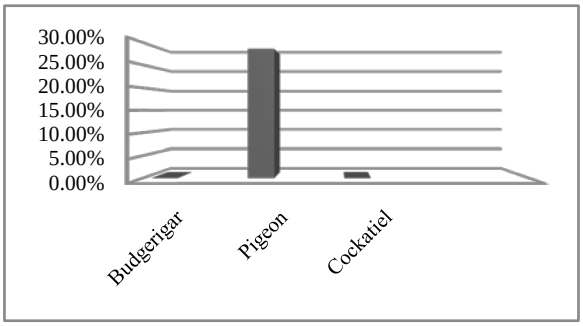

Figure 5. Prevalence of *mec*A gene of *S. aureus* isolates in pigeon, budgerigar, cockatiel.

3.3. Antibiogram Profile of *S. aureus* Isolates

The *nuc* gene-positive *S. aureus* isolates were found to be resistant to methicillin (100%), vancomycin (71.43%), intermediate to ampicillin (57.14%), Ciprofloxacin (57.14%), sensitive to Cotrimoxazole (85.71%), Tetracycline (71.43%), and chloramphenicol (57.14%) (Table 3, Table 4 and Figure 6).

Table 3. Antibiogram profile of S. aureus isolates

Antimicrobial agent	No. of isolate (%)		
	R	I	S
Ampicillin	3 (42.86%)	4 (57.14%)	0 (0%)
Azithromycin	1 (14.29%)	3 (42.86%)	3 (42.86%)
Chloramphenicol	0 (0%)	3 (42.86%)	4 (57.14%)
Ciprofloxacin	3 (42.86%)	4 (57.14%)	0 (0%)
Cotrimoxazole	0 (0%)	1 (14.29%)	6 (85.71%)
Gentamicin	2 (28.57%)	3 (42.86%)	2 (28.57%)
Methicillin	7 (100%)	0 (0%)	0 (0%)
Streptomycin	1(14.29%)	3 (42.86%)	3 (42.86%)
Tetracycline	2 (28.57%)	1(14.29%)	5(71.43%)
Vancomycin	5 (71.43%)	1 (14.29%)	1 (14.29%)

*R=Resistant, I=Intermediate, S=Sensitive

Table 4. *S. aureus* isolates resistance to number of antibiotics

No. of antibiotics resistance	No. of samples (Total= 7)
Resistance to seven antibiotics	1
Resistance to five antibiotics	1
Resistance to three antibiotics	2
Resistance to two antibiotics	2
Resistance to one antibiotic	2

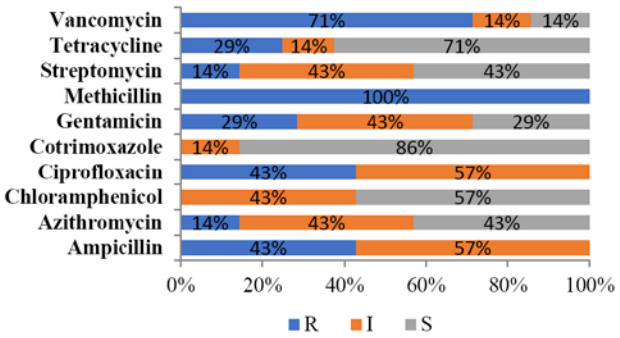

Figure 6. Antibiogram profile of *S. aureus* isolate

4. Discussion

Pets have been a major conduit for zoonotic infections to infect humans since the dawn of humanity [23]. *S. aureus*, an opportunistic pathogen that usually inhabits the skin and mucosa of healthy individuals, can produce a variety of infections, such as food poisoning, skin diseases, wound colonization, and respiratory infections, and poses a unique capacity to induce clotting [24]. This study aimed to isolate and identify the pathogenic *S. aureus* from pet birds. This study also focused on the antimicrobial resistance patterns of isolated bacteria. In the current study, Staphylococcus spp. was found to be 20.71% of the isolated bacteria from pet birds. *Staphylococcus* spp. is ubiquitous in aviaries and, under favorable conditions, acts as an opportunistic pathogen. It may cause omphalitis, femoral head necrosis, infected hocks, and stifle joints secondary to bumblefoot in pet birds.

The molecular detection of *Staphylococcus* spp. was done by using the *tuf* gene. The nuc gene primers were used to confirm the *S. aureus* isolates, as reported by Kalorey et al. [20] and Haque et al. [25] and. The current study found that pet pigeons (7.10%) had a higher prevalence of pathogenic *S. aureus* than budgerigar (3.57%) and cockatiel (1.79%), respectively. Because of this, pet birds could be a significant reservoir for pathogenic staphylococci infections, which may be passed from animals to humans via zoonotic transmission. As a result, pet birds could be considered a major reservoir for pathogenic staphylococci infections that can be transmitted to humans through zoonotic interaction transmission. In contrast, pet birds could also play a major role in transmitting staphylococci because of their frequent with their owner as well as their environment, as described by Jung et al. [26], Damborg et al. [27], Maia et al. [28], and Truong et al. [29]. Aside from that, habits like grooming of birds and hand-feeding of pet birds could raise the risk of zoonotic disease transmission to owners [27,30]. Therefore, this indicates a high possibility of co-colonization of *S. aureus* between pet birds and their owners.

Methicillin (100%), Vancomycin (71.43%) resistance was observed in the isolated *S. aureus*, and intermediate resistance to Ampicillin (57.14%) and Ciprofloxacin (57.14%) was seen. Cotrimoxazole (85.71%), tetracycline (71.43%), and chloramphenicol (57.14%) were sensitive. Bagheri et al. [31], Gharsa et al. [32], Ho et al. [33], Dressler et al. [34], and Loeffler et al. [35] found antibiogram profiles that were almost identical. Because resistant isolates could be superbugs, methicillin and vancomycin resistance in *S. aureus* is a big issue for civilization. To confirm the resistance, this study also looked at the distribution of bacterial resistance genes in *S. aureus* isolates. For *S. aureus*, the prevalence of the *mec*A genes was 28.57% in our investigation; however, no isolates tested positive for the *mec*C, *van*A, *van*B, or *van*C genes. Akter et al. [36] also used *mec*A and *mec*C primers to detect the presence of methicillin resistance genes in their study. In another study, Shahid et al. [37] detected vancomycin resistance in S. aureus from a food processing environment using *van*A, *van*B, and *van*C primers, which may result from contaminated human hands [38,39].

It can be concluded that if these bacteria can enter the food chain, it can cause serious illness to individuals, and treatment for those patients using those antibiotics would not be successful.

5. Conclusion

Pet birds are the closest friends ever to their owners because they are able to create a strong emotional bond with their owners while also representing their social norms and physical well-being. As a result, the number of people raising pet birds around the world is increasing. The goal of this study was to examine the prevalence of *S. aureus* as well as their antibiotic resistance patterns. *S. aureus* isolates were identified using morphological, staining, culture, and biochemical features. PCR was used to identify pathogenic bacteria with the antibiogram profile of positive isolates. *S. aureus* had a higher prevalence (7.10% in pigeons), followed by 3.57% in budgerigar and 1.79% in cockatiel, respectively. The prevalence of the *mec*A gene in *S. aureus* was 28.57% in pigeons. But no *mec*A gene was found in budgerigar or cockatiel. *S. aureus* isolates were resistant to methicillin (100%) and vancomycin (71.43%). Resistance to vancomycin (71.43%) of *S. aureus* isolates from pet bird samples is a noteworthy outcome of this study that is alarming for civilization. However, cotrimoxazole (85.71%) and tetracycline (71.43%) are sensitive to S. aureus treatment. In Bangladesh, there has been an increase in the demand for pet birds for cage-rearing. Drug-resistant germs progressively grow in pet birds. The aim of the current investigation was to extract and characterize bacterial pathogens from domesticated birds using their antibiogram. As a result of this research, we now have a clear image of pet birds (pigeons, budgerigars, and cockatiels) carrying harmful germs, posing a risk of illness transmission to their owners. To protect the health of both humans and their pets, professional veterinarians should check and vaccinate pet birds on a regular basis.

Conflict of Interest

The authors assert that they do not possess any conflicts of interest.

Acknowledgments

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