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Characterization, prevalence and antibiogram study of *Staphylococcus aureus* in poultry

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ABSTRACT

Objective: To reveal the presence of methicillin resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) in poultry samples and to determine the antibiogram pattern against five antibiotics.

Methods: Samples from different poultry farm of Chittagong city, Bangladesh were examined for *S. aureus* by different biochemical tests and confirmed as MRSA by identifying the presence of *mecA* gene using PCR. Antibiotic resistance pattern in *S. aureus* was determined by antibiotic disk diffusion method.

Results: In this study, a total of 60 samples (30 from nasal swabs and 30 from cloacal swabs) were used, of which 54 were confirmed as *S. aureus* by different biochemical tests. Among these, 12 were confirmed as MRSA by detecting *mecA* gene using PCR. During antibiogram study, both nasal and cloacal samples showed the highest resistance against penicillin-G and the lowest resistance was observed against neomycin.

Conclusions: Based on the present study, it can be said that different antibiotics are used extensively in poultry that leads to MRSA and is alarming for human health.

1. Introduction

Staphylococcus aureus (*S. aureus*) is an opportunistic pathogen in human and other different animal species. The pathogen is mainly related to food poisoning and is the third largest cause of food related illness throughout the world [1–3]. *S. aureus* can cause a number of infectious diseases such as dermatitis, pneumonia, meningitis, osteomyelitis in human, bovine mastitis in cattle and bumble foot disease in poultry [4]. Methicillin resistance in this bacterial species is very alarming for human health, as it has shown potential for zoonotic transmission [5]. In Germany, zoonotic transmission of methicillin-resistant *S. aureus* (MRSA) from livestock to humans occurs mostly because of occupational livestock contact [6]. MRSA was found positive in 26 persons who worked in Dutch poultry slaughterhouses out of 466 tested persons. This indicates a higher risk of exposure of MRSA compared to general Dutch people [7].

MRSA was first reported in 1961 [8]. MRSA is mediated by penicillin binding protein PBP2a, which is a 78 KDa protein. This protein is often heterogeneously expressed in staphylococci [9–11]. It shows low affinity for β-lactum antibiotics. The *mecA* gene is responsible for encoding this protein [10] and found on a large mobile genetic element named as the staphylococcal chromosomal cassette *mec* (SCC*mec*) [12,13]. Until now at least 8 SCC*mec* types (SCC*mec* I to SCC*mec* VIII) have been identified [12–14]. MRSA has been reported in a variety of meats including raw chicken, turkey, pork, veal, beef, mutton or lamb and rabbit [15–18]. Prevalence of MRSA was the highest in turkey (35.3%), followed by chicken (16.0%), veal (15.2%), pork (10.7%) and beef (10.6%) [19]. During a prevalence study of MRSA in food and food products of poultry in Germany, MRSA was found in 37.2% samples [20]. In Spain, 318 raw food samples were examined and identified only five MRSA isolates [21]. Similar result was found in a study in the USA, only 1.8% was MRSA

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positive out of 114 tested samples [22]. Detection of MRSA has also been reported in some countries in different proportions, for example, Netherlands 2.5%, Canada 6.4%, Italy 3.8%, and Spain 1.6% [23]. In Asia, few reports are available on livestock-associated MRSA, which is probably due to shortage of very few data and diagnostic facilities. The prevalence of nasal MRSA colonisation among pig farmers varied from 5.5% in Malaysia to 15% in China and 19.2% in Taiwan [24].

Extensive use of antimicrobial drug in human and in animal farming for therapeutic and preventive purpose, is a major cause for the prevalence of drug resistance among food born pathogens [25]. Different antimicrobial agents such as penicillin, erythromycin, tetracycline are extensively used in poultry for treating staphylococcal and other infections, which leads to development of drug resistant strains of pathogens [26–28].

The objective of this study is to determine the prevalence of *mecA* gene in *S. aureus* collected from nasal swab and cloacal swab of poultry sample as well as to determine the frequency of resistance and sensitivity to five antimicrobial agents in these samples. Poultry sector is a significant source of economic development in Bangladesh. Extensive use of different antibiotics leads to development of MRSA in our poultry, which is a global problem as well. This study will help to determine the presence of MRSA in poultry to ensure quality meat as well as to prevent losses in poultry industry due to infection of *S. aureus*.

2. Materials and methods

2.1. Sample collection area

The samples were collected from different poultry farms located in urban and peri-urban areas of Chittagong city, Bangladesh. Nasal and cloacal swabs were used as samples from broiler chicken of these farms.

2.2. Isolation and identification of S. aureus by phenotypic observation

During collection of samples, buffered peptone water (HIMEDIA, India) and mannitol salt agar (HIMEDIA, India) were used as bacterial culture media. *S. aureus* was identified by Gram staining, slide coagulase and catalase test.

2.3. Molecular characterization of MRSA by PCR amplification

For PCR, colony PCR was performed. A single colony from fresh bacterial culture was mixed in 50 μL of autoclaved distilled water and mixed well. PCR was performed in a 15 μL reaction tube with 3 μL DNA sample, 7.5 μL PCR mixture (Thermo Scientific, USA), 1 μL from each forward and reverse *mecA* primers (BioServe Biotechnology, India), and 0.2 μL *Taq* DNA polymerase (Thermo Scientific, USA). Amplification was performed with initial denaturation at 94 °C for 5 min, followed by 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min and final extension at 72 °C for 5 min. A total of 35 PCR cycles were run for the amplification.

The amplification of *mecA* gene was done by two primers mecA_fw (5′-AAAATCGATGGTAAAGGTTGGC-3′) and mecA_rv (5′-AGTTCTGCAGTACCGGATTTGC-3′) and target amplicon was 533 bp.

2.4. Electrophoresis of PCR product

The PCR amplicons were separated on 1% agarose gel in 1 × TAE buffer. Four microlitres of PCR product and 3 μL of 1 kb ladder (RBC Bioscience, Taiwan) was loaded on gel well. After that, the gels were documented under a UV transilluminator.

2.5. Antibiogram study of S. aureus

Antibiogram profile was determined by disc diffusion assay. Five antibiotics erythromycin (15 μg), gentamycin (10 μg), neomycin (30 μg), penicillin-G (10 μg) and tetracycline (30 μg) (Micro Master, India) were used for this experiment. Isolates from each sample were first incubated in Luria–Bertani broth for overnight, which was then spread on Mueller–Hinton agar (HIMEDIA, India) plate. The antibiotic discs were then placed on the Petri plate and incubated for 16–24 h at 37 °C. Results were collected in mm by measuring clear zone around each antibiotic.

3. Results

3.1. Strain confirmation

A total of 60 samples (30 nasal swabs and 30 cloacal swabs) were inoculated in mannitol salt agar. Results from Gram staining, catalase and coagulase test showed that 100% nasal samples and 86.67% (26 out of 30) cloacal samples were *Staphylococcus* sp. positive.

3.2. Prevalence of MRSA by PCR

A total of 56 samples (30 from nasal swabs and 26 from cloacal swabs) were subjected to PCR for detection of the presence of *mecA* gene. Out of these samples, 12 samples of *S. aureus* showed the presence of *mecA* gene (Figure 1), which means that these bacteria were MRSA.

From 30 nasal swabs, 7 samples of *S. aureus* showed positive result for MRSA, and the percentage was 23.33%. The percentage

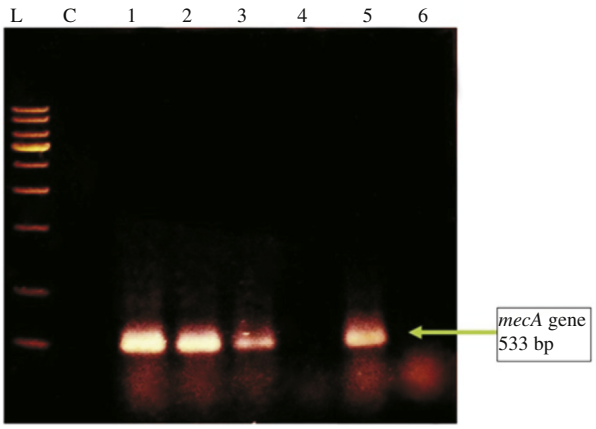

Figure 1. PCR result after electrophoresis.
Lane L: Ladder (1 kb); Lane C: Negative control; Lanes 1 to 6: Samples after PCR. Lanes 4 and 6 showed negative result.

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