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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

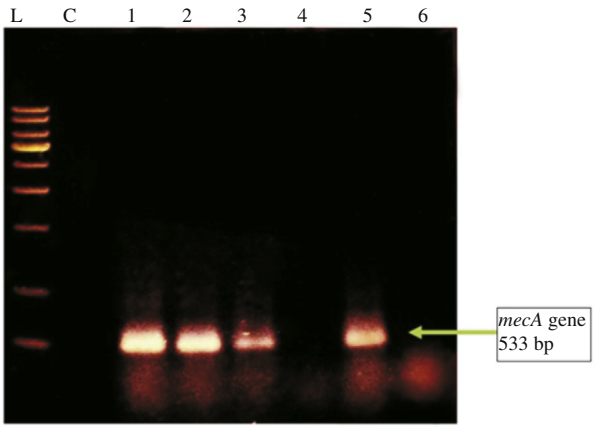

Figure 1. PCR result after electrophoresis.
Lane L: Ladder (1 kb); Lane C: Negative control; Lanes 1 to 6: Samples after PCR. Lanes 4 and 6 showed negative result.

was 19.23% for cloacal swabs, as out of 26 sample, 5 samples showed positive result.

3.3. Antimicrobial resistance of S. aureus isolates

Following the guidelines of National Committee and Clinical Laboratory Standards, antimicrobial susceptibility pattern of *S. aureus* isolates were determined by using disk diffusion assay. The antimicrobial sensitivity and resistance pattern of all isolates were studied against 5 antibiotics and depicted in Table 1. For nasal swabs, isolates showed highest resistance against penicillin-G (93.33%), which is followed by erythromycin, tetracycline, gentamycin and neomycin, respectively. Very few isolates were sensitive to these antibiotics and gentamycin (50%) showed the highest sensitivity.

Table 1
Antibiogram profile of *S. aureus* isolates [*n* (%)].

Name of antibiotics	Types of samples	Resistant	Sensitive
Erythromycin (15 μg)	NS	26 (86.67)	1 (3.33)
	CS	19 (73.08)	2 (7.69)
Gentamycin (10 μg)	NS	7 (23.33)	15 (50.00)
	CS	9 (34.62)	11 (42.31)
Neomycin (30 μg)	NS	7 (23.33)	12 (40.00)
	CS	7 (26.92)	8 (30.77)
Penicillin-G (10 μg)	NS	28 (93.33)	2 (6.67)
	CS	25 (96.15)	1 (3.85)
Tetracycline (30 μg)	NS	25 (83.33)	1 (3.33)
	CS	21 (80.76)	4 (15.38)

NS: Nasal swab; CS: Cloacal swab.

For the cloacal swabs, a total of 26 isolates were tested for the same antibiotics. Isolates showed lowest resistance against neomycin (26.92%), while the highest resistance was found against penicillin-G (96.15%). For sensitivity, 42.31% was shown by gentamycin, which is the highest. On the other hand, penicillin-G showed the lowest percentage of sensitivity (3.85%).

4. Discussion

To appraise the potential health hazard, it is important to detect the occurrence of MRSA in poultry samples. In the present study *mecA* gene PCR assay was used to identify MRSA positive samples. Out of 56 samples, 12 showed positive result in PCR. Thus the prevalence of MRSA was 21.43% in the total tested samples, which comprises 23.33% for nasal swabs and 19.23% for cloacal swabs separately. The percentage of antimicrobial resistance is increasing over time and consequently higher percentage of antibiotic resistance was found in recent poultry sample than in old sample [28]. Recurrent use of different antibiotic agents in poultry industry could be the cause of elevation in MRSA percentage.

Usage of different antimicrobial agents such as penicillin, erythromycin, and tetracycline is very common for the treatment of staphylococcal infection [27,29]. Recently the percentage of MRSA is increasing in an alarming rate. In the Netherlands, the percentage of MRSA was 16% in chicken meat while in Korea it was 13% [19,30]. In our study, we found 93.33% resistance against penicillin-G for the samples from nasal swabs, which was the highest among all five antibiotics. For the cloacal swabs, the percentage was 96.15% for the same antibiotic. Moreover, multidrug resistant *S. aureus* in poultry meat have been reported in the USA [31]. During the antibiogram study, 92.9% resistance was reported against tetracycline in poultry meat [32]. We also found a high percentage of resistance against tetracycline that comprised 83.33% and 80.76% for nasal swabs and cloacal swabs, respectively. In our study, least resistance was shown for gentamycin and neomycin compared to the other three antibiotics. Similar result was also found for other poultry isolates, 14.8% and 17.3% resistance against gentamycin and neomycin, respectively [28].

In poultry, penicillin, tetracycline and erythromycin are extensively used antimicrobial agents for the treatment of staphylococcal infections [27,29]. In the present investigation, we found more resistance against these antibiotics compared to gentamycin and neomycin.

In conclusion, the findings of the study dictate that both the prevalence and frequency of MRSA in poultry are alarming and increasing day by day with the increasing trends of usage of antibiotics. This study was based in Chittagong only. Similar studies can be extended to other areas of Bangladesh to determine the horizontal intensity of prevalence and frequency of MRSA. Further studies may also be done to determine the speed and alacrity of zoonotic transmission of MRSA that may help in assessing the risks posed by MRSA to human health.

Conflict of interest statement

We declare that we have no conflict of interest.

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