Molecular Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA) in Poultry in Bangladesh: Having Public Health Significance


**ABSTRACT**

Methicillin-resistant *Staphylococcus aureus* (MRSA) has a major public health concern. It can be identified throughout the chain of production for poultry, which raises questions regarding potential transmission from farm to consumer. MRSA has zoonotic significance and can be transmitted to humans and poultry. Several studies have been carried out on MRSA on poultry, but this study was conducted to find out the whole scenario of MRSA at the farm level. A total of 100 samples were collected randomly from different poultry farms and retail shops in Khulna city to investigate this study. MRSA was isolated and identified by culturing antibiotic susceptibility testing, and polymerase chain reaction (PCR). Among the 100 samples, 57% were positive for *S. aureus* and 80.70% of the isolated *S. aureus* showed hemolysis on blood agar. Among the 57 isolates, 78.94% were MRSA (oxacillin) and 19.29% were vancomycin-resistant *Staphylococcus aureus* (VRSA) phenotypically. Surprisingly, 59.64% of *S. aureus* results showed a positive mecA gene. It is also concerning that 60% of broiler meat and 53.84% of farm personnel were infected with MRSA. The present study revealed that MRSA could be transmitted from poultry to humans.

**Keywords:** MRSA, Public health, Poultry, VRSA.

**I. INTRODUCTION**

*Staphylococcus aureus* is an opportunistic pathogen that causes numerous skin and food-borne illnesses in animals, poultry, and humans [1]. It is well established that *S. aureus* has a potential zoonotic significance and poses a serious foodborne illness risk to public health on a global scale [2], [3]. *S. aureus* could be isolated as a normal microflora from chicken skin, feathers, respiratory tracts, and intestinal tracts, just like in humans and other animals [4], [5]. However, various clinical problems, including dermatitis, arthritis, osteomyelitis, synovitis, tenosynovitis, femoral head necrosis, bumble-foot, and omphalitis, may be linked to this pathogen [6].

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common human and animal pathogen that has been frequently identified in clinical studies [7]. MRSA first appeared in the world in 1961 in England [8]. MRSA transmission from animals to humans through workplace livestock interaction. The 78 KDa protein known as PBP2a,
which binds to penicillin, is the mediator of MRSA [9]. This protein is encoded by the mecA gene, which is located on a substantial mobile genetic material known as the staphylococcal chromosomal cassette mec (SCCmec) [10].

Antibiotic-resistant bacteria in animals are of great concern due to their potential impact on human transmission [11]. Several types of meat, including raw chicken meat, have been linked to MRSA [12]. S. aureus can acquire many resistance genes and grow more virulent due to the widespread use of antimicrobial agents in the poultry sector [13]. As a result, the efficacy of preventative and control measures could be restrained by the transmission of these resistance genes and the resistant bacteria to humans through poultry [14]. Very few studies have been carried out on MRSA in poultry in Bangladesh [15]. The hemolytic activity of the isolated bacteria was detected following growth on blood agar [16].

C. Antibiotic Sensitivity Test

An antibiotic sensitivity test was performed by disk diffusion on Mueller-Hinton agar (Hi Media, India) plates having a concentration of bacteria equivalent to 0.5 McFarland standards [17]. The plates were incubated at 37 °C aerobically for 18–24 hours to observe the results. Results of the antibiotic sensitivity tests were recorded as sensitive, intermediate, sensitive, or resistant, and the zone of growth inhibition was compared with the zone size interpretative tables provided by the Clinical and Laboratory Standards Institute [18]. The antibiotic disc was used to determine the antibiogram of oxacillin (methicillin) at a dose of 1μg/disc. In addition, ciprofloxacin (5μg), ceftriaxone (30μg), amoxicillin (30μg), trimethoprim-sulfamethoxazole (25 μg), and vancomycin (30μg) also used in the antibiogram study. Any isolate showing resistance to oxacillin will be considered MRSA and resistance to vancomycin will be considered VRSA.

D. Molecular Detection of Methicillin-resistant S. aureus (MRSA)

Boiling was used to recover the genomic DNA from S. aureus [19]. S. aureus detection was confirmed using polymerase chain reaction (PCR) with the nuc gene as the target. [20]. A final 25 μl reaction including, 12.5 μl of master mixture 2X (Promega, USA), 2 μl of genomic DNA (about 30 ng), 1 μl (100 pmol) of individual primer, and 8.5 μl nuclease-free water, was used for PCR. PCR Products were examined by electrophoresis on 1.5% agarose gel. The gel was stained with ethidium bromide and examined with an ultraviolet transilluminator (Biometra, Germany). A 100 bp DNA ladder (Promega, USA) was used as a molecular weight marker.

E. Statistical Analysis

To calculate the frequencies of MRSA, all the obtained data were entered into an excel spreadsheet (MS-2013) and descriptive statistics were done using SPSS software (SPSS-26.0, IBM, USA).
III. RESULT

The prevalence of MRSA in poultry farms was identified by phenotypic and genotypic observations. The yellow color colony characteristics of *S. aureus* were observed on MSA (Fig. 2). These colonies showed Gram-positive clustered cocci shape by Gram staining. Additionally, a zone of hemolysis was observed on blood agar. These results indicated the existence of *S. aureus*.

Table II showed that among 100 samples, 57% were positive for *S. aureus*, with the highest prevalence in poultry litter (70%). Among 57 isolates of *S. aureus*, 80.70% produced hemolysis on blood agar. Poultry litter isolates showed the highest degree (85.71%) of hemolysis. Table II also showed various degrees of MRSA in broiler meat (60%), cloacal swabs (66.67%), feces (50%), and hand washing of farm personnel (53.84%).

Fig. 3 shows the phenotypic results of MRSA in different poultry samples. Among the 57 isolates, 78.94% were MRSA and 19.29% were VRSA phenotypically (Table III). The various degrees of resistance to ciprofloxacin (36.84%), ceftriaxone (10.52%), amoxicillin (26.31%), and trimethoprim-sulfamethoxazole (35.08%) (Table III).

![Fig. 2. Yellow colonies of *S. aureus* in Mannitol salt agar and Gram-positive cocci with cluster arrangement of *S. aureus*.](image)

![Fig. 3. Antibiotic susceptibility of the isolated *S. aureus* from poultry samples on Mueller Hinton agar plates each with five antibiotic discs.](image)

![Fig. 4. Molecular detection of *nuc* gene of *S. aureus*. PCR amplification of *nuc* gene of *Staphylococcus aureus*. M=100 bp size DNA marker, Lane 1: negative control, Lane 2: positive control, Lane 3 to 5: representative samples of *S. aureus* isolated from different poultry samples.](image)

![Fig. 5. Molecular detection of *mecA* gene of methicillin-resistant *S. aureus*. PCR amplification of *mecA* gene of methicillin-resistant *Staphylococcus aureus* (MRSA). M=100 bp size DNA marker, Lane 1: negative control, Lane 2: positive control, Lane 3 to 5: representative samples of MRSA isolated from different poultry samples.](image)

![TABLE II: PREVALENCE OF METHICILLIN RESISTANT *S. AUREUS* IN POULTRY](table)

<table>
<thead>
<tr>
<th>Sample (n)</th>
<th>Positive for <em>S. aureus</em> (nuc) (%)</th>
<th>Prevalence of MRSA (mecA) (%) among the <em>S. aureus</em></th>
<th>Hemolysis on blood agar (%) among the <em>S. aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Broiler meat (20)</td>
<td>10 (50.00)</td>
<td>6 (60.00)</td>
<td>8 (80.00)</td>
</tr>
<tr>
<td>Cloacal swab (20)</td>
<td>12 (60.00)</td>
<td>8 (66.67)</td>
<td>10 (83.33)</td>
</tr>
<tr>
<td>Feces (20)</td>
<td>8 (40.00)</td>
<td>4 (50.00)</td>
<td>6 (75.00)</td>
</tr>
<tr>
<td>Litter (20)</td>
<td>14 (70.00)</td>
<td>9 (64.28)</td>
<td>12 (85.71)</td>
</tr>
<tr>
<td>Hand washing (20)</td>
<td>13 (65.00)</td>
<td>7 (53.84)</td>
<td>10 (76.92)</td>
</tr>
<tr>
<td>Total (100)</td>
<td>57 (57.00)</td>
<td>34 (59.64)</td>
<td>46 (80.70)</td>
</tr>
</tbody>
</table>

![TABLE III: RESISTANT PATTERN OF *S. AUREUS* ISOLATED FROM POULTRY](table)

<table>
<thead>
<tr>
<th>Sources (n)</th>
<th>Resistant to applied antibiotics (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OX</td>
</tr>
<tr>
<td>Broiler meat (10)</td>
<td>7 (70.00)</td>
</tr>
<tr>
<td>Cloacal swab (12)</td>
<td>10 (83.33)</td>
</tr>
<tr>
<td>Feces (8)</td>
<td>6 (75.00)</td>
</tr>
<tr>
<td>Litter (14)</td>
<td>12 (85.71)</td>
</tr>
<tr>
<td>Hand washing (13)</td>
<td>10 (76.92)</td>
</tr>
<tr>
<td>Total (57)</td>
<td>45 (78.94)</td>
</tr>
</tbody>
</table>

Here, n= Number of *S. aureus*, OX = Oxacillin, CIP = Ciprofloxacin, CRO = Ceftriaxone, AX = Amoxicillin, SXT = Trimethoprim-sulfamethoxazole, VA=Vancomycin.

Molecular detection of *S. aureus* was confirmed by PCR (Fig. 4 & 5). Overall, 59.64% *mecA* genes were found in isolated poultry samples, with the highest percentage (66.67%) found in cloacal swabs (Table II). Table II also confirmed MRSA in various poultry samples such as broiler meat (60%), feces (50%), and litter (66.28%). MRSA was also detected in human samples at 53.84% (Table II).

IV. DISCUSSION

MRSA has the potential to be harmful to both human and animal health. MRSA is frequently found in poultry farms [1]. In this study, we observed the overall prevalence of MRSA in various poultry samples was 59.64%. Several studies on MRSA in poultry have been carried out around the world. Previously, El-Tawab et al. [6] detected 66.6% MRSA from chicken samples in Giza, Egypt. Recently, Benrabia et al. [22] reported 33.5% MRSA in laying hens and 15.9% MRSA in broilers in Algeria. Earlier, Olayinka et al. [5] also reported 39.8% MRSA from poultry farm isolates in Zaria, Nigeria. In the current study, we found 60% MRSA from broiler meat samples. Earlier, Parvin et al. [23] reported 43.5% MRSA from frozen meat samples in Bangladesh. Similarly, 43% of MRSA was reported from raw chicken meat in Malaysia [24]. During this study, 66.67% of the cloacal swabs were infected with MRSA, whereas 32.60% were found in Nigeria [25]. Surprisingly, 53.84% of the poultry farmers’ hand wash samples were contaminated with MRSA, whereas 83.3% were reported to health workers in Brazil [26].
Different classes of antibiotics are frequently used for the treatment of poultry in Bangladesh [27]. Most of them are β-lactam antibiotics, fluoroquinolones, and trimethoprim-sulfonamides. In this study, we observed 78.94% methicillin (oxacillin) resistance to \textit{S. aureus} from poultry isolates, where the highest resistance was found in the case of poultry litter (85.71%). Previously, Parvin \textit{et al.} [23] and Hossain \textit{et al.} [28] detected 42.1% to 97.7% oxacillin-resistant \textit{S. aureus} in poultry samples in Bangladesh. Recently, Benrabia \textit{et al.} [22] reported 100% oxacillin-resistant \textit{S. aureus} from poultry isolates in Algeria. Earlier, EL-Adaway \textit{et al.} [29] observed 100% resistance to oxacillin in \textit{S. aureus} isolates from broilers in Germany. Ciprofloxacin is widely used for the cure of different poultry diseases in Bangladesh [27]. Overall, 36.84% resistance was found in \textit{S. aureus} isolates against ciprofloxacin during this study, while 50% resistance was observed in the case of broiler meat samples, which is very alarming for consumers. On the other hand, 50% resistance to ciprofloxacin against \textit{S. aureus} was also observed by Benrabia \textit{et al.} [22] in poultry species in Algeria. Earlier, EL-Adaway \textit{et al.} [29] also observed 55.6% resistance to ciprofloxacin in \textit{S. aureus} isolates from broilers in Germany.

Ceftriaxone is a semi-synthetic third-generation antibiotic that is effective in treating many septicemic diseases of poultry [27]. In this study, we observed 10.52% ceftriaxone resistance against \textit{S. aureus} isolated from poultry samples. Previously, Ugwu \textit{et al.} [30] reported 65.91% ceftriaxone resistance in \textit{S. aureus} from poultry in Nigeria. We also found 26.31% amoxicillin-resistant \textit{S. aureus} from poultry samples where 50% was identified from feces. Parvin \textit{et al.} [23] reported 87% amoxicillin resistance to \textit{S. aureus} from frozen chicken meat samples in Bangladesh. Previously, Otalu \textit{et al.} [13] and Sallam \textit{et al.} [31] reported 23% and 77.8% amoxicillin-resistant \textit{S. aureus} from retail chicken in Nigeria and Egypt, respectively. Overall, 35.08% of trimethoprim-sulfamethoxazole resistance against \textit{S. aureus} was observed during this study, while 50% was detected from poultry litter. Previously, 27.8% to 38.55% of trimethoprim-sulfamethoxazole resistance to \textit{S. aureus} was documented in Nigeria and Algeria [13, 22].

Vancomycin is not commonly used for the treatment of vancomycin-resistant infections. But various degrees of vancomycin resistance were found during antibiotic susceptibility testing of poultry samples. For decades, vancomycin has been frequently used where MRSA is found [32, 33]. In this study, we found 19.29% VRSA from poultry, with the highest (37.5%) resistance observed from feces. Recently, El-Ghany [1] reported 54% VRSA from poultry in northwest Algeria. Earlier, Otalu \textit{et al.} [13] also reported 46.2% VRSA from poultry samples in Nigeria.

The present study also confirmed the molecular detection of \textit{S. aureus}, where 57% were found positive for the \textit{mecA} gene. Recently, Shahid \textit{et al.} [34] also detected 50.62% \textit{mecA} gene of \textit{S. aureus} from food processing environments in Bangladesh. Earlier, Akilu \textit{et al.} [24] reported 77% \textit{mecA} gene of \textit{S. aureus} from raw chicken meat samples in Malaysia. We also found 59.64% positive \textit{mecA} gene of methicillin-resistant \textit{S. aureus} from poultry samples. Previously, Parvin \textit{et al.} [23] detected the 43.5% \textit{mecA} gene of MRSA from frozen broiler meat samples in Bangladesh. Earlier, varying degrees (37.2%-100%) of the \textit{mecA} gene of MRSA were found positive in poultry samples in Egypt and Germany [6], [27], [29], [35].

Multidrug-resistant genes of MRSA found in poultry farming in Bangladesh result in the burden of medical costs and great health concerns for humans and animals. More host-adaptive evolutionary alterations may occur in animal-adapted clones, which might lead to the epidemic spread of new and more virulent MRSA strains among humans. Additional investigations are still required to determine the sources of MRSA infections in humans and the validity of animal-to-human transmission findings.

V. CONCLUSION

MRSA found in poultry and poultry farm personnel is a major health issue that indicates the zoonotic significance of MRSA. In this study, we found MRSA and VRSA from broiler meat samples, which reveals the direct threat to the consumer’s health. The findings underscore the necessity of mitigating actions based on the farm-to-fork principle at all points in the poultry production chain to prevent or eradicate MRSA transmission in humans, poultry, and farms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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