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Detection and Molecular Characterization of Methicillin-Resistant *Staphylococcus aureus* Obtained from Poultry and Poultry House Environment of Anand District, Gujarat, India

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A B S T R A C T

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the important emerging pathogen worldwide responsible for causing wide range of disease in human and animals. It is responsible for causing nosocomial, community infection but it has been reported to cause livestock infection also. The main aim of the study was to investigate the occurrence of MRSA in raw poultry meat sold at in and around Anand City, Gujarat. A total 23 *S. aureus* isolates obtained from 250 poultry and poultry house environment samples were taken in this study. All the 23 isolates were confirmed as *S. aureus* by PCR. Among 23 isolates, four (1.6%) isolates yield positive growth on MeReSa agar giving characteristics appearance of light pink colour colony indicating MRSA positive strain. All these 4 isolates were found positive for *mecA* gene by PCR which is responsible for Methicillin resistance in *S. aureus*. The presence of MRSA in poultry meat is of great concern for public health importance.

Introduction

Staphylococcus aureus (*S. aureus*) is one of the pathogenic organisms of genus *Staphylococcus* which cause disease in human as well as animals. It is an also important agent of food poisoning all over the world (Suleiman *et al.*, 2013). In human, *S. aureus* is also responsible for causing wide range of disease such as pneumonia, bloodstream infections, skin and soft tissue infections, endocarditis and osteomyelitis as well as toxin-mediated syndromes like toxic shock and food poisoning (Shittu *et al.*, 2011).

From last few decades antibiotic resistance in the bacteria is of great concern for public health over the world. However, Human as well as animal isolates of *S. aurues* were frequently showing resistance to penicillinase-resistant penicillins and organisms showing such type of resistance are known as Methicillin resistant *Staphylococcus aureus* (MRSA) (Wang *et al.*, 2013). Methicillin

resistant of *S. aureus* is mainly mediated by *mecA* gene which encode for penicillin-binding protein 2a (PBP2a) and it has low affinity for all beta-lactam antimicrobials agents (Kawano *et al.,* 1996). They normally remain present on the skin and nose of human and animals as commensal organisms and its treatment with antibiotic such as Methicillin is ineffective. So it is difficult to get rid of MRSA infection. They are resistance to most of the commonly used antimicrobial agents, including the aminoglycosides, macrolides, chloramphenicol, tetracycline, and fluoroquinolones (Lee, 2003; Batabyal *et al.,* 2012). From last few decades MRSA has been become important bacterial pathogen responsible for causing nosocomial and community onset infections and in the recent year, it has gain also importance in the livestock infection over the world wide (Alzohairy, 2011; Huber *et al.,* 2010).

The extensive and indiscriminate use of antibiotics in the food of animal origin leads to increase in the resistance among commensal as well as opportunistic *S. aurues* (Yurdakul *et al.,* 2013). Few survey of different food animal product, it has been came to known that chicken and chicken products are widely known as important reservoir for *S. aureus* and MRSA and they have been considered as important vehicles for transmition of *S. aureus* from chicken to human population (Wang *et al.,* 2013). Usually chicken are reared in close proximity to the human being and the presence of MRSA in them leads to great public health importance. It also invariably increases cost of treatment in the human being. So it is essential to monitor the spread of MRSA that will have great public health concern (Otalu *et al.,* 2011).

In India, there are few reports regarding the prevalence, detection and molecular characterization of MRSA in poultry meat. The present study was carried out to determine the prevalence of MRSA in chicken and molecular detection of *mecA* gene in MRSA isolates.

Materials and Methods

Bacterial isolates

A total of 23 *S. aureus* isolates were recovered from 250 poultry and poultry house environment samples which includes tracheal swabs (50 samples), cloacal swabs (50 samples), skin swabs (50 sample), meat (50 samples) and environment sample (25 soil samples and 25 butchers' hand swabs) collected from different retail poultry meat shops located in and around Anand. All *S. aureus* isolates were identified by colony morphology Gram's stain as well as biochemically using methods described by Sneath *et al.,* (1986).

Identification of MRSA based on MeReSa Agar Base Medium (Hi-Media, M1594-500G)

For the identification of the MRSA among the 23 isolates of *S. aureus*, MeReSa Agar medium (Hi-Media, M1594-500G) was used. All the positive isolates of *S. aureus* which were confirmed by PCR using species specific primer for *sau* gene (Riffon *et al.,* 2001), were streaked on MeReSa Agar medium and incubated for 18-24 h at 37 °C. After complete period of incubation characteristic appearance of light pink colour colonies on the MeReSa Agar medium were considered as MRSA.

PCR detection of mecA gene of *S. aureus*

Extraction of DNA

The DNA of MRSA isolates was extracted by boiling method as previously described by Van Eys *et al.,* (1989). Briefly, loopful of culture was taken in microcentrifuge in 100 µl

of sterilized DNAse and RNAse-free milliQ water (Millipore, USA). Then vortexed and samples were heated at 95 °C for 10 min, cell debris was removed by centrifugation and 3 µl of the supernatant was used as a DNA template in PCR reaction mixture.

A PCR reaction was carried using that would detect the *sau* gene primers, species specific gene for *S. aureus* and *mecA* gene primer for detection of MRSA isolates. The *sau* gene primers, Forward: 5'GGACGACATTAGAC GAATCA 3' and Reverse 5'-CGGGCACCTA TTTTCTATCT-3' (1318 bp) while the *mec*A primers were: Forward 5'-AAAATCGATG GTAAAGGTTGGC-3' and Reverse: 5'-AGT TCTGCAGTACCGGATTTGC-3' (533 bp). PCR amplification was performed in a total reaction volume of 25 µl. The reaction mixture contained 12.5 µl (2 X) of Emerald Amp GT PCR mastermix and 10 pmol of each forward and reverse primer, 7.5 µl nuclease free distilled water and 3 µl of DNA template. A positive control PCR reaction was also included in this assay. The reaction was performed in the thermal cycler with pre-heated lid (Lid temp.105 °C). The cycling conditions for PCR comprised of an initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 60 s and final extension of 5 min at 72 °C was employed. The amplified products were analysed on agarose gel electrophoresis through 1% agarose gel and visualized under gel documentation system.

Results and Discussion

S. aureus is an opportunistic pathogen and a frequent coloniser of many animal species as well as humans (Persoons *et al.,* 2009). It can cause a wide range of different infections in humans and poultry (Quinn *et al.,* 2000). Antimicrobial agents are extensively used in the food producing animals especially poultry for disease prevention and growth promotion and this may be the reason for developing resistance in the bacterial pathogen (Barber *et al.,* 2003; Otalu *et al.,* 2011). Methicillin-resistant *Staphylococcus aureus* (MRSA) is important pathogen of concern in humans, responsible for causing nosocomial and community acquired infection in the recent year (Diederen and Kluytmans, 2006).

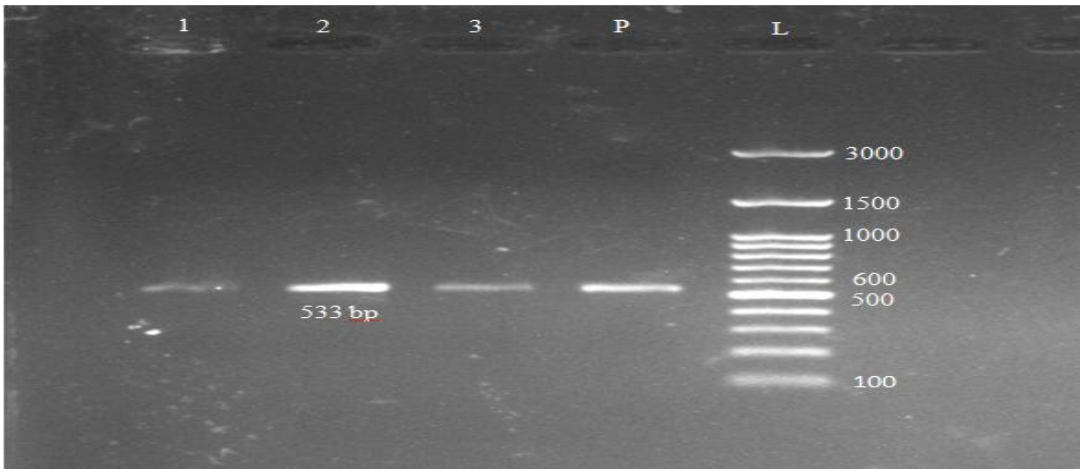
So it is important to carry out screening the prevalence of MRSA in population for early prevention and control of community acquired infection. Hence the present study was carried out for detection of MRSA in poultry and poultry house environment in and around Anand city.

Isolation and identification of MRSA were done based on colony morphology on MeReSa agar medium. Colony showing characteristic appearance light pink colour were consider as MRSA. Out of twenty three isolates, 4 (1.6%) isolates showed growth on MeReSa agar for MRSA positive

In the present study, all the twenty three isolates of *S. aureus* were streaked on MeReSa agar medium and appearance of light pink colour colonies on growth were considered as MRSA positive. Out of 23 *S. aureus* isolate, 4 (1.6%) isolates were grown on MeReSa agar medium which were also found positive for *mecA* gene by PCR (Figure 1).

Detection of *mecA* gene by PCR is gold standard method for identification of MRSA strain (Loeffler and Lloyd, 2010). According to sample wise study highest incidence of MRSA was recorded from tracheal swabs (6.0 %) followed by Butchers' hand swabs (4.0 %) they were also positive for *mecA* gene.

Fig.1 Electrophorosis pattern of *mecA* PCR assay



Lanes 1, 2, 3: positive amplification of 533 bp for *mecA* gene
Lane P: *mecA* gene positive control
Lane L: DNA ladder 100 bp

The finding of the present study i.e. 1.6 % (4/250) prevalence was in concurrent with the finding of Wang *et al.,* (2013) and Abdalrahman *et al.,* (2015) who reported 1.7% (20/1152), 1.8% (02/114) prevalence of *mecA* gene in MRSA respectively. Similarly, Hanson *et al.,* (2011) reported 1.2% overall prevalence of *mecA* gene MRSA isolated from retail meat (pork, beef, chicken, turkey) but in contrast to this they did not found any *mecA* isolates from Poultry. In Bhargava *et al.,* (2011) reported 3.9% (3/76) prevalence of *mecA* gene from chicken isolates which was somewhat higher than the present findings.

Febler *et al.,* (2011) reported 37.2% (32/86) were positive for *mecA* gene of MRSA which include 25.0% (6/24) samples from fresh chicken meat, 21.1% (4/19) samples from chicken meat products. Similarly, Enany *et al.,* (2013) detected 20.0% (3/15) isolates were positive for *mecA* gene which is higher than the present finding. In present finding 4.0% of (1/25) *mecA* reported from buthchers' hands swab which is in contrast with the findings of Helal *et al.,* (2015) who reported 66.6% (38/ 57) human nasal swabs were MRSA.

To the best of our knowledge, this study demonstrated for the first time the presence of *mec*A positive MRSA from chickens in Anand, Gujarat. From the present study it has been concluded that presence of MRSA in poultry meat is indicating that there is lack of adequate hygiene, bad sales practices and poor meat handling in the retail shops which leads to increase the potential public health hazard.

There is also wide spread use of antibiotic in poultry for growth promotion and disease prevention which leads to in the resistance among commensal bacteria. To control prevalence of MRSA, Strict hygienic and preventive measures are needed among animals and human populations and during food processing to avoid colonization of MRSA isolates.

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