DETECTION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) FROM ANIMAL AND HUMAN ORIGIN IN BANGLADESH BY POLYMERASE CHAIN REACTION

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) is defined by the presence of the meca gene, which is considered to have been transferred horizontally from unknown bacterial species to S. aureus. The meca gene which encodes an additional \(\beta\)-lactam-resistant penicillin-binding protein (PBP), termed PBP-2a (PBP-2') with reduced binding affinity for \(\beta\)-lactam compounds. We investigated distribution of the meca gene in a total of 94 clinical strains of S. aureus isolated from both man and animal admitted in Bangladeshi medical hospital as well as Veterinary clinic. The meca gene was detected by PCR in 25% of human clinical isolates of S. aureus, whereas not a single meca gene was detected in animal isolates of S. aureus.

Key words: Methicillin-resistant Staphylococcus aureus (MRSA), meca gene, Polymerase Chain Reaction (PCR), Animal & Human

INTRODUCTION

Antibiotic resistance in S. aureus was almost unknown when penicillin was first introduced in 1943. By 1950, 40% of hospital S. aureus isolates were penicillin resistant and by 1960, this had risen to 80% (Chambers, 2001). Today, S. aureus has become resistant to many commonly used antibiotics. In the UK, only 2% of all S. aureus isolates are sensitive to penicillin with a similar picture in the rest of the world, due to a penicillinase (a form of \(\beta\)-lactamase). The \(\beta\)-lactamase-resistant penicillins (methicillin, oxacillin, cloxacillin and flucloxacillin) were developed to treat penicillin-resistant S. aureus and are still used as first-line treatment.

Methicillin was the first antibiotic in this class to be used (it was introduced in 1959), but only two years later, the first case of methicillin-resistant S. aureus (MRSA) was reported in England (Jevons, 1961). Methicillin-resistant Staphylococcus aureus (MRSA) is a specific strain of the Staphylococcus aureus bacterium that has developed antibiotic resistance to all penicillins, including methicillin and other narrow-spectrum \(\beta\)-lactamase-resistant penicillin antibiotics (Foster, 1996). The resistant strain, MRSA which was first discovered in the UK in 1961, is now widespread, particularly in the hospital setting. MRSA may also be known as oxacillin-resistant Staphylococcus aureus (ORSA) and multiple-resistant Staphylococcus aureus. Despite this, MRSA generally remained an uncommon finding even in hospital settings until the 1990s when there was an explosion in MRSA prevalence in hospitals where it is now endemic (Johnson \textit{et al}., 2004). Worldwide, an estimated 2 billion people carry some form of S. aureus; of these, up to 53 million (2.7% of carriers) are thought to carry MRSA. In the United States, 95 million carry S. aureus in their noses; of these 2.5 million (2.6% of carriers) carry MRSA (Graham \textit{et al}., 2006).

The genetic determinant of methicillin resistance in MRSA is the acquired gene meca, which encodes the low-affinity penicillin-binding protein 2A (PBP2A), which can function as a surrogate trans-peptidase in the presence of high concentrations of \(\beta\)-lactam antibiotics that inactivate the four high-affinity PBPs native to S. aureus (de Jonge and Tomasz, 1993). It has been established that the production of an additional penicillin-binding protein PBP-2' (PBP-2a), with low-affinity for beta-lactam antibiotics, is mainly involved in the mechanism of methicillin resistance of S. aureus (Utsui and Yokota, 1985).

While the PBP-2', which is encoded by a chromosomal structural gene designated as meca, is usually induced by beta-lactam antibiotics, it is known to be constitutively produced in some MRSA(Song \textit{et al}., 1987 and Ubukata \textit{et al}., 1990).
There are limited reports on the epidemiological aspects of nosocomial infections in the animal hospitals and laboratory settings (Hartmann et al., 1997, Koterba et al., 1986 and Tomlin et al., 1999). Although, faculty of reports on nosocomial spread of an MRSA infection in a veterinary hospital could be found (Seguin et al., 1999), there have been Veterinary reports of MRSA infections in dairy herds with mastitis (Devriese and Hommez, 1975 and Revill, 2003) and in companion animals (Koterba et al., 1986; Tomlin et al., 1999 and Cefai et al., 1994) and of an isolated incident in a horses and horse personnel (Hartmann et al., 1997 and Weese et al., 2005). There is a Press Headlines such as “Hospital Superbug” MRSA spreads to animals (Revill, 2003) and MRSA on the risk in U. K. Veterinary clinics (Veterinary Times, October 2004) have focused attention on the potential risk of MRSA to companion animal health. Transmission of MRSA between humans and horses has also been suspected in a Veterinary teaching hospital in the United States (Seguin et al., 1999) and in Canada (Weese et al., 2005). The environment contributes to MRSA transmission (Udo et al., 1996) and transmission of MRSA to humans also caused by consuming contaminated food products of animals (Lee, 2003). The research on the detection as well as prevalence of MRSA in animal population & differences in prevalence of MRSA between animal and human origin has never been studied in Bangladesh previously. Therefore, this research will focus on the distribution and prevalence of MRSA in animal and human origin in Bangladesh.

MATERIALS AND METHODS

Isolation and Identification of S. aureus

The bacteria was isolated & identified by cultural characteristics, colony morphology, Gram’s stain and biochemical tests.

Detection of MRSA by PCR

MRSA was detected by PCR, as described previously by Kobayashi et al. (1994).

Extraction of DNA of S. aureus

A total of 100 µl TNE buffer was taken in eppendorf tube in which bacterial sample was added and mixed by vortexing, centrifuged at 10000 rpm for 1 minute. The supernatant was removed by micropipette & added 10 µl achromopeptidase enzyme(disrupting solution) with the pellet, mixed well by pipetting and incubated the tube at 40-42°C in water bath for 10 minutes. Removed from water bath, added 50 µl of 0.5 M KOH in the tube and mixed by vortex, kept at room temperature for 5 minutes. Finally 50 µl of 1M-Tris-Hcl (pH 6.76) was added and mixed by vortex, centrifuged at 10000 rpm for 1 minute and collected supernatant which contains DNA used for PCR.

Polymerase Chain Reaction (PCR) for detection of mecA gene from S. aureus

A total amount of 79 µl de-ionized distilled water (DDW) was taken to the eppendorf tube, added 10 µl X10 reaction/PCR buffer (Roche Diagnostics, Germany) & 8 µl of 2.5 mM dNTP (Takara, Japan) to the tube. Then added 2 µl mecA primer,1 µl supernatant DNA sample and 0.5µl Taq Polymerase (5 units/µl, Roche Diagnostics, Germany) to the tube. Gently mixed by vortex and then kept tubes in thermal cycler. A thermal cycler was used to amplification of DNA. The cycling program included 30 cycles of a denaturing step at 94°C for one minute, an annealing step at 55°C for one minutes, and an extension step at 72°C for two minutes. After completion of cycling program reactions were held at 4°C. Based on the nucleotide sequences of mecA gene (Song et al., 1987 and Berger-Bächli et al., 1989), the oligonucleotide primers were designed (Table 1) and target genes were synthesized by PCR.

Preparation of 1% Agarose gel for electrophoresis

Two grams agarose was dissolved into a 200 ml TAE buffer in a flask and was heated by microwave oven for 1-2 minutes up to transparent. A total of 20µl of 10 mg/ml ethidium bromide was added to 200 ml melted agarose and then melted agarose was poured on the gel casting tray and placed the comb of appropriate teeth and number and allowed to solidify on the bench (one hour at room temperature).
Amplified DNA sample preparation and electrophoresis
The comb was gently removed. The solidified gel was transferred to the electrophoresis chamber containing sufficient amount of TAE buffer with 30µl ethidium bromide keeping the gel horizontally. Loading dye (as required) placed on a piece of parafilm using adjustable micropipette (0.5 to 20µl) and 1µl of DNA size marker was mixed with drop of dye and loaded to the first hole of the gel. A total of 10µl of amplified DNA sample was added to next drop of dye and mixed well by pipetting subsequently loaded to the next hole and continued up to last hole of the gel following same procedure. The electrophoresis chamber was covered and the electrophoresis apparatus were connected to the power supply. Electrophoresis was carried out at 110 volt for 25 minutes to get the dye third fourth of the length. When DNA migrated sufficiently as judged from the migration of Bromophenol blue of loading buffer, the power supply was switched off and examined to detect the band under UV illumination (Figure 1).

Documentation of the DNA samples
After electrophoresis, the gel was taken out carefully from the gel chamber and the gel gently placed on the UV transilluminator in the dark chamber of the image documentation system. The UV light of the system was switched on; the image was viewed on the monitor, focused and saved in a dictate, as well as printed on thermal paper. The positive sample was detected by visualized band on the gel.

RESULTS AND DISCUSSION
A total of 100 animal origin and 100 human origin specimens were included in this study. Among 100 animal origin specimens Staphylococcus aureus was 54 (54%). All the total animal origin Staphylococcus aureus were examined by PCR but no MRSA was detected.
The human origin samples were collected from hospitalized patients. Among 100 human origin specimens 40 were *Staphylococcus aureus* (Table 2). All the *S. aureus* of human origin specimens were examined by PCR. Out of 40 *S. aureus*, MRSA were 10 (25%). The detection rate of MRSA (n=10) isolated from different human origin specimens were given in Table 2. Majority (7.5%) of the MRSA were isolated from pus from skin infection followed by surgical wound swab, burn ulcer exudates and oral swab (5% each) and vaginal swab (2.5%).

<table>
<thead>
<tr>
<th>Type of specimen</th>
<th>Total no. specimen examined</th>
<th>Detection of <em>S. aureus</em></th>
<th>MRSA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical wound swab</td>
<td>50</td>
<td>17</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Burn ulcer exudate</td>
<td>14</td>
<td>4</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Aural swab</td>
<td>11</td>
<td>8</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Pus from skin infection</td>
<td>19</td>
<td>9</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>Diabetic ulcer exudate</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Vaginal swab</td>
<td>1</td>
<td>1</td>
<td>1 (2.5%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
<td><strong>40</strong></td>
<td><strong>10 (25%)</strong></td>
</tr>
</tbody>
</table>

Methicillin-resistant *S. aureus* (MRSA) has become increasingly prevalent worldwide. Strengthening surveillance and screening of high-risk patients appears as an important component of effective infection control programme to limit the spread of MRSA in hospitals. In this context accurate detection of MRSA is essential. Antimicrobial susceptibility by disc diffusion or broth microdilution methods cannot detect MRSA those express low levels of oxacillin resistance. Recently many methods for detection of MRSA have been evaluated and are widely used in bacteriological laboratory. One of them has been designed as PCR detection of MRSA by detecting mecA gene. The present study attempted the detection of MRSA by PCR technique.

Two chromosomal mec regulator genes mecRI and mec1 have been identified (Hirasmatu et al., 1992 and Tesch et al., 1990). Surveys of the distribution of mec regulator genes among clinical isolates of methicillin-resistant staphylococci indicated that mecI encodes the repressor protein of the mecA gene and it is deleted or mutated in methicillin-resistant strains (Suzuki et al., 1993). Although the mechanism of regulation of the mecA gene has not been completely elucidated, the presence of the mecA gene in staphylococci has been considered as a molecular basis for the identification of MRSA or methicillin-resistant coagulase negative staphylococci (CNS), even though the strain appears methicillin-sensitive by the measurement of minimum inhibitory concentration (MIC) (Hirasmatu et al., 1992). On the basis of this findings, attempts have been made to identify MRSA by polymerase chain reaction (PCR) amplification of mecA gene fragments derived not only from isolated strains but also from clinical specimens directly (Higashiyama et al., 1993, Mukakami et al., 1991 and Tokue et al., 1991).

The mecA gene and the associated large (40 to 60-kb) mec element (Fontana, 1985; Hartman and Tomasz, 1986; Matsuhashi et al., 1986; Matthews et al., 1987; Reynolds and Brown, 1985 and Tesch et al., 1988) are not native to *S. aureus* but were acquired from an extra species source by an unknown mechanism (Beck et al., 1986 and Pattee, 1990). The nature of the extra species source, i.e., the evolutionary origin of mecA and the formation of the mec element, has remained largely a matter of speculation (Archer and Niemeyer, 1994; El Kharroubi et al., 1991; Hirasmatu, 1995 and Piras et al., 1993).

In the present study all the animal origin *S. aureus* (n=54) were examined by PCR for mecA gene but no mecA positive strain was detected. The probable reason behind this outcome that there is no oxacillin preparation in our market for animal therapy. But Kwon et al. (2006) isolated three pre-MRSA, one silent mecA-carrying methicillin susceptible *S. aureus* (smMSSA) from retail chicken meat, and three MRSA from hospitalized dogs in Korea although methicillin (oxacillin) was not used in animal husbandry or in animal hospitals in Korea that was not in agreement with present study. Therefore, further extensive study is required for elucidate this issue.

Methicillin-resistant *S. aureus* (MRSA) represents a major challenge to hospitals in all countries due to the emergence and spread of isolates with decreased susceptibilities to several antibiotic classes, in addition to methicillin and the other members of the β-lactam family. Molecular typing techniques applied to international collections of MRSA isolates have contributed to the understanding of the epidemiology and evolution of this infectious agent.
Detection of methicillin-resistant S. aureus

The prevalence of MRSA differs strongly throughout the countries. In the present study, no MRSA in animals was found while in human the isolation rate is 25%. In 1999-2000, 20% of the European blood isolates were MRSA which is agreement with present study. Another study from Bangladesh in human reported an isolation rate of MRSA as 12.5% (Hossain et al., 2002) which is not in agreement with present study, which also indicates that the incidence of MRSA in our country is increasing day by day. The increasing incidence of MRSA observed in this study might be due to the fact that our specimens were taken from tertiary hospital where there is no authentic antibiotic policy to treat infectious patients. As a result, indiscriminate use of antibiotics is not less common. In addition, hospital environment are not adequately hygienic. Overcrowding of patients and attendants favors the spread of infectious agents. So hospitals acquired infection either in surgery or in medical wards are quite high. Due to infection, patients stay becomes prolong in hospital keeping under antibiotic therapy. All these factors mentioned above might be sufficient cause to increase the acquisition of resistance property among strains of S. aureus.

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REFERENCES
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