Abstract: Methicillin-resistant Staphylococcus aureus (MRSA) more serious problem in dairy animals suffering from mastitis. In this study collated 130 sample of mastitis milk and 130 from nasal worker. The S. aureus characterized phenotypically by biochemical and API Staph and genotypically by sau gene specific species. (47.69%) percentage of isolation in mastitis milk, (82.25%) biotype C and (4.83%) biotype B that origin from bovine and (12.90%) biotype A origin from human, while isolation S. aureus(44.61%) from nasal carrier, the biotype A recoded 91.37% and 8.63% biotype C origin from bovine. The isolates were tested using agar disc diffusion method for oxacillin and cefaxiton and confirmatory mec A gene by PCR(28. 12%) was appeared MRSA from bovine and (35.48%) from human isolated, all of MRSA have all of virulence genes ctfA gene, Spa gene and Nuc gene for their antimicrobial susceptibility to 10 different antimicrobial drugs. Most of MRSA isolates were found to be multi-drug resistant.

Keywords: biotypes, S. aureus MRSA

1. Introduction

S. aureus is considered as a significant pathogen with related virulence factors such as slime factor (biofilms), PVL (Panton Valentine Leucocidine) and some enzymes (proteases, lipases, and elastase), which enable it to destroy host tissues and metastase to other sites. MRSA infection from mastitis cases is partly related with failure in dosage therapy and choice of antimicrobial substance.

Methicillin-resistant S. aureus (MRSA) includes S. aureus have acquired gene, called mecA, this gene encoding to resistance of methicillin and possibly to some beta-lactam antibiotics. MRSA was first reported as a nosocomial pathogen in 1961, soon after methicillin was introduced into human medicine to treat penicillin-resistant staphylococci. MRSA clones have particularly been detected in animal populations. Although MRSA is mostly associated with the acquiring mecA gene, the role of inappropriate antibiotics use should also not be under estimated in formation of bacterial resistance and multidrug resistant strains. MRSA infection from mastitis cases is partly related with failure in dosage therapy and choice of antimicrobial substance.

The emergence of (MRSA) poses a serious public health threat. Strains of resistant to b-lactam antibiotics are known as (MRSA). Resistance of methicillin is caused by the gain of the mecA gene. PBP2A gene encodes for alternative Penicillin-binding protein, which has a low affinity for beta-lactam antibiotics. SCCmec Staphylococcal Cassette Chromosome mec , mecA gene one of a large movable genetic. MRSA are acquired multidrug resistant, MRSA have been give an account to resist most antibiotics like macrolides, fluoroquinolones, chloramphenicol, tetracycline and aminoglycosides. In veterinary medicine the extension with treatment the antibiotics are used for some different purpose such as enhance efficiency of feeding and enhance growth of animals. This different purpose enhance multiple drugs resistant to zoonotic bacterial pathogens. Community-acquired infections in every region of the world is associated with S. aureus recognized as causing health care. S. aureus that able to produce Enterotoxins in milk possess a potenciyriskiness to healthy of consumers.

Commercial identification panels such as Vitec, Sensititre, BACTEC, API-Staph identification systems have been used to identify Staphylococcus to sub-species inboth human and animal isolates for many years. MecA detection by PCR is accepted as the gold standard method for identifying MRSA.

In the control of mastitis, the improper use of antimicrobial agents on dairy farm animals is a major concern as it leads to the emergence of resistant zoonotic bacterial pathogens.

In veterinary medicine the extension with treatment the antibiotics are used for some different purpose such as enhance efficiency of feeding and enhance growth of animals. This different purpose enhance multiple drugs resistant to zoonotic bacterial pathogens dissemination.

Community-acquired infections in every region of the world is associated with S. aureus recognized as causing health care. S. aureus that able to produce Enterotoxins in milk possess a potenciyriskiness to healthy of consumers.

The aims of this study was isolation, characterization, biotyping of S. aureus and study some virulence molecular detection of MRSA from cases of mastitis milk mastitis and worker or cattleman handling with infected cattle and determine the reservoir for MRSA infection cattle and humans and vice –versa.

2. Materials and Methods

Samples collection, this study was performed between October 2013 and July 2015 from Waste Province. Milk samples (130) CMT was scored between I- IV isolation of staphylococcus was attempted depend on clinical sign and decrease milk production were tested by California mastitis test (CMT) were graded as negative, trace, weak, distinct, or strong positive, (clinical and subclinical mastitis). Worker
sample collect from human handling with bovine, 130 samples were taken by nasalswab under sterile conditions for further bacteriological analysis.

The specimens were inoculated onto Mannitol salt agar MSA Himedia - India; and primary cultures incubated at 37°C for 24 h. All colonies were purified by sub culturing onto MSA medium.16

Biochemical testes
The identification of the bacteria was performed by a tube coagulate test, manniott fermentation on Mannitol salt agar and coagulate test tube and detection of clumping with plasma on slide, the production of hemolysis of isolates was determined by cultivation of the bacteria on blood agar plates and vagues proskuor VP, urease oxidase Latex agglutination (MASTSTAPH); considered as S. aureus with finally detection by API Staph.17,18,19

DNA isolation
KAPA Express Extract KK7100 (50 rxns). Transfer DNA containing supernatant to a fresh tube. And may be diluted in TE buffer for long term storage at -20°C. (KAPA BIOSYSTEMS).

Genotypic characterization
Kit KAPA Taq Ready Mix DNA polymerase contain Taq DNA polymer bases (0.5 U /1.25 U per 25 ul) Reaction buffer with Mg +2 and o.4 mM each dNTP with or without dye. (KAPA BIOSYSTEMS).

A ladder (KAPA BIOSYSTEMS) size of amplicons KAPA Universal ladder contain (100 ng/µl) 1 x1 ml KAPA with Mg +2 and o.4 mM each dNTP with or without dye. (KAPA BIOSYSTEMS).

Thermolucnase (nuc), Reaction mixtures (25 µl) included 2 µl template DNA, 20 µl of master mix, 10 pmol of each of the 2 primers 279 bp. Amplification and primer described by Stepan et al.21 PCR cycles 37 denaturation (94°C for 1 min), annealing (55°C for 0.5 min), and extension (72°C for 1.5 min), final extension (72°C for 3.5 min). Amplified products were separated by agarose gel (1.7% agarose) used for electrophoresis at 5 V/cm for 3 h.

Cefoxitin sensitivity testing
This test was done according.22 Kirkby and Bauer, (1966), by disc-diffusion method, staphylococcal isolates were tested for their sensitivity to Cefoxitin (cx 30 mg). A zone of inhibition with a diameter of ≤ 21 mm was considered as an indication for resistance to methicillin. Out of the 64 samples enrolled in the current study there were only 14 samples showed resistance to cefoxitin (30 mg) can be considered as methicillin resistant Staphylococcus aureus.

Oxacillin Disk Diffusion Test
Oxacillin disks (1 µg. Oxoid) were used was performed on all isolates of S. aureus using the Kirby-Bauer disk diffusion method. Zones of inhibition were determined in accordance with procedures of the CLSI standard guidelines. 23 According to CLSI were . S. aureus isolates were considered susceptible to oxacillin inhibition zones more than 13 mm after incubation on 2% NaCl Mueller Hinton agar at 35°C for 18-24 h. All isolates of S. aureus using the Kirby-Bauer disk diffusion method to detection antibiotic sensitivity, zones of inhibition were determined in accordance with procedures of the CLSI standard guidelines Ciprofloxacin, CIP, 5 µg. Clindamycin, DA, 2 µg, Cloxacillin, CX, 10 µg. Erythromycin, E, 15 µg. Gentamycin, CN, 10 µg, Penicillin, P, 10 µg. Streptomycin, S, 10 µg. Tobramycin,
PCR amplification for detection of mecA gene

All S. aureus isolates resistance to cefoxitin (30 mg) were screened for mecA gene by PCR. The mecA gene was amplified using primers as described by Murakami et al. For mecA gene, reaction mixtures (25 µl) included 2 µl template DNA, 20µl of master max 2 pmol concentrations of forward and reverse primers. The cycling parameters were as follows: an initial denaturation at 94°C for 5 min; followed by 40 cycles of 94°C for 30 s, 55°C for 30 s, and 70°C for 1min; and with a final extension at 72°C for 5 min.

PCR products were visualized on 2% agarose gel with ethidium bromide dye under UV transilluminator. Amplicons of 533 bp were consistent with mecA gene amplification.

Biotyping

Biotyping was based on three properties-hydrolysis of Tween 80, pigmentation on Tween 80 Agar. Tween 80 1% v/v was incorporated in Nutrient Agar, and the test organism was spread over an area approximately 1 cm in diameter. Plates were incubated at 37°C for 2-3 days. A positive test was denoted by a halo of fatty acids around the inoculum. This medium enhanced pigmentation of S. aureus anidolates were described as gold, buff or cream.

Virulence factors characterization S. aureus virulence factors were phenotypically characterized in vitro. Biotypes A, B, C and D were identified by culturing isolations in brain and heart agar with violet crystal (1:10000) at 37°C. Positive colonies were determined for presenting violet color. Different biotypes were considered according to color and growth. Biotype A growth positive to violet crystal, biotype B white, biotype C yellowish and no growth for biotype D.

3. Statistical Analysis

S. aureus frequency isolation comparison, virulence factors expression and frequency of association, was determined by χ2 test based on a cut point for positive and negative results. Statistical significance was established at a 5% level using the statistical SSPS system

4. Results

In this study was collected 130 sample of bovine mastitis and 130 from human worker in bovine farm, in bovine mastitis the percentage of isolation was appeared (47.69%) and the most strain of isolation biotype c (82.25%) and some strain was shown biotype A and(12.90%) biotype B (4.83%).

While the percentage of human isolation was shown (44.61%), the most strain was recorded biotype A (91.37%) and (8.6%) was appeared biotype C without recorded any strain type B this results depended on growth on crystal violet and hydrolysis of tween 80 and pigment productiontable.1.

<table>
<thead>
<tr>
<th>Biotypes</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>NO</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine strain</td>
<td>8</td>
<td>12</td>
<td>3</td>
<td>4.83</td>
<td>51</td>
</tr>
<tr>
<td>Human strain</td>
<td>53</td>
<td>91.37</td>
<td>0</td>
<td>0</td>
<td>8.6</td>
</tr>
</tbody>
</table>

Growth on crystal violet

<table>
<thead>
<tr>
<th>Violate</th>
<th>White</th>
<th>Yellow</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Hydrolysis of tween 80

Pigment production

Cream buff variable

Figure 1: The percentage of S. aureus isolation and biotypes differentiation in bovine and human.

All isolation strain was testing in the some virulence factor, all strain can detection by used Sua gene that specific spcies detection of S. aureus. When study of ctf A gene the percentage was appeared 69.35% in bovine strain and 72.41% in human strain, while the spa gene was shown 62.35% in 950bp and 4.68% in 390bp in bovine strain and 77.58% in 950bp and 8.62% in human strain, mce gene was shown 87.93% in bovine strain while 87.93% in human strain with significant differences between virulence factor.

After the test of resistance to methicillin mec A gene the bovine strain was shown 28.12%, while the 35.48% in human strain Table 2.

Table 2: Number and percentage of some virulence genes in the S. aureus.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sua gene</th>
<th>ctfA gene</th>
<th>spa gene</th>
<th>Nuc gene</th>
<th>Mec A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>530 bp</td>
<td>985 bp</td>
<td>950 bp</td>
<td>390 bp</td>
<td>278 bp</td>
</tr>
<tr>
<td>62</td>
<td>62</td>
<td>43</td>
<td>40</td>
<td>3</td>
<td>53</td>
</tr>
<tr>
<td>Percentage</td>
<td>100%</td>
<td>69.35%</td>
<td>62.5%</td>
<td>4.68%</td>
<td>82.81%</td>
</tr>
<tr>
<td>Human</td>
<td>58</td>
<td>42</td>
<td>45</td>
<td>5</td>
<td>51</td>
</tr>
<tr>
<td>Percentage</td>
<td>100%</td>
<td>72.41%</td>
<td>77.58%</td>
<td>8.62%</td>
<td>87.93%</td>
</tr>
</tbody>
</table>

The all strain test to antibiotic sensitivity to Cefoxitin (cx 30 mg) and Oxacillin disks (1 µg,) the strain of MRSA was shown high sensitivity to cefoxitin comparison to oxacillin, the number of strain resistance to cefoxitin was appeared carry the mec A gene when used PCR technique, table 3.

The all isolation strain was test by antibiotic sensitivity by used 10 different antibiotic he high percentage of resistance to Cefoxitin, Cloxacin, Oxacillin, Penicillin followed by Streptomycin and Erythromycin and the high sensitivity was shown in Vancomycin and Clindamycin in both bovine and human strain table 4, 5. With highly significant differences in p<0.001. The multidrug resistance more than 9 antibiotic of bovine strain (50% of MRSA) while in human strain the multidrug resistance was appeared in (40.90), Table 6.

Table 3: The sensitivity of Oxacillin and cefoxitin comparison with PCR

<table>
<thead>
<tr>
<th>Strain</th>
<th>OX disk</th>
<th>False positive</th>
<th>Cefoxitin disk</th>
<th>False positive</th>
<th>PCR</th>
<th>False positive</th>
<th>True MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine 62</td>
<td>23</td>
<td>5</td>
<td>18</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Sensitivity rate</td>
<td>21.73%</td>
<td>0%</td>
<td>0%</td>
<td>29.03%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human 58</td>
<td>33</td>
<td>11</td>
<td>23</td>
<td>1</td>
<td>22</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Sensitivity rate</td>
<td>33.33%</td>
<td>4.34%</td>
<td>0%</td>
<td>37.93%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
as the geographical difference, type of clinical sample, number of samples, specificity and sensitivity of methodology and types of strains (MRSA or MSSA).

In this study was determine methicillin resistant staphylococci a zoonotic importance associated with mastitis in cattle and their role in transmission to animal handlers.

Our study confirms the usefulness of PCR for the detection of antibiotic resistance genes associated with *S. aureus* infections. The PCR offers a rapid, simple, and accurate identification of antibiotic resistance profiles. Laboratory methods used to detect multidrug resistant bacteria such as MRSA should have high sensitivity and specificity.

Some study combined in same line, a total of 151 (31.45%) *S. aureus* isolates were identified by API-Staph® detection, (41.05%) isolates were determined as resistant to Cephoxitin (30 ìg) demonstrates the distribution of *mecA* carrying *S. aureus* isolates and their locations in Turkey.

Other study reviled the results combined with this study, A total of 235 clinical mastitis milk samples revealed 116, (49.36%) samples positive for *S. aureus*, of which 12 samples (10.34%) were MRSA.

However, improper use of antibiotics creates problems such as the emergence of bacterial resistance to antibiotics.

One of these problems, the occurrence of methicillin resistance, has been observed frequently in recent years.

Therefore, infections by MRSA require rapid and accurate diagnosis for elimination at an early stage, because these strains can cause severe damage to infected sites and may be widespread in the environment.

In most routine microbiological settings, the detection of methicillin resistance among staphylococcal isolates is based on phenotypic assays such as the disk diffusion test and MIC determination. Genetic confirmation of positive findings based on detection of the *mecA* gene has also been reported.

MRSA strains were multi-drug resistant which might be due to production of betalactamase and PB2a (penicillin binding protein).

The disk diffusion and broth dilution method require at least 24 h for evaluation of the results. However, the detection of antibiotic resistance genes such as *mecA* gene by PCR techniques is considered the gold standard method.

A good correlation between phenotypic antibiotic susceptibility patterns and genotypic analysis by PCR was also reported. The critical para meters for success of a PCR for the detection of multidrug resistant bacteria asMRSA are cost, reliability and practical, fast, accuracy and sensitivity and results were obtained within 4 hours. In veterinary microbiology, numerical techniques applicable to diagnosed used to characterize *S. aureus* strains that important cause of bovine mastitis and Rapid detection necessary to

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**Table 4:** The sensitivity and resistance of different antibiotic in bovine strain

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive</th>
<th>Percentage</th>
<th>Resistance</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin, Cloxacillin, Oxacillin, Penicillin</td>
<td>0</td>
<td>0%</td>
<td>22</td>
<td>100%</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>6</td>
<td>27.27%</td>
<td>16</td>
<td>72.73%</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>7</td>
<td>31.81%</td>
<td>15</td>
<td>68.19%</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>7</td>
<td>31.81%</td>
<td>15</td>
<td>68.19%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>7</td>
<td>31.81%</td>
<td>15</td>
<td>68.19%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>9</td>
<td>40.90%</td>
<td>13</td>
<td>59.10%</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>14</td>
<td>63.63%</td>
<td>8</td>
<td>36.37%</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>14</td>
<td>63.63%</td>
<td>8</td>
<td>36.37%</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>15</td>
<td>68.19%</td>
<td>7</td>
<td>31.81%</td>
</tr>
</tbody>
</table>

X2=35.552 P= 0.00021

**Table 5:** The sensitivity and resistance of different antibiotic in human strain

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive</th>
<th>Percentage</th>
<th>Resistance</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin, Cloxacillin, Oxacillin, Penicillin</td>
<td>0</td>
<td>0%</td>
<td>18</td>
<td>100%</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>4</td>
<td>22.22%</td>
<td>14</td>
<td>77.78%</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>4</td>
<td>22.22%</td>
<td>14</td>
<td>77.78%</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>4</td>
<td>22.22%</td>
<td>14</td>
<td>77.78%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>3</td>
<td>16.66%</td>
<td>15</td>
<td>83.44%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>9</td>
<td>50%</td>
<td>9</td>
<td>50%</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>13</td>
<td>72.22%</td>
<td>5</td>
<td>27.78%</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>11</td>
<td>61.11%</td>
<td>7</td>
<td>38.89%</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>13</td>
<td>72.22%</td>
<td>5</td>
<td>27.78%</td>
</tr>
</tbody>
</table>

X2=43.438 P= 0.0001

**Table 6:** The multidrug resistance of different antibiotic in human strain

<table>
<thead>
<tr>
<th>Resistance to antibiotic</th>
<th>Number of strain</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nine antibiotic or more above</td>
<td>9</td>
<td>50%</td>
</tr>
<tr>
<td>Eight antibiotic</td>
<td>2</td>
<td>11.11%</td>
</tr>
<tr>
<td>Sven antibiotic</td>
<td>4</td>
<td>22.22%</td>
</tr>
<tr>
<td>Six antibiotic</td>
<td>1</td>
<td>5.55%</td>
</tr>
<tr>
<td>Five antibiotic</td>
<td>2</td>
<td>11.11%</td>
</tr>
<tr>
<td>Four antibiotic</td>
<td>2</td>
<td>100%</td>
</tr>
</tbody>
</table>

X2=42.800 P= 0.0003

**Table 7:** The multidrug resistance of different antibiotic in bovine strain

<table>
<thead>
<tr>
<th>Resistance to antibiotic</th>
<th>Number of strain</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nine antibiotic or more above</td>
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</tr>
<tr>
<td>Eight antibiotic</td>
<td>3</td>
<td>13.63%</td>
</tr>
<tr>
<td>Sven antibiotic</td>
<td>1</td>
<td>4.54%</td>
</tr>
<tr>
<td>Six antibiotic</td>
<td>5</td>
<td>22.72%</td>
</tr>
<tr>
<td>Five antibiotic</td>
<td>4</td>
<td>18.18%</td>
</tr>
<tr>
<td>Four antibiotic</td>
<td>22</td>
<td>100%</td>
</tr>
</tbody>
</table>

X2=48.000 P= 0.0002

5. Discussion

Mastitis is the most common cause for antibiotic use in dairy herds. Effectiveness of current treatments and ability to control infectious diseases in both animals and humans may become hazardous.

In general, variation in the prevalence of genes between these studies might be attributable to different factors such as the geographical difference, type of clinical sample, number of samples, specificity and sensitivity of methodology and types of strains (MRSA or MSSA).

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Strains producing TNase by S. aureus encoding by nuc genelease bacteria. S. aureus TNase has of nuc gene has potential for the rapid diagnosis of S. aureus infections with contain species-specific sequences and amplification. The tests of susceptibility antimicrobial are provide guide to the veterinarian to help in selecting treatment of mastitis that caused by S. aureus. Many studies are available to recorded MRSA and multi-resistant drug of S. aureus strains and has also been reported in some cases in veterinary medicine.

Different strains genetic variability and phenotypical expression of virulence factors related to severity of clinical signs in S. aureus mastitis.

The host specificity of S. aureus strains of diverse origin related with analyses of the genotype distributions of demonstrated. It seems that the occurrence of some S. aureus strains lineages is restricted to animals.

In the study of Vishnupriya et al. that collect 158 samples from mastitic milk and 126 nasal swabs from the animal handlers when analysis of phenotypic and genotypic six MRSA isolates (three bovine and three human) Control of mastitis staphylococci may occur by control to milk from harbor organisms that causes by potentially pathogenic to humans because this bacteria most commonly isolated from subclinical mastitis. S. aureus strains produce penicillinase and more than 80% of thus strain carry β-lactam and resistance to different antibiotic such as methicillin, (MRSA) have become a serious nosocomial infection worldwide.

MRSA isolation from bovine mastitis so milk could be a source of MRSA infection to human beings. Prevalence of MRSA among the two groups were found to be 8/19 (42.1%) and 2/21 (9.5%) respectively healthy medical students and healthy nursing students. No report that recoded to systematic study explain relatedness of MRSA between animals and humans and vice-versa.

Different animals such horses and dogs with lesions, and dogs and cats that were carriers MRSA strains and isolated from cows with mastitis. Transmission of MRSA between humans and animals (e.g., dogs, horses, pigs) has been reported.


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