ABSTRACT

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Staphylococcus aureus is a well known pathogen of man and animals. Despite the development of antimicrobial agents the staphylococcal infection still remains an important cause of morbidity and mortality. The spectrum of disease produced by this organism varies from pyogenic infections to toxin-mediated phenomenon. It also causes serious infections in man including deep-seated abscesses, bacteraemia, endocarditis, osteomyelitis, food poisoning etc. The main reservoirs of infection are infected cases and carriers who spread infection in a hospital or in a community through droplets in air or through fomites. Carriage in nose plays an important role in its epidemiology and pathogenesis.

In animals too it causes a wide spectrum of diseases like intramammary infections in lactating animals, sporadic septicaemia in pigs, abscesses, synovitis, arthritis, osteomyelitis, dermatitis in poultry and ducks. Bovine mastitis is one of the most important bacterial diseases in dairy cattle throughout the world, and it is responsible for great economical losses to milk producers as well as to the milk processing industries. *S. aureus* can also multiply in many food items.

Various global studies are available to characterize the *Staphylococcus aureus* at molecular level, however, the studies are fragmentary from India on the current aspect and especially in *Staphylococcus aureus* isolates obtained from samples of animal origin. Therefore, the present study was undertaken with the aims to evaluate the phenotypic and genotypic characters of *Staphylococcus aureus*, whicl might provide an understanding of the distribution of the clones and might aid in the development of the steps to control *S. aureus* infections in humans as well as in animals.

A total of 102 *Staphylococcus aureus* from human clinical specimens and 100 from animal-originated (both animal-clinical and animal-originated food) samples were randomly selected for the study. All were tested phenotypically according to the standard methodology and confirmed as *S. aureus*.

Among 102 **human clinical isolates,** maximum number of isolates were resistant to penicillin (98.03%) followed by cotrimoxazole (69.61%), tetracycline (68.63%), amoxycillin (64.7%), ciprofloxacin (60.79%), erythromycin (54.9%), amikacin (35.3%), **oxacillin (32.35%),** cefaclor (32.35%), ceftriaxone (32.35%), ceftazidime (32.35%), cefepime (32.35%), chloramphenicol (23.53%) and gentamicin (27.45%). While none of the isolates were found resistant to vancomycin and teicoplanin. Out of these 102 **clinical isolates,** 80 (78.4%) were β-lactamase producers as identified by iodometric method.

The isolates from **animal-origin samples** showed resistance to penicillin (93%), erythromycin (51%), tetracycline (49%), ciprofloxacin (39%), cotrimoxazole (54%), chloramphenicol (34%), amikacin (27%), **oxacillin (27%),** cefaclor (27%), ceftriaxone (27%), ceftazidime (27%), cefepime (27%), gentamicin (20%), and amoxycillin (19%). Only one isolate was found susceptible to all 16 antibiotics and none of the isolates was found resistant to vancomycin and teicoplanin. Out of 100 **animal-origin isolates**

69 (69%) were found β-lactamase producers by iodometric method. Interestingly, both human and animal isolates showed multidrug resistance patterns.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen worldwide and is often difficult to detect due to the heterogeneous nature of expression of oxacillin resistance. All the human and animal isolates were tested for oxacillin resistance by disc diffusion method, screen agar plate method, and for detection of *mec* A gene by PCR. Out of 102 **human clinical isolates,** 33 were oxacillin resistant by disc diffusion method, 32 by screen agar plate method and 34 isolates were found positive for *mec* A gene by PCR whereas 29% isolates from **animal-origin samples** were identified as MRSA by PCR, 27% by disc diffusion method and 26% by oxacillin agar plate method. The results of our study on PCR of MRSA seem to be promising for early and reliable identification of MRSA.

Bacteriophage typing was performed in 102 **human clinical isolates**. Out of these isolates tested, only 55 (53.9%) isolates could be typed by the conventional set of phages at RTD. 47 non-typeable isolates were further tested at 100 RTD, they showed 16.67% more typeability. The total typeability at 1 RTD and 100 RTD was observed in 70.58% of clinical isolates including 10 methicillin-producing strains.

MRSA phage groups were used to type methicillin-resistant *Staphylococcus aureus* isolates of clinical specimens. A total of 14 (41.18%), out of 34 MRSA, were found typeable at RTD and 100 RTD using MRSA phages. The non typeable isolates at RTD could be typed at 100x RTD and on addition 16.6% isolates were typed. Out of 100 **animal-origin isolates** none of the isolates were typeable at routine test dilution and at 100 RTD. Only two isolates (one from raw milk and the other from buffalo meat) could be typed by using MRSA phage groups at 100 RTD which belonged to phage group II.

All 202 *Staphylococcus aureus* isolates were found positive for the presence of thermostable nuclease (*nuc)* gene by polymerase chain reaction.

Coa gene was detected by PCR and twelve different electrophoretic patterns were observed in human clinical isolates, whereas 7 electrophoretic patterns were observed in animal-originated isolates.

The **molecular typing** of the **human-clinical isolates** and **animal-origin isolates** was carried out by **Coa-RFLP**, after the digestion with *Alu 1* enzyme. Twelve types of ***coa* -RFLP** patterns were observed in **human-clinical isolates**. Among these 12 patterns, 7 group patterns, namely, group I, group II, group IV, group V, group VI, group VIII and group XI were noticed in MRSA isolates. Rest of the group patterns were in MSSA isolates. In 100 **animal-origin isolates,** 7 types of ***coa* –RFLP** patterns were observed. Four group patterns, namely, group II, group III, group I, and group VII, were noticed in 29 MRSA isolates from animal-origin samples. Rest of the group patterns were in MSSA isolates. In the present study, *S. aureus* isolates from animal-origin samples and human clinical specimens were found to differ in their gene patterns whereas animal clinical specimens and animal-origin food samples were showed mixed Coa-RFLP gene patterns. Antibiotic resistance patterns in relation to *Coa* – RFLP pattern could not be inferred in isolates obtained from food samples due to variability of resistance patterns; 40 different types of patterns.

We feel that *Coa*-RFLP technique could be incorporated as diagnostic tool to confirm the MRSA. Since the *Coa*-RFLP patterns of the MRSA strains were unique and distinct from the MSSA strains from both human and animal isolates of *Staphylococcus aureus*. *Coa*-RFLP is performed with primers, homologous to a conserved region with in the *coa* gene, in order to amplify the sequence encoding the C-terminals region of this molecule. Since the number of repetitive sequence varies within the *coa* gene, the resulting PCR products of individual strains can be of different lengths. Therefore we found different length and different patterns of *S. aureus* strains in *coa* RFLP. It is noted that there is extensive polymorphism with *coa* gene circulating in human and animal strains. We believe that the heterogeneity observed for the *coa* gene has a potential discriminatory power for further epidemiological studies of medical and veterinary importance.

For the detection of enterotoxin genes. namely enterotoxin A, enterotoxin B and enterotoxin C, PCR assays were used. A total of 202 isolates (102 human clinical isolates and 100 animal-origin isolates) were tested for the production of enterotoxin A, B and C. Of the 102 **human-clinical isolates,** 3 were found enterotoxigenic, out of which one stool sample showed presence of enterotoxin A, one vomitus sample showed enterotoxin B while one urine sample showed enterotoxin C.

Out of 100 **animal-origin isolates,** a total of 13 were found enterotoxigenic. Out of which 2 were positive for enterotoxin A (1 from animal clinical raw milk sample and 1 from buffalo meat); 3 were positive for enterotoxin B (1 from animal clinical raw milk sample, 1 from a sweet, and 1 from cottage cheese) and 8 were found positive for enterotoxin C (1 from animal clinical raw milk, 1 from paneer, 3 from goat meat, and 3 from buffalo meat).

Conclusions:

On the basis of this study, following **conclusions** were drawn:

- Multidrug resistance was noticed in both human clinical and animal-origin isolates.
- None of the isolate was found resistant to vancomycin and teicoplanin in both clinical and food isolates.
- Phage typing of the **clinical isolates** showed that only 70.58% clinical isolates were typeable using conventional phages at 1 RTD and at 100 RTD including 10 methicillin-producers. Amongst these, maximum number (35.3%) of isolates belonged to phage group III.
- Majority of the MRSA isolates were found non-typeable by conventional sets of phages. By using a set of MRSA phages they showed a typeability of 41.8% (14/34).
- In **animal-origin**, only 2% isolates were found typeable using MRSA phages at 1 RTD and 100 RTD both of them belonged to group II.
- MRSA were detected in **human-clinical isolates** by disc diffusion method in 32.35%, by screen plate agar method in 31.41% and by PCR in 33.33%.
- 29% isolates from **animal-originated samples** were identified as MRSA by PCR, 27% by disc diffusion method and 26% by oxacillin agar plate method.

- All the coagulase positive *Staphylococcus aureus* isolated from clinical and animal-origin samples were found Tnase and Nuc gene producers.
- 12 Coa-RFLP patterns were observed in **human clinical isolates**. Whereas 7 types of *coa* –RFLP patterns were observed in **animal-origin samples**. Knowledge about the genetic variability within different populations may help in the identification of the most likely source of an isolate.
- Coa-RFLP patterns were different for **clinical and animal-originated food isolates** and suggest a divergence between *Staphylococcus aureus* isolates of human and bovine origin.
- Results of coagulase gene typing demonstrated that the MRSA and MSSA strains from **clinical specimens** could be grouped into 7 and 5 *Coa*-RFLP patterns, respectively. However, in **food isolates** 4 from MRSA and 3 from MSSA *Coa*-RFLP patterns were observed.
- The MRSA and MSSA strains did not share similar PCR-RFLP patterns and this could be initialized as a diagnostic tool to differentiate MRSA from MSSA. It offers an attractive option to be considered for rapid epidemiological analysis of *S. aureus* strains.
- 102 **human clinical isolates** were tested for commonly encountered enterotoxins; three of them were found enterotoxin producers, out of which 1 was positive for enterotoxin A, 1 for enterotoxin B and 1 for enterotoxin C.
- Out of 100 **animal-origin isolates**, a total of 13 were found enterotoxogenic. Of which 2% were positive for enterotoxin A; 3% were positive for enterotoxin B and 8% were found positive for enterotoxin C.

In nutshell, diversity between clinical and food isolates of *Staphylococcus aureus* was noticed and that the incidence of methicillin resistance was quite high in this collection of isolates. Concomitant high resistance to other classes of antibiotics was also noted. Phage typing was found to be of low discriminatory value whereas Coa-RFLP could discriminates a fairly large numbers of bacterial isolates and suggest that Coa-RFLP could be used as an epidemiological typing method for *Staphylococcus aureus.* Enterotoxin A and B were detected in our collection of clinical isolates, whereas all three enterotoxins were prevalent in food isolates with enterotoxin C being the predominant type.