Epidemiological Survey Of Staphylococcus Aureus Isolated From Clinical Sample Of Bagalkot District

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Abstract
The \textit{Staphylococcus aureus} is the most prevalent species isolated from in patient isolates, irrespective of origin, and the second most prevalent from outpatient isolates in a laboratory-based surveillance. The epidemiology is an important study the spared of the infection in given location. Keeping Bagalkot district as the area of spared we try to study infection. In present study, 310 clinical samples were isolated from different places of Bagalkot district, for convenience, five Zones are made; namely A, B, C, D and E. Out of 310 clinical samples, 223 from males and 87 from females were collected. After performing all biochemical tests 180 isolates were confirmed as \textit{S. aureus}. Therefore the rate of infection is very high in Bagalkot district.

Keywords: Epidemiology, \textit{S. Aureus}, Nosocomial Infection, Hemolysis Coagulase

1. Introduction
\textit{Staphylococcus aureus} is a facultative anaerobic gram positive bacteria appears as grape-like clusters when observed under simple microscope and has large round and golden-yellow colonies often with haemolysis were observed when grown on blood agar plates \cite{1}. 
\textit{S. aureus} is catalase-positive which is able to convert hydrogen peroxide (H$_2$O$_2$) to water and oxygen, which makes the catalase test useful to distinguish staphylococci from enterococci and streptococci \cite{2}. A small percentage of \textit{S. aureus} can be differentiated from most other staphylococci by the coagulase test, \textit{S. aureus} is primarily coagulase-positive because it can produce the enzyme coagulase that causes clot formation. \textit{S. aureus} causes a variety of superlative (pus-forming) infections and toxinoses in humans. It causes superficial skin lesions
such as boils and furuncles: more serious infections such as pneumonia, mastitis, meningitis, and urinary tract infections; and deep-seated infections such as osteomyelitis and endocarditis [3]. *S. aureus* is a major cause of hospital acquired (nosocomial) infection of surgical wounds and infections associated with indwelling medical devices. *S. aureus* causes food poisoning by releasing enterotoxins into food and toxic shock syndrome by release of super-antigens into the blood stream [4]. *S. aureus* became a major public health problem worldwide [5]. *S. aureus* infections may spread through contact with pus from an infected wound, skin-to-skin contact with an infected person by producing hyaluronidase that destroys tissues, and contact with objects such as towels, sheets, clothing or athletic equipment used by an infected person. Deeply penetrating *S. aureus* infections can be severe. Prosthetic joints put a person at particular risk for septic arthritis, *Staphylococcal* endocarditis (infection of the heart valves) and pneumonia [6]. Risk factors for MRSA colonization or infection in the hospital include prior antibiotic exposure, admission to an intensive care unit, surgery, and exposure to an MRSA colonized patient [7]. In theyear 1942, two years after the introduction of penicillin for medical use, the first penicillin-resistant *S. aureus* isolates were observed. Since 1960, approximately 80% of all *S. aureus* isolates have been penicillin-resistant. In addition, *S. aureus* developed methicillin resistance in 1961, only two years after its introduction. The resistance to methicillin and all other β-lactam antibiotics developed due to the acquisition of the *mecA* gene. Pencillin binding protein (PBPs) are transeptidases that catalases the formation of cross-bridges in bacterial cell wall peptidoglycan. In methicillin-sensitive *S. aureus* (MSSA), β-lactam antibiotics bind to the native PBPs of the cell wall, disrupting the synthesis of the peptidoglycan layer and resulting in the death of the bacterium. Since PBP2a has a low affinity for all β-lactam antibiotics, synthesis of the peptidoglycan layer is not disrupted and MRSA can continue to grow normally [8].

### 2. Materials and Methods

#### 2.1. Sample Collection Zones:
Isolation and study of epidemiology of *Staphylococcus aureus* from the infected patients were chosen from five places of Bagalkot district, Karnataka. The Bagalkot district temperature varies between 25 to 43°C. The places were classified in to five Zones as A (District Govt. Hospital, Bagalkot), B (Sri Hangal Kumareshwara Hospital and Research Centre, Bagalkot), C (Miskin Diagnostic Laboratory) D (Govt. Hospital Hunugunda) and E (Govt. Hospital Jamakhandi). Clinical samples were collected regularly from above mentioned places during January 2007 to November 2010.

#### 2.2. Isolation of *Staphylococcus aureus* from different clinical samples:

##### 2.2.1. Collection of Samples:
The clinical samples were collected form the patients who were infected with *Staphylococcus aureus* in and around Bagalkot region of Karnataka state, India. The clinical specimens like pus, cerebrospinal fluid (CSF), blood, urine, biomedical waste were selected as the sources of organisms and carried in 18.2% peptone water to the laboratory. Mannitol salt agar was used as a selective media for primary isolation of the *Staphylococci*.

##### 2.2.2. Media used for isolation and Characterization of *Staphylococcus aureus*:
For the present study different culture media like, Nutrient agar, Mannitol salt agar (MSA), Brain heart infusion agar (BHI) and Blood agar were used. Nutrient agar/broth was prepared for the appropriate
growth of *Staphylococcus aureus* culture, which could be the source of the strains for inoculation in subsequent steps. MSA plates were prepared and streaked; the inoculations could be done either from the peptone water containing organisms or from the nutrient broth culture. The blood agar ingredients were dissolved and autoclave at 121°C for 15 min. Cool to 45-50 °C and 50 ml of sterile defibrinated sheep blood was added aseptically. Mixed thoroughly, avoiding accumulation of air bubbles and immediately poured into sterile petri plates.

2.3. Colony Characterization and Microscopic Observation:
The isolated colony from mannitol salt agar (MSA) was picked up and prepared the smear on grease free glass slides, which was dried and heat fixed. Gram-staining was performed according Christiana Gram which allows better differentiation of organisms. The microscopic observation showing spherical cocci, 1-1.5 μm in diameter occurring singly, in pairs, short chains >7 cocci, or in small groups resembling a cluster of grapes were considered as *Staphylococci*.

2.4. Biochemical Characterizations of *Staphylococcus aureus*:
For the characterization of isolates, HiStaph Identification kit (Kb-004) from Hi-Media Mumbai, was used. For biochemical tests, control organisms *S. aureus* MTTC Nos. 3160 and 9542 respectively were procured from IMTECH, Chandigarh, India. A set of biochemical tests were performed that would confirm *Staphylococcus aureus* strains among the isolates. Tests were performed in order to characterize the *Staphylococcus aureus* in respect to various biochemical properties.

3. Results

3.1. Isolation and Characterization of *S.aureus*
The present investigation was carried out during the period from January 2007 to November 2010. During this period of time epidemiologically important *Staphylococcus aureus* was isolated form different clinical samples. An effort was made to collect the sample from five different places of Bagalkot region. For convenience, places were classified into five Zones. A total of 310 clinical samples, from 223 males and 87 females were collected. For isolation and identification of *Staphylococcus aureus*, samples were inoculated on to the selective medium viz: Manitol Salt Agar (MSA), Brain Heart Infusion Agar (BHI) and Blood Agar. Distinguishing colonies grown on both media at 37°C were picked and confirmed by microscopic and biochemical characterization. Out of 310 clinical samples inoculated, 180 (58.06%) strains were confirmed as *Staphylococcus aureus* and were further characterized. The microscopic studies for all strains displayed gram positive cocci, arranged in clusters, non motile and non spore forming. The results of biochemical reactions of the isolates were positive for Voges Proskauer’s, alkaline phosphate, O-nitrophenyl-β-D-galactopyranoside (ONPG), and Catalase production. The isolates were also found positive for all the carbohydrates used in the HiStaph Identification kit (Kb-004). The growth on selective media indicated the fermentation of mannitol salt agar (β-haemolysis of sheep blood cells on blood agar and production of DNase and coagulase confirms it as coagulase positive. The Biochemical reaction by Histaph identification kit (Kb-004).

3.2. Epidemiology of *Staphylococcus aureus*:
In the present study, the incidence of *Staphylococcus aureus* were distributed Zone wise, gender wise, economic group wise and age group wise, are presented in the following sections.

### 3.2.1. Zone wise incidence of *Staphylococcus aureus*:

The incidence of *Staphylococcus aureus* of different Zones is shown in. It is evident that the incidence of *Staphylococcus aureus* colonization is very high in Zone-D 72.00% followed by Zone-E 66.66%. In Zone-A 62.5% isolation rates were observed. The lowest rate of incidence was observed in Zone-C and Zone-B with 53.76% and 52.85% respectively. In all these Zones *Staphylococcus aureus* colonization in females is lesser than the males. Out of 310 samples, the overall rate of incidence observed in both male and female is 41.93% and 16.12%, respectively. Out of 180 positive isolates, the incidence rate was more in pus sample (76.00%), followed by 57.50%, in blood, 50.00% in soft tissue abscess, 42.30% in urine, 40% in cerebrospinal fluid (CSF) and 33.33% in biomedical waste.

### 3.2.2. Economic group-wise incidence of *Staphylococcus aureus*:

The incidence of *Staphylococcus aureus* distribution among the various economic groups (LIG below 50, 000 MIG 50,000 to 2, 00, 000 and UIG More than 2, 00, 000). The overall recovery from the total samples collected, indicated that the *Staphylococcus aureus* colonization was higher, among LIG (66.66%), MIG 63.63% and UIG 50.0% from Zone-A. In Zone-B colonization was high in LIG (54.83%), 53.84% and 50.0% in UIG and MIG respectively. In Zone-C colonization was high in LIG (56.33%), 47.61% and 52.63% in UIG and MIG respectively. In Zone-D colonization was high in population group LIG (78.57%), 33.33% and 66.66% in UIG and MIG, respectively. In Zone-E colonization was high in MIG (73.33%), followed by UIG (72.0%) and lower colonization was observed in UIG (60.0%). The average incidence of *Staphylococcus aureus* among three economic groups was, LIG (61.11%), 57.57% and 50.00% in MIG and UIG, respectively.

### 3.2.3. Age wise incidence of *Staphylococcus aureus*:

There was a clear indication of the increase in *Staphylococcus aureus* incidence with age more than 40 years and between 6-20 years when compared to that age group 21-40 years in both male and female individuals. In Zone-A, maximum rate of *Staphylococcus aureus* incidence was recorded in > 40 years (72.22%) age group followed by 6-20 years (60.00%) and minimum incidence was recorded in adults between 21-40 years (57.14%). In Zone-B maximum rate of *Staphylococcus aureus* incidence was recorded in > 40 years (64.51%) age group followed by 6-20 years (44.44%) and minimum incidence was observed in 21-40 (41.66%) years of age. In Zone-C, maximum rate of *Staphylococcus aureus* incidence was recorded in > 40 years (61.29%) age group followed by 59.37 in age group 6-20 years and minimum incidence was recorded in adults between 21-40 years (34.28%). In Zone-D maximum rate of *Staphylococcus aureus* incidence was recorded in > 40 years (84.61%) age group followed by 70.00% in age group 6-20 years and minimum incidence was observed between 21-40 years (50.00%). In Zone-E maximum rate of *Staphylococcus aureus* incidence was recorded in > 40 years (68.18%) age group followed by 60.00% in 21-40 years age group and minimum incidence was observed in age group between 6-20 years (57.14%). The average incidence of the *S. aureus* infections in three age groups was...
found to be highest in age group >40 years (66.43%) followed by 56.12% in 6-20 of age group and least incidence was observed in age group 21-40 42.42%.

4. Discussion

In present study, 310 clinical samples were isolated from different places of Bagalkot district, for convenience, five Zones are made; namely A, B, C, D and E. Out of 310 clinical samples, 223 from males and 87 from females were collected. After performing all biochemical tests using standard kit, and specific test like blood haemolysis, catalase, coagulase and DNase, 180 isolates were confirmed as S. aureus. The prevalence rate of S. aureus in Bagalkot District was observed around 58.06%. The prevalence of S. aureus in hospital varies considerably from one region to another. Geographic spread of S. aureus between countries and continent has been reported previously and proven by molecular evidences. The previous studies showed that prevalence of S. aureus varies widely not only in India but also in other countries. In USA the prevalence rate varies as 68%. In Saudi Arabia it was 70% as reported [9]. Similar study in Pakistan varied widely, in 2002 it was reported 45% and 40% prevalence studied by [10]. In other study, the high prevalence rate of around 64% to 65% was observed by [11]. In other study reported that total of 54 (36%) S. aureus isolates were isolated from 150 urine samples collected. The other report by of the S. aureus isolates, 37 (69%) were methicillin-resistant [12]. Traditionally, S. aureus infections have been acquired almost exclusively in hospitals, long-term care facilities, or similar institutional settings. The highest colonization of S. aureus was observed in Zone-D with 72% followed by Zone-E with 66.66% and the lowest incidence was observed in Zone-B with 52.85%. Out of 180 positive isolates, the incidence rate was more in pus samples (76.00%), out of which, 50% of the patients suffering from the type-II diabetes and are suffering from wound, foot ulcers and pus forming pimples.

The incidence of S. aureus isolates were categorized based on economic groups. The economic groups were divided into LIG (Lower Income Group) MIG (Middle Income Group) and UIG (Upper Income Group). The incidence of S. aureus were observed in Zone-A with LIG (66.66%) and MIG (63.63%) compared to LIG and MIG, UIG were little less incidence (50%) in Zone-A. In Zone-B the colonization was high in LIG (54.83%), 53.84% and 50% in UIG and MIG respectively. In Zone-C colonization was high in LIG (56.33%), 47.61% and 52.63% in UIG and MIG respectively. In Zone-D colonization was high in population group LIG (78.57%), 33.33% and 66.66% in UIG and MIG respectively. In Zone E colonization was high in MIG (73.33%) followed by LIG (72%) and lower colonization was observed in UIG (60%) (Table-10 and Fig: 7), the average incidence of S. aureus among three economic groups was highest in LIG (62.11%), 57.57% and 50.00% in MIG and UIG respectively. Our result revealed that the rate of incidence was more in LIG compared to MIG and UIG 57.57% and 50.00% respectively.

Age group wise incidence of S. aureus colonization studies, says that age group above 40 and between 6 to 20 years, the individuals are more prone for S. aureus infections. The highest rate of incidence was recorded in above 40 years, in Zone-D with (84.61%), followed by Zone-A with 72.00%, in Zone-E with 68.18%, in Zone-B 64.51% and in Zone-A 50.0% and in Zone-C 61.29%. The age between 6 to 20 years, the highest incidence was recorded in Zone-D (70.00%) followed by in Zone-A 60.00%, Zone-C 59.37%, Zone-E 57.14% and in Zone-B 44.44%. The age between 21-40 years in all five Zones least incidence was observed. In our investigation the highest incidence was observed in the age
group of above 40 years (66.43%) and age group between 6-20 years, where as least incidence was observed in the middle age group.

5 Conclusion

310 samples were collected from 5 different Zones of Bagalkot district, and named as Zone-A (40 Samples), Zone-B (70 Samples), Zone-C (130 Samples), Zone-D (25 Samples) and Zone-E (45 Samples). In the present study a total of 180 S. aureus isolates isolated from 310 clinical samples, out of which 223 from males and 87 from females. The 56.06% of S. aureus isolated from total 310 clinical samples. The isolates are confirmed by all biochemical tests. The incidence of S. aureus in 5 Zones and the highest percentage of incidence were observed in Zone-D with 72% and lowest incidence was observed in Zone-B with 52.85%. Out of 180 positive clinical isolates, maximum isolates from pus samples (76.00%) and minimum from in biomedical waste (33.33%). The incidence of S. aureus based on economic group was observed, and the highest percentage incidence was observed in LIG (Lower Income Group) with 62.11% and lowest with UIG (Upper Income Group) with 50.00%. The results of age wise distribution of S. aureus in all five Zones were studied and the percentage of incidence was more in the age group of above 40 (66.43%) years and age group between 6 to 20 (56.12%) years.

6 References


