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Identification of *Staphylococcus aureus* in Pus samples and its Anti-microbial Susceptibility against Imipenem, Tobramycin and Linezolid

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Abstract – *Staphyloccocus. aureus is* a significant human pathogen and causes wound infection and also infection of soft tissue. *Staph.aureus* is leading causing hospital acquired infection. The study was to determine the frequency of *Staph.aureus* in different ward of Sheikh Zayed Hospital Rahim Yar khan. To find the prevalence of *Staph.aureus* in operated and non-operated patients. Also the determination of *Staph.aureus* resistance against Imipenem,Tobramycin and Linezolid. This study was conducted in Microbiology Department Sheikh Zayed Hospital Rahim Yar Khan from Janurary to March. 50 different samples of patients are included in this study. Staph.aureus was isolated by culture, Gram staining and biochemical test catalase and coagulase. 50 samples were collected in which 64% is operated and36% are non-operated. In this study *Staph aureus* resistance against Imipenem,Tobramycin and Linezolid is 14%,42% and 10% respectively**.** *Staph.aureus* infections are more common in surgically operated patients. This is due to lack of proper sterilization and poor hygiene problem. *Staph.aureus* resistance against against Imipenem and Tobramycin is high due to poor intake of antibiotics.

Keywords – *Staph aureus,* Imipenem,Tobramycin , Linezolid ,Gram staining

1. Introduction

Staphylococcus is gram-positive cocci that are microscopically observed as individual organisms, in pairs, and in irregular, grapelike clusters. The term *Staphylococcus* is derived from the Greek term *staphyle*, meaning "a bunch of grapes." Staphylococci are nonmotile, non–spore-forming, and catalase-positive bacteriais as a part of human flora found in the axillae, the inguinal and perineal areas, and the anterior nares [1].

Though approximately a third of the population is colonized with *S. aureus*, colonization by strains of *S. aureus* that are resistant to methicillin (methicillin-resistant *S. aureus*, MRSA) is less common [2, 3]. Persistent carriage is more common in children than in adults. Nasal carriers may be divided into persistent carriers with high risk of infection and intermittent or noncarriers with low risk of infection [4].

Wenzel et al (1995) found that, among healthy adults, carrier rates of 11-32% were detected in the general population, and a prevalence of 25% was detected in hospital personnel [5]. Persistent nasal carriage depends on host genetic determinants [6]. Wertheim HF et al (2005) stated that between 25% and 35% of healthy human individuals carry S. aureus on the skin or mucous membranes [7], Community-associated methicillin-resistant *S aureus* (CA-MRSA) is less often found in the anterior nares than are methicillin-S*S aureus* (MSSA) and hospital-acquired methicillin-resistant *S aureus* (HA-MRSA) [8,9,10]. A 2006 study found the overall U.S. healthcare-associated MRSA prevalence to be 46.3 per 1000 hospital inpatients (34 infections and 12 colonizations per 1000 inpatients [11].

In March 2005 data from the Surveillance Network-USA, MRSA accounted for 59.2%, 55%, and 47.9% of all non-ICU inpatient, ICU inpatient, and outpatient S. aureus specimens, respectively. Data from the same network showed that by 2005 inpatient MRSA rates had surpassed 50% in all regions of the country except for New England (49.9%). Outpatient MRSA rates ranged from lows of 36.3% in the Mid-Atlantic

region and 37.6% in New England to a high of 63% in the East South Central region. Rates were highest (55.9%) in inpatient lower respiratory specimens and lowest (37.6%) in outpatient skin and soft tissue specimens [12]. Rates in Japan, Israel, the United Kingdom, and the rest of Europe are more comparable to that of the United States [13, 14] *S. aureus* colonizes the skin, particularly in the perineal area and the rectum. It also colonizes the pharynx, [15] gut, [16] and vagina [17, 18]. The organism may cause disease through tissue invasion and toxin production [19].

The organism also elaborates toxins that can cause specific diseases or syndromes and likely participate in the pathogenesis of staphylococcal infection [15].

2. Materials and Method

For this research work, 6 month cross sectional study was conducted in the Department of Microbiology Sheikh Zayed Hospital Rahim Yar Khan. 50 samples of pus were taken from different ward in Sheikh Zayed hospital Rahim Yar Khan. The sample were taken from Male surgicleward1and 2, labour room, gyne1and 2, female surgical ward1 and 2 and All the patients which were suspected to had Staphylococcal infections, on antibiotics treatment more than seven days and those who had age below 5 years and above 60 years were included

2.1. Isolation of Staph.aureus

Growth of *Staph.aureus* is obtained by inoculation of sample on Blood agar media and it is confirmed by Gram staining and different biochemical tests catalase and coagulase[12].

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2.2. Blood Agar Media

Blood Agar media was prepared and sterilized by autoclave. Samples were inoculate on this media and were overnight incubated at 37 ^{0}C. *Staph.aureus* produces golden yellow colonies which are further identify by biochemical tests and Gram staining.

2.3. Biochemical Tests

The *Staph.aureus* isolates were identified by the standard Morphological and culture characteristics by performing Gram stain, Catalase and Coagulase test.

2.4. Gram stain

Thin smear of the isolates was prepared on clean glass slides using a sterile loop. The slides were air dried and then heat fixed by passing it through a flame and performed Gram staining, smear was covered with crystal violet stain for 60 seconds then poured off and covered the smear with Lugol's iodine solution for 30 seconds. The iodine solution was poured off and the smear was decolorized with acetone iodine decolorizer until the ceased to come out of the smear. The slide was thoroughly washed with water. The slide was counter stained with diluted carbol fuchsin for 30 Seconds. Washed with water, blotted with absorbent paper and air dried. Organisms that retained the crystal violet-iodine dye complexes, after Decolorizing with acetone-iodine, stain purple and were termed as Gram positive and those that lost that complex and become red due to counter stain (carbolfuchsin) were termed Gram negative [13].

2.5. Catalase Test

This test is useful in distinguishing organisms that have the ability to produce catalase from those that lack this enzyme.This test was performed from a blood free non inhibitory medium for example Nutrient agar. Catalase is an enzyme that decomposes hydrogen peroxide into water and oxygen.Hydrogen peroxide forms as one of the oxidative end products of aerobic carbohydrate metabolism.

$$2H_20_2 \text{ ----------} \rightarrow 2H_20 + 0_2$$

A small amount of culture to be tested was picked with glass rod and inserted into the 3% hydrogen peroxide solution in a clean tube.The production of gas bubbles indicated a positive test.

2.6. Coagulase test

This test was used to identify *Staph.aureus* which produces the enzyme coagulase. Coagulase causes plasma to clot by converting fibrinogen to fibrin. Place a drop of distilled water on each end of slide. Emulsify the colony of the organism in each of the drops to make two thick suspensions. Loopful plasma is added to one of the suspension and mix gently. Clumping is produced within 10 seconds due to the presence of organism. No plasma added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping [14].

Clumping within 10 seconds……..*Staph.aureus*
No clumping…….no bound coagulase.

2.7. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility test was used for infectious organisms due To their resistance against many Antibiotic.Staph.aureus also produces resistance against Methicillin Vancomycin and Tobramycine.The resistance against Imipenum,Tobramycin and Linezolid was observed by disk diffusion technique in this Study.

2.8. Media Preparation

Muellar-Hinton agar was prepared by dissolving 19g of agar in 500ml of distilled water and microwaved for 2 minutes for complete Solubilization. Agar was autoclaved at 121c for 15 minutes.Twenty Mililiter of MHA was poured in each Petri dish of 90mm diameter. One plate was incubated overnight at 35-37 ^{0}C as sterility control. Rests of plates were stored at 4 ^{0}C [15].

2.9. Inoculums preparation and procedure

3-4 morphological identical colonies of *Staph.aureus* were Picked Up and mixed in saline.Then the bacterial suspension was adusjusted to0.5 McFarland standard**.** Mueller-Hinton Agar plate was dry before inoculation of bacterialColonies. Staph.aureus colonies were inoculated on the dry Mueller-Hinton Plate. Imipenum,Tobramycin disks of 10ug and Linezolid disks of 30ug were applied within 15 minutes of inoculation. Disks were firmly apply on the agar plates. The

plate were inverted and incubated for 16-20h. Zone of inhibition was measured by a ruler in milimeter. And results were interpreted according to CLSI_2007. The zone size for Imipenum and Tobramycin which was less than 15mm was called as "Resistant" and which was greater than 15mm was called as "Sensitive". While for Linezolid zone size which was less than 21mm was called as "Resistant" and which was greater than 21mm was called as "Sensitive" [16].

3. Results and Discussion

In this study the patient at the age of 30 are most infected with *Staph.aureus* infection. The percentage of the age of 30 is 18%.Females are more infected with *Staph.aureus* Infection as compared to male.The ratio is 62% females and 38% males of *Staph.aureus* Infection. This study shows that surgically operated patient were more prone to *Staph.aureus* Infection as compared to non operatedpatient.The ratio of *Staph.auerus* infection of Surgically operated patient in Sheikh Zayed Hospital Rahim Yar khan is 64% and non-operated are 36%. *Staph.aureus* is mostly present in pus sample.50 sample were collected and the ratio of presence of *Staph.aureus* in pus sample in this study is 64%.It shows That *Staph.aureus* mainly produces the pus producing infection. The resistance of *Staph.aureus* against Imipenem is 14% in this study.Out Of 50 sample 7 are resistant against Imipenem and 43 are sensitive.The maximum Zone of inhibition of Imipenem against *S.aureus* in this study is 38mm and most frequent zone of inhibition is 16mm.Tobramycin has resistance against Imipenem 42%. Out of 50 samples 21 were resistant and 29 were sensitive. Zone of inhibition was 15mm and maximum zone size was 26mm. The resistance of *Staph aureus* against Linezolid was 10% and 90% samples were sensitive.ximum zone size was 36mm and minimum zone size was 15mm [17]

Table1. Frequency of Gender Distributions

Gender	Frequency	Percent
Female	31	62%
Male	19	38%

Table 2. Sensitivity and Resistance of Antimicrobial drugs

Antimicrobial Drugs	Frequency		Susceptibility	
	Sensitive	**Resistance**	**Sensitive %age**	**Resistance %age**
Imipenem	43	7	86%	14%
Tobramycin	29	21	58%	42%
linezolid	45	5	90%	10%

Stap. aureus in different wards of Sheikh Zayed Hospital Rahim Yar khan. Fifty isolates of *Staph. aureus* were collected from the Sheikh Zayed Hosopital. The clinical samples were collected from both genders male and female. This study shows that females are more infected with *staph.aureus* infection. In fifty isolate of *staph.aureus* 62% isolated from female patient and 38% from male. This study reveals that operated Patient are more infected with Staph.aureus as compared to non-operated. The main goal of study was to find out the *Staph.aureus* rsistance against Imipenum, Tobramycin and Linezolid Tobramycin is an aminoglycoside antibiotic used to treat various types of bacterial infections .tobramycin provides action against susceptible bacteria. *In vitro* studies have demonstrated that tobramycin is active against susceptible isolates of the following bacteria: *Staphylococcus aureus* (includes penicillin-resistant isolates [18], SMITH SHADOMY ANDCAROL KIRCHOFF et al described in his study that isolates of 50 *Staph. aureus* gave zone size of inhibition with diameters of 21 to 23.5 mm with the remaining 10 isolates given zones of 24 mm or larger [19]. While in our study among 50 isolates of *Staph Aureus* 5 isolates showed zone size 24mm or more than 24mm while remaining had zone size less than 15 mm .This may be because of this fact that microorganisms develop resistance with passage of time .Imipenum is an intravenousβ-lactamantibiotic It has an extremely broad spectrum of activity against many gram negative and gram positive organisms including staph aureus[20],W. Michael Scheld*†John M. Keeley et al reported in their studies thaImipenem was very active in vitro against 36 Staphylococcus aureus isolates from cases of infective endocarditis among them 22 isolates were senstive and showed zone sizes more than 15mm [21,22]. While in our study out of 50 isolates 35 isolates showed more than 15mm zone size [44]. A study conducted in the department of onpathology and medicine general hospital Boston, USA Linezolid was much effective againt many Grgam positive organisms including MRSA; In this study 90% *Staph Aureus* were sensitive to Linezolid [23].

4. Conclusion

The ratio of *Staph aureus* infection in Surgical operated patient is greater as compared to non-operated due to lack of proper sterilization. The resistance of *Staph.aureus* against Imepenem, tobramycine and linezolid is 14%, 42% and 10% respectively.So linezolid is best choice of Antibiotic against *Staph.aureus*.

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