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# ISOLATION, IDENTIFICATION AND SCREENING OF VANCOMYCIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN CLINICAL SPECIMEN ISOLATES FROM BALLARPUR AREA HOSPITALS

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**ABSTRACT:**
The virulent strains of *Staphylococci* and *Streptococci* commonly cause skin infections in human. Virulent *Staphylococcus aureus* usually cause nosocomial infections such as Boils, Post-operative sepsis, Septicemia, Abscess etc. Previously most of the virulent strains of S. aureus were found be susceptible to Penicillin antibiotic. However, in late 1980s, about 50% strains of S. aureus were emerged as Penicillin resistant. In late 1980s Methicillin was applied to treat Staphylococcal infections. However, within 10-15 years, 50 % isolates became resistant to Methicillin and resulted endemics in hospitals worldwide. In late 1980s, about 50% strains of S. aureus were emerged as Methicillin resistant. In early 1990s the use of a Glycopeptide antibiotic, 'Vancomycin' started against Penicillin resistant strain of S. aureus. Increase in the use of Vancomycin again resulted in to the emergence of Vancomycin resistant S. aureus in late 1990s. With reference to the above facts, the present review study was carried out to isolate, identify and screen out the Vancomycin antibiotic resistant S. aureus isolates from clinical specimens such as blood, pus, abscess, wound and carbuncle etc., collected from the patients in hospitals in Ballarpur City area. The screening of Vancomycin resistant S. aureus was made by a standard Baur-Kirby method. Out of 50 clinical specimen isolates analyzed about 40 % pathogenic S. aureus isolates were found to be resistant to Vancomycin antibiotic.

**Keywords:-** *Staphylococcus aureus*, Vancomycin, Antibiotic resistance, Baur-Kirby.

## INTRODUCTION :

Staphylococci are widely distributed in nature and considered as ubiquitous in occurrence. They are found to be present in water, soil, food and air. Many species of Staphylococci have been reported as commensal flora of human skin (Rayon Cruz et al., 2005). Staphylococcus aureus is a Gram-positive coccus that occur in grape like cluster and produce golden- yellow pigment when cultivated on nutrient agar medium. It is considered as an opportunistic pathogen, usually causing two types of infections in human beings;

Invasive or Suppurate ii) Toxigenic or Non-Suppurate.

Pathogenic S. aureus was first observed in human pyogenic lesions and reported as commonest cause of nosocomial localized suppurate lesions such as boils, abscess, furuncle, carbuncle, impetigo etc. It also causes Pneumonia, Osteomyelitis, Urinary tract infection, Endocarditis, Pharyngitis etc. (Fridkin, 2001). Musher, (1977) reported that, pathogenic S. aureus causes secondary infections such as Ulcer, Wound and Burn sepsis also associated with food poisoning due to the production of enterotoxin. Scalded skin syndrome is also caused by virulent S. aureus.

Recently, S. aureus have evolved resistance to both synthetic and traditional antibiotics, Saha B, et al., (2008). The ability of pathogenic S. aureus to survive in hospital environment (cause nosocomial infections), also the ability to develop resistance against Penicillin and other antibiotics has gain importance as a human pathogen. About 50 years ago (early 1980s),



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Penicillin antibiotic was commonly used to cure the diseases caused by S. aureus. However, within 10 years use of penicillin antibiotic, about 50% isolates turned resistant to it (Sabath, 1987). In late 1980s Methicillin was applied to treat Staphylococcal infections. However, within 10-15 years, 50 % isolates became resistant to Methicillin and resulted endemics in hospitals worldwide (Tiwari and Sen, 2006). Then the use of a Glycopeptide antibiotic i.e. 'Vancomycin' started in early 1990s against Penicillin and Methicillin resistant strains of S. aureus. But it was again observed in late 1990s that, about 34 % of S. aureus isolates became resistant to Vancomycin antibiotic. The first case of Vancomycin resistant S. aureus strain was reported in Japan in 1996 (Rayon Cruz et al., 2005).

The specific mechanism associated with the development of Penicillin, Methicillin and Vancomycin resistant strains of S. aureus is not yet clear. (Perichon B and Courvalin P., 2006). However, the emergence of Vancomycin resistance seriously threatened the treatment of Staphylococcal infections.

The present review investigation was therefore carried out for the isolation, identification and to find out recent percentage of Vancomycin resistant S. aureus strain from clinical specimen isolates in Ballarpur area hospitals.

**MATERIALS AND METHODS:**

**Collection of Clinical Specimens:**

About 100 clinical specimens such as blood, pus, wound swab, burn swab and urine were collected in sterilized glass containers from patients in different hospitals and pathological laboratories of Ballarpur city (Maharashtra). Collected specimens were separately transferred in to sterile nutrient broth medium containing 9.5 % Sodium chloride (Saline) in test tubes. The tubes were incubated at 37 O C for 24 hours to obtain the culture.

Isolation and Identification of Pathogenic S. aureus isolates:

To obtain pure culture of pathogenic S. aureus isolates, the nutrient broth culture of clinical specimens was cultivated on selective media viz. Mannitol Salt Agar (MSA, Himedia – M118), which favors the growth of only pathogenic S. aureus. After incubation the colony characteristics developed on MSA was observed and noted. The well isolated colonies on MSA were picked up and grown separately in Nutrient agar slant and Nutrient broth medium to obtain pure culture of isolates.

The Morphological identification of isolates was made by performing Gram staining using nutrient agar slant culture whereas motility was tested by Hanging drop preparation using nutrient broth culture.

The Biochemical identification was done by standard conventional procedure i.e. Glucose, Lactose, Mannitol fermentation, IMViC test, Catalase and Coagulase test (Bergey D., 1986).

Screening of Vancomycin Antibiotic Resistant S. aureus isolates:

The screening of Vancomycin resistant S. aureus isolates was performed by a standard Disc diffusion method of Baur-Kirby et al., (1996), using Muller Hinton Agar medium (MHA, Himedia-M173). The disc containing 30 mcg concentration of standard Vancomycin antibiotic was used against 0.5 ml broth culture of isolates over MHA medium to screen out resistant S. aureus isolates. After incubation at 37O C for 24 hours, the isolates showing zone of inhibition less than 11 mm were considered as Vancomycin resistant.

**RESULTS AND DISCUSSION:**

In the present study, total 100 clinical specimens, such as blood, pus, wound swab, burn swab and urine were collected from the patients in different hospitals and pathological laboratories of Ballarpur city area. Staphylococcus aureus isolates were isolated



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and identified up to species level by applying different conventional Morphological, Biochemical and Selective media cultivation studies (Bergy, 1996) as shown in the Table – 1.

The Morphological characterization and identification of pathogenic S. aureus was done by Gram's staining and Motility test by hanging drop preparation. The isolates which found to be Gram positive Cocci arranged in clusters like grapes and Non-motile were considered as pathogenic S. aureus.

The Cultural identification was made by cultivation on Selective media viz. Mannitol Salt Agar (MSA, Himedia–M118). The isolates growing on MSA medium and producing yellow zone around the colonies were considered as pathogenic S. aureus.

The Biochemical identification was made by Glucose, Lactose, Mannitol fermentation, IMViC test, Catalase and Coagulase test. The isolates fermenting Glucose, Lactose and Mannitol, producing acid only but no gas. The isolates giving MR and VP test positive but Indole and CU test negative also showing Positive Catalase and Coagulase test were considered as pathogenic S. aureus.

Out of 100 clinical specimens collected, about 50 pathogenic S. aureus isolates were obtained after standard Morphological, Biochemical and Selective media cultivation studies (Table 1).

Screening of Vancomycin Antibiotic Resistant S. aureus isolates:

Further, 50 isolates of S. aureus were screened out for Vancomycin resistance by standard disc diffusion method of Baur-Kirby (1996) using Muller-Hinton agar (Himedia–M173) and 30 mcg/ml concentration of Vancomycin. The isolates showing Zone of Inhibition below 11 mm were considered as resistant to Vancomycin. (Table 2).

The results obtained in the above Table 2 shows that, out of 50 pathogenic S. aureus isolates screened out, 20 (40 %) exhibits Vancomycin Resistance whereas 30 (60 %) were found to be Sensitive to Vancomycin antibiotic (Table 2).

Tiwari and Sen (2007) reported 30 % Vancomycin resistant S. aureus isolates. Ameen A.J. (2011), reported 32 % Vancomycin Resistant S. aureus from Post-Operative Pus Sample.

This indicate that, the epidemiology of Vancomycin resistant S. aureus in our country is also increasing over the last few decades. The incidences of infection by Vancomycin resistant S. aureus isolates keep changing every year and is on rise as compared to last few years, (Vidhani et al., 2001).

The specific mechanism associated with the development of Penicillin, Methicillin and Vancomycin resistant strain of S. aureus is not clearly understood. Previously Van-A gene that encodes Vancomycin resistance in Enterococci was considered to be responsible for it. Another hypothesis says that the cell wall composition of S. aureus may be responsible (Hamamatsu 1998). Sabath, (1987), reported that, plasmid mediated drug resistance has acquired special significance. S. aureus became resistant to Penicillin and Methicillin (β-Lactam antibiotics) due to the production of plasmid coded penicillinase (β-Lactamase) enzyme, which rapidly inactivate β-lactam ring responsible to kill S. aureus. Perichon B. et al., (2006) also reported similar mechanism.

A Glycopeptide antibiotic Vancomycin treatment was considered to be the best therapeutic drug of choice for Penicillin and Methicillin resistant S. aureus strains. But the emergence of Vancomycin resistance in S. aureus isolates seriously threatens the treatment of Staphylococcal infections.

**CONCLUSION:**

This study concludes, the emergence of 40% Vancomycin antibiotic resistant S. aureus



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strains in Ballarpur region and suggest to provide another more effective therapy against Staphylococcal infections.

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**Table 1. Isolation, Characterization and Identification of Pathogenic *S. aureus* isolates:**

| No. of Specimens Collected | Gram's staining & Motility Study | Growth on Selective media (MSA-M118) | Sugar Fermentation Tests | IMViC Test | Catalase & Coagulase Test | No. of pathogenic *S. aureus* isolates obtained |
|---|---|---|---|---|---|---|
| 100 | Gram positive cocci, arranged in bunches like grapes. Non-motile | Yellow zone around colonies | Glucose - A<br>Lactose - A<br>Mannitol - A | Indole - ve<br>MR + ve<br>VP + ve<br>CU - ve | + ve<br>+ ve<br>+ ve<br>+ ve | 50 |

**[A=Acid production, MR=Methyl Red test, VP=Voges-Proskauer test, CU= Citrate Utilization test]**

**Table 2. Screening of Vancomycin Antibiotic Resistant *S. aureus* isolates by Baur-Kirby Disc Diffusion Method.**

| No. of Specimens Collected | No. of pathogenic *S. aureus* isolates obtained | Antibiotic used for screening | No. of Sensitive Isolates | Percentage of Sensitive Isolates. | No. of Resistant Isolates | Percentage of Resistant Isolates |
|---|---|---|---|---|---|---|
| 100 | 50 | Vancomycin | 30 | 60 % | 20 | 40 % |



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