



Identification of Vancomycin Antibiotic Resistant *Staphylococcus Aureus* from Clinical Specimen Isolates, from Ballarpur Area.

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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 susceptible to Penicillin antibiotic. 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 glycopeptide antibiotic, 'Vancomycin' started against 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 this view, the present study was carried out to identify antibiotic Vancomycin resistant *S.aureus* isolates from clinical specimens such as blood, pus, abscess, wound, burn etc. from hospitals in Ballarpur area.

Isolation and Identification of Vancomycin antibiotic resistant *S.aureus* was made by a standard Bauer-Kirby method. Out of 50 clinical specimen isolates analyzed about 60% were found to be Sensitive whereas 40 % were Resistant to antibiotic Vancomycin.

Key Words: *Staphylococcus aureus*, Antibiotic resistance, Vancomycin, Bauer-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, (Bauer, Kirby and Sherris, 1996).

Staphylococcus aureus is a Gram positive spherical bacterium (Cocci) that occur in grape like cluster and produce golden-yellow pigment when cultivated on nutrient agar medium. It is considered as an opportunistic pathogen causing two types of diseases in human beings; i) Invasive or Suppurative ii) Toxicogenic or Non-suppurative.

It was first observed in human pyogenic lesions and reported as commonest cause of nosocomial localized suppurative lesions such as boils, abscess, furuncle, carbuncle, impetigo etc. It also causes Pneumonia, Osteomyelitis, Urinary tract infection, Endocarditis and Pharyngitis (Fridkin, 2001).

The ability of *S. aureus* to survive in hospital environment (nosocomial infections), also the ability to develop resistance against Penicillin and other antibiotics has gained importance as a human pathogen. It may result secondary infections of 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* (Musher, 1977).

About 50 years ago (early 1980s), Penicillin antibiotic was used to cure the diseases caused by *S.aureus*. However within 10 years about 50%

isolates turned resistant to it, (Sabat, 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, 'Vancomycin' started in early 1990s against Penicillin and Methicillin resistant strains of *S. aureus*. But it was observed in late 1990s that, about 34 % isolates became resistant to Vancomycin. The first case of Vancomycin resistant *S.aureus* was reported in Japan by Bauer, Kirby and Sherris, (1996).

The specific mechanism associated with the development of antibiotic Penicillin, Methicillin, and Vancomycin resistant strains of *S.aureus* is not yet clear. However the emergence of antibiotic Vancomycin resistance seriously threatened the treatment of Staphylococcal infections.

The present investigation was therefore carried out to find out recent percentage of antibiotic Vancomycin resistant *S.aureus* from clinical specimen isolates from hospitals in Ballarpur area.

MATERIALS AND METHODS:

i) 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 to sterile nutrient broth medium containing 9.5 % Sodium chloride in test tubes. The tubes were incubated at 37 ° C for 24 hours to obtain the culture.

ii) Isolation and Identification of *S. aureus* Isolates:

To obtain pure culture of *S.aureus* isolates, the nutrient broth culture of clinical specimens was cultivated on selective media viz. Baird-Parker Agar (BPA, Himedia-M043S). After incubation the colony characteristics developed on BPA was observed and noted. The well isolated black colonies on BPA were picked up and grown separately in nutrient agar slant and nutrient broth medium to obtain pure culture of isolates.

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. Further biochemical identification was done by standard conventional procedure i.e. Glucose, Lactose and Mannitol fermentation and IMViC test, (Bergey David G, (1996).

iii) Identification of Antibiotic Vancomycin Resistant *S.aureus* Isolates:

The detection of Vancomycin resistant *S.aureus* isolates was performed by a standard Disk diffusion method of Bauer-Kirby (1996), using Muller Hinton Agar (MHA, Himedia-M173) medium. A 30 µg concentration of Vancomycin was used to screen out resistant *S.aureus* isolates. The isolates showing zone of inhibition less than 12 mm were considered as Vancomycin resistant whereas those showing zone of inhibition 12 mm and more were considered as sensitive .

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 in Ballarpur area. *Staphylococcus aureus* isolates were isolated and identified up to species level by applying different conventional morphological, biochemical and selective media cultivation studies (Bergey David, 1996) as shown in the

Table - 1.

Table 1. Isolation and Identification of *S.aureus* Isolates:

No.of specimens collected	No.of isolates obtained	Gram's staining & Motility	Growth on Selective media (BPA-M043S)	Sugar fermentation	IMViC test
100	50	Gram positive cocci, arranged in clusters like grapes and non-motile	Black colonies	Glucose... A Lactose ... A Mannitol .. A	Indole .. - ve MR ... + ve VP ... + ve CU ... - ve

Note: A= Acid production, MR= Methyl red test, VP= Voges-Proskaur test, CU= Citrate Utilization test

Morphological identification was done by Gram's staining and hanging drop preparation. All the isolates found to be Gram positive cocci, arranged in clusters like grapes and non-motile. Cultural identification was made by cultivation on Selective media viz. Baird-Parke rAgar (Himedia-M043S). All the isolates grow on BPA and produce black colonies. Biochemical identification was made by Glucose, Lactose, Mannitol fermentation and IMViC test. All isolates ferment Glucose, Lactose and Mannitol, producing acid only but no gas. They give MR and VP test positive but Indole and CU test negative.

Out of 100 clinical specimens collected, 50 *S.aureus* isolates were obtained after standard

morphological, biochemical and selective media cultivation studies (**Table-1**). Further, 50 isolates of *S.aureus* were screened out to detect antibiotic Vancomycin resistance by standard disc diffusion method of Bauer-Kirby (1996) using Muller-Hinton agar (Himedia-MM0443) and standard 30 mcg concentration of Vancomycin. The isolates showing zone of inhibition below 12 mm were considered as resistant to Vancomycin whereas those isolates showing zone of inhibition, 12 mm and more were considered as sensitive. Out of 50 isolates of *S.aureus* screened out, 20 (i.e. 40 %) exhibits Vancomycin resistance whereas 30 (i.e. 60 %) were sensitive to Vancomycin (**Table-2**).

Table 2: Identification of Vancomycin Antibiotic Sensitivity/ Resistance against *S.aureus* Isolates.

No.of <i>S.aureus</i> Isolates	No. of Vancomycin Sensitive Isolates	Percentage (%)	No.of Vancomycin Resistant Isolates	Percentage (%)
50	30	60 %	20	40 %

Tiwari and Sen, (2006) in his studies reported 30 % Vancomycin resistant *S.aureus* isolates. This result indicates that, the epidemiology of antibiotic 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 are on increasing as compared to last few years (Vidhani and Mathur, 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 (Hiramatsu, 1998). However studies made by Tiwari and Sen, (2006) ruled out this assumption. Sabath, (1987) has 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) which rapidly inactivate β -lactam ring responsible to kill *S.aureus*.

A glycopeptide antibiotic Vancomycin treatment was considered to be the best therapeutic drug of choice in place of penicillin and methicillin resistant *S.aureus* strains. But the emergence of antibiotic Vancomycin resistance in *S.aureus* isolates seriously threatens the treatment of staphylococcal infections.

CONCLUSION

The emergence of antibiotic Vancomycin resistance seriously threatened the treatment of

Staphylococcal infections. This study concludes that identification of antibiotic Vancomycin resistant *S.aureus* in Ballarpur region is a serious issue as far as public health point of view. This study also suggests to provide another more effective therapy against Staphylococcal infections.

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