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ISOLATION AND IDENTIFICATION OF MULTI-DRUG RESISTANT *STAPHYLOCOCCUS AUREUS* FROM POST-OPERATIVE PUS SAMPLE

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ABSTRACT

Staphylococcus aureus can cause a wide range of infections ranging from minor skin abscesses to more serious invasive diseases. The development and spread of bacterial strains that are resistant to antibacterial drugs has emerged as a global problem. The appearance of antibiotic resistant bacteria over the past decades has been regarded as an inevitable genetic response to the strong selective pressure imposed by antimicrobial chemotherapy, which plays a crucial role in the evolution of antibiotic resistant bacteria. The present study was aimed to isolate and determine the antibiotic susceptibility pattern of *Staphylococcus aureus* strain against some conventional and traditional antibiotics. Post surgical pus samples were collected from nearby Hospital and species identification was confirmed by Gram staining and standard biochemical tests. Antibiotic susceptibility tests were carried out by determination of Minimum inhibitory concentration, minimum bactericidal concentration, DAD test and BHI vancomycin screening agar test. From this study, it was observed that isolated *S. aureus* strains are pathogenic; 50% of strains were resistant to penicillin G, ampicillin and erythromycin; 40% strains were resistant to cephotaxime, gentamycin, streptomycin, tetracycline, chloramphenicol, norfloxacin, methicillin and vancomycin. Our study exposed the appearance of VRSA in Midnapore surroundings which is very alarming and may soon become a comprehensive problem, unless antimicrobial agents are used more wisely.

Keywords: *Staphylococcus aureus*, vancomycin, antibiotic emergence, MDR, VRSA

1. INTRODUCTION

Staphylococcus aureus is an important mammalian pathogen that has long been recognized for its propensity to cause serious and invasive diseases. The disease-causing *Staphylococcus aureus* produced a golden yellow pigment, whereas the non-disease causing strain was generally white **[1]**. *S. aureus* commonly causes boils, carbuncles, furuncles and impetigo, but after gaining access to the blood, may also be a major cause of endocarditis, osteomyelitis, pneumonia, toxic shock syndrome and septicemia **[2]**. In healthy individuals, the carrier rate of *S. aureus* range between 15% to 35% with a risk of 38% of individuals developing infection followed by a further 3% risk of infection when colonized with methicillin-susceptible *Staphylococcus aureus* (MSSA) **[3]**. Certain groups of individuals are more susceptible to *S. aureus* colonization than others including health-care workers, nursing home inhabitants, prison inmates, military recruits and children **[4]**. In a study, conducted in 2007 by the University of the Witwatersrand and the University Hospital of Geneva, health-care workers accounted for 93% of personnel to patient transmission of methicillin-resistant *S. aureus* (MRSA) **[5]**. Previously several outbreaks have been reported in Northern-Taiwan in 1997 that suggested MRSA transmission associated with health-care workers, including surgeons **[6]**. In 2007, a prevalence of more than 50% of MRSA strains isolated from Cyprus, Egypt, Jordan and Malta was reported that was attributed due to overcrowding and poor hand-hygiene facilities in the hospitals **[7]**.

Vancomycin has been the most reliable therapeutic agent against infections caused by meticillin-resistant *Staphylococcus aureus*. However, in 1996 the first MRSA to acquire resistance to vancomycin was isolated from a Japanese patient. The patient had contracted a post-operative wound infection that was refractory to long-term vancomycin therapy. Subsequent isolation of several vancomycin resistant *S aureus* (VRSA) strains from USA, France, Korea, South Africa, and Brazil has confirmed that emergence of vancomycin resistance in *S aureus* is a global issue. Vancomycin resistance is acquired by mutation and thickening of cell wall due to accumulation of excess amounts of peptidoglycan. This seems to be a common resistance mechanism for all VRSA strains isolated in the world so far **[8]**. In 2002, a newly reported VRSA was isolated from the catheter tip of a renal dialysis patient in Michigan. The isolate contained both the *mecA* gene for methicillin resistance and the *vanA* gene for vancomycin resistance **[9]**.

The possible emergence and dissemination of VRSA strains is a serious health threat and makes it absolutely necessary to optimize prevention strategies and fast detection methods. Till today only seven VRSA have been found all over the world. First in USA in 2002 **[10]**, second in Michigun in 2002 **[9]**, third in Pennsylvania in 2002 **[11]**, fourth in New York in 2004 **[12]**, fifth in New York in 2005 **[13]**, sixth in Kolkata, India in 2005 **[14]**, and seventh in Midnapore, India in 2011 **[1]**. Vancomycin resistant emergence of *Staphylococcus aureus* in developed and developing country is becoming a global problem. From the above background this study was aimed to isolate and determine the antibiotic emergence pattern of pathogenic *Staphylococcus aureus* strain against some conventional and traditional antibiotics.

2. MATERIALS AND METHODS

2.1 Culture media and Chemicals

Luria broth, nutrient agar, tryptic soy broth, Mueller-Hinton broth, brain heart infusion broth, agar powder, beef extract, DNase agar, mannitol salt agar, blood agar, crystal violet, lugol's iodine, safranine N, N, N', N'-tetramethyl-*p*-phenylenediamine dihydrochloride, rabbit plasma, lysostaphin and antibiotic discs were purchased from Himedia, India. Sodium chloride (Nacl) was procured from Merck Ltd., Mumbai, India. All other chemicals were from Merck Ltd., SRL Pvt., Ltd., Mumbai and were of the highest grade available.

2.2 Collection and transport of sample

Pus samples were collected from surgical ward of Vidyasagar Institute of Health (Paramedical College and Hospital), Midnapore, West Bengal, India during a two month period. Samples were obtained using cotton tipped swabs from the pus of deep-seated wounds of patients. Swabs were transported to the laboratory in autoclaved Luria broth (LB) within 30 minutes of collection **[15]**.

2.3 Culture of microorganisms

Pus containing swabs, kept in LB were shaken in a shaking incubator at 37°C for overnight. Bacterial cultures were grown on Nutrient agar (NA) media and purified by a single colony isolation technique on NA containing 10% sodium chloride **[14]**. Isolates were sub-cultured from primary media to nutrient agar plates and incubated at 35°C in 5 to 7% CO_2 for 16 to 24 hr. A thick suspension of each strain was then prepared by transferring three to six isolated colonies into approximately 0.5 ml of sterile 0.85% NaCl solution at room temperature. This suspension was used to perform the latex agglutination test and the slide coagulase test **[16]**. *E. coli* ATCC 23509 and *S. aureus* ATCC 25923 strains were used as negative and positive control for this study. These strains were obtained from Microbiology laboratory of Midnapore

Medical College and Hospital. These strains were stored in agar slants at 4°C for further studies as reference strain.

2.4 Species Identification

Identification of the clinical isolates was performed by traditional biochemical tests, including Gram staining; oxidase, catalase, coagulase, latex agglutination, motility, thermonuclease (DNase), mannitol fermentation tests; lysostaphin susceptibility and haemolysis activity **[17]**.

2.4.1 Gram staining

Gram staining of isolates was performed according to Duguid, 1996 **[18]**. Briefly, on a glass slide bacterial smear was prepared from broth culture and heated gently to fix. The slide was flooded with 0.5% crystal violet and left for 30 sec. The slide was tilted and poured sufficient 1% Lugol's iodine to wash away the excess stain. The slide was covered with fresh iodine and allowed to act for 30 sec. The slide was tilted and washed off the iodine with 95-100% ethanol until colour ceases to run out of the smear. The slide was rinsed with water and 0.1% safranin was poured on it and left to act for 2 min. The slide was washed with water and blotted to dry and observed under microscope.

2.4.2 Oxidase test

Oxidase test of isolates was performed by filter paper method according to Snell *et al*., 1999 **[19]**. Briefly, a piece of filter paper was soaked in 1% N, N, N', N'-tetramethyl-p-phenylenediamine dihydrochloride solution. With a disposable loop some fresh growth of isolates from NA plate was scraped and rubbed onto the filter paper and examined for the blue colour within 10 seconds.

2.4.3 Catalase test

Both tube and slide catalase test of isolates was performed according to MacFaddin, 2000 **[20]**. For tube catalase test, 200µl of 3-6% hydrogen peroxide (H_2O_2) was taken in a test tube. A colony of isolates was taken from NA plate with disposable loop and rubbed onto the inside wall of the test tube and examined the vigorous bubbling within 10 seconds. For slide catalase test, 2-3 colonies of isolates was taken from NA plate with sterile loop and spotted onto the centre of a glass slide. One drop of 3-6% H_2O_2 was added on it and observed the vigorous bubbling within 10 seconds.

2.4.4 Coagulase test

Slide coagulase test of isolates was performed according to Doern, 1982 and Baird- Parker *et al*., 1976 **[16, 21]**. Briefly, One drop of the bacterial suspension was mixed with one drop of citrated rabbit plasma (Himedia) on a cleaned glass slide. The slide was gently rocked for 5 to 10 sec and examined for the presence of clumping.

2.4.5 Motility test

Mortility test of isolates was performed according to Tittsler and Sandholzer, 1936 **[22]**. Briefly, semi solid agar tube (beef extract-0.3%, pancreatic digest of casein-

1.0%, Nacl-0.5% and agar-0.4%) was prepared. Tubes were inoculated with a pure culture of isolates by stabbing the center of the column of medium to greater than half the depth and incubated for 24-48 hr at 35±2°C in an aerobic atmosphere. The growth of organisms was observed, whether it was through the line of inoculation or spread out from the inoculation.

2.4.6 Thermonuclease test

Thermonuclease test of isolates was performed according to Lachica *et al*., 1971 **[23]**. Standard DNA hydrolysis tests were made with DNase test agar containing 0.005% methyl green indicator. Colonies of isolates were grown in tryptic soy broth (TSB). The DNase test agar plates were inoculated by making a 1.5-cm-diameter ring of each isolate on plates (100 by 15 mm) that were divided into several sections per plate. After 24 to 30 hr of incubation at 35°C, DNA hydrolysis was observed by clearing of the methyl green around the ring. Observations were best made when the plates were placed against a yellow background and illuminated with fluorescent light.

2.4.7 Mannitol fermentation test

All isolates were inoculated onto MSA and plates were incubated at 37°C for 24-48 hr. Mannitol fermentation was observed and recorded **[24]**.

2.4.8 Lysostaphin susceptibility

Lysostaphin susceptibility of isolates was performed according to Schindler and Schuhardt, 1965 **[25]** with some modification. Culture of isolates was inoculated into 200µl of phosphate saline buffer and emulsified. One half of the suspended cells was transferred to another tube and mixed with 100µl of phosphate saline buffer to serve as negative control. 100µl of lysostaphin solution was added to the original tube to give a concentration of 25 mg lysostaphin per ml of cell suspension. Both tubes were incubated at 35°C for up to 2 hr and examine the clearance of turbidity. A positive test, as indicated by clearing of turbidity in the tube containing lysostaphin, sometimes occurred after 20 min of incubation. The control remained turbid. If there was failure of clearing after 2 hr of incubation, the test was recorded as negative.

2.4.9 Pathogenicity test of clinical isolates by haemolysis test

All isolates were inoculated onto blood agar and plates were incubated at 37°C for 24 hr. A clearing zone surround the bacterial colony was observed and recorded **[17]**.

2.5 Antibiotic susceptibility testing

2.5.1 Determination of Minimum Inhibitory Concentration

The MIC values of penicillin G, ampicillin, cephotaxime, gentamycin, streptomycin, tetracycline, erythromycine, chloramphenicol, norfloxacin, methicillin

and vancomycin were determined by a broth dilution method using Mueller–Hinton broth (MHB), as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) **[26]**. About $5x10^4$ cells in MHB were treated with different concentrations of antibiotics and shaken for 16 hr at 37°C. The minimum concentration at which there was no visible turbidity was taken as the MIC of that antibiotic.

2.5.2 Determination of Minimum Bactericidal Concentration

The MBC value of antibiotics was determined according to Okore, 2005 **[27]** with some modification. This is an extension of the MIC Procedure. Antibiotics treated bacterial culture showing growth or no growth in the MIC tests were used for this test. Bacterial culture used for the MIC test were inoculated onto the Mueller–Hinton agar and incubated at 37°C for 24 hr. Microbial growth or death were ascertained via no growth on Mueller–Hinton agar plate. The minimal concentration of the antibiotic that produced total cell death is the MBC.

2.5.3 Disc Agar Diffusion (DAD) test

Susceptibility of isolates to penicillin G, ampicillin, cephotaxime, gentamycin, streptomycin, tetracycline, erythromycine, chloramphenicol, norfloxacin, methicillin and vancomycin was determined by the disc agar diffusion (DAD) technique according to Acar, 1980 and Bauer *et al*., 1966 **[28, 29]**. The test bacterium taken from an overnight culture (inoculated from a single colony) was freshly grown for 4 hr having approximately 10^6 C. F. U ml^{-1}. With this culture, a bacterial lawn was prepared on Mueller-Hinton agar. Filter paper discs of 6-mm size were used to observe antibiotic susceptibility patterns against 11 antibiotics [amount of antibiotic per disc in microgram (µg); penicillin G (10 units), ampicillin (10), cephotaxime (30), gentamicin (10), streptomycin (10), tetracycline (30), erythromycine (15), chloramphenicol (30), norfloxacin (10), methicillin (5) and vancomycin (30). Antibiotic discs were obtained commercially from Himedia. The diameter of zone of bacterial growth inhibition surrounding the disc (including the disc), was measured and compared with a standard for each drug. This gave a profile of drug susceptibility vis-à-vis antibiotic resistance **[29]**.

2.5.4 Inoculation on BHI vancomycin screen agar

Isolates were inoculated on Brain Heart Infusion screen agar according to Tiwari and Sen, 2006 **[30]**. 6 µg/ml vancomycin containing BHI agar screen plates were prepared. Inoculum suspensions were prepared by selecting colonies from overnight growth on nutrient agar plates. The colonies were transferred to sterile saline to produce a suspension that matches the turbidity of a 0.5 McFarland standard. The final inoculum concentration of 10^5 to

10^6 CFU per spot was prepared by adding the sterile saline to the bacterial suspension. These suspensions were inoculated onto BHI screen agar plates and were incubated for 24 hr at 35°C in ambient air. Any visible growth indicated the vancomycin resistance.

3. Results

3.1 Species Identification

The clinical isolates were identified using standard biochemical tests. Purification of bacterial culture by a single colony isolation technique on Nutrient Agar containing 10% sodium chloride exhibited several types of colony. **Table 1** illustrates, 47.62% isolates were Gram positive and 52.38% isolates were Gram negative; 100% of gram positive isolates are oxidase positive, catalase positive and coagulase positive; all isolates were non-motile; 100% of gram positive isolates had thermonuclease activity, mannitol fermentation activity, haemolytic activity (α haemolysis-35% and β haemolysis-65%) and were susceptible to lysostaphin.

3.2 Antibiotic Susceptibility Testing

3.2.1 Minimum Inhibitory Concentration (MIC) of isolated *S. aureus* strains

The MIC values of penicillin G, ampicillin, cephotaxime, gentamycin, streptomycin, tetracycline, erythromycine, chloramphenicol, norfloxacin, methicillin and vancomycin for isolates were determined. In each set of experiment, bacterial control tubes showed no growth inhibitory effect of antibiotics. These MIC values were compared with the NCCLS breakpoints of Minimum Inhibitory concentration for *Staphylococcus aureus*. It was observed that of MIC values of penicillin G, ampicillin and erythromycin for 50% of isolated strains; cephotaxime, gentamycin, streptomycin, tetracycline, chloramphenicol, norfloxacin, methicillin and vancomycin for 40% of isolated strains are beyond the sensitive range **(Figure 1)**.

3.2.2 Minimum Bactericidal Concentration (MBC) of isolated *S. aureus* strains

The MBC values of penicillin G, ampicillin, cephotaxime, gentamycin, streptomycin, tetracycline, erythromycine, chloramphenicol, norfloxacin, methicillin and vancomycin for isolates were determined. In each set of experiment, bacterial control plates showed no growth. It was observed that of MBC values of penicillin G, ampicillin and erythromycin for 50% of isolated strains; cephotaxime, gentamycin, streptomycin, tetracycline, chloramphenicol, norfloxacin, methicillin and vancomycin for 40% of isolated strains are beyond next two concentrations of MIC values **(Figure 2)**.

4.3 Disc Agar Diffusion (DAD) test of isolated *S. aureus* strains

The antibiotic-resistance profile, as determined by DAD test, revealed that out of 10 Gram positive isolates, 50% strains

were resistant to penicillin G, ampicillin and erythromycin and 40% strains were resistant to cephotaxime, gentamycin, streptomycin, tetracycline, chloramphenicol, norfloxacin, methicillin and vancomycin **(Figure 3)**.

4.4 Growth of suspected VRSA strains on BHI vancomycin screen agar

Out of ten Gram positive clinical isolates 40% strains were grown in BHI vancomycin screening agar **(Figure 4)**.

4. DISCUSSION

Staphylococcus aureus is a major human pathogen causing large variety of infections worldwide and predominates in surgical wound infections with prevalence rate ranging from 4.6% - 54.4% **[17, 31- 32]**. *S. aureus* causes superficial skin infections to life-threatening diseases such as endocarditis, sepsis and soft tissue, urinary tract, respiratory tract, intestinal tract, bloodstream infections **[33, 34]**. The development and spread of bacterial strains that are resistant to antibacterial drugs has emerged as a global problem **[35]**. In recent years, *Staphylococcus aureus* become resistance to both synthetic and traditional antibiotics. Treatment of antibiotic resistant bacteria is a therapeutic problem. Susceptibility pattern is useful to determine the future challenges of effective therapy.

Throughout the study, sixteen pus samples were collected from surgical ward of Vidyasagar Institute of Health (Paramedical College and Hospital), Midnapore, West Bengal, India. Samples were transported to the laboratory within 30 minutes of collection and species identification was carried out by Gram staining and standard biochemical tests. In this study, 47.62% of clinical isolates were Gram positive and 52.38% isolates were Gram negative **(Table 1)**. Gram negative clinical isolates were not involved in this study as it is commonly known that *Staphylococcus aureus* is gram positive. Clinical isolates were gram positive, which may be due to thicker and denser peptidoglycan layers of their cell walls; iodine penetrates the cell wall of these isolates and alters the blue dye to inhibit its diffusion through the cell wall during decolourisation **[18]**. Our results showed that 100% of gram positive isolates were oxidase positive, catalase positive and coagulase positive **(Table 1)**. Isolates were oxidase positive that may due to the presence of N, N, N', N'-tetramethyl-p-phenylenediamine dihydrochloride as artificial electron acceptor which takes the electron from cytochrome oxidase in the electron transport chain and changes colour to a dark blue. Oxidase positivity of clinical isolates distinguishes them from *Micrococcus* spp. **[19]**. Clinical isolates were catalase positive which may be due to the production of catalase enzyme by isolates which catalyzes H_2O_2, a potent oxidizing agent into water and oxygen. Catalase positivity of clinical isolates

distinguishes them from *Streptococcus* spp. **[20]**. Isolates were coagulase positive which may be due to the production of coagulase enzyme by isolates, that reacts with prothrombin and form staphylothrombin which causes blood to clot by converting fibrinogen to fibrin. Coagulase positivity of isolates distinguishes them from other *Staphylococcus* spp. except *Staphylococcus aureus* and represents them as possible *Staphylococcus aureus* **[21]**.

It is evident from our study that all gram positive isolates were non-motile **(Table 1)**. Non-motility of clinical isolates may be due to absence of flagellum **[22]**. Our results also demonstrated that, 100% of gram positive clinical isolates have haemolytic activity (α haemolysis-35% and β haemolysis-65%), thermonuclease activity, mannitol fermentation activity and are susceptible to lysostaphin **(Table 1)**. Isolates have haemolytic activity that may be due to the production of haemolysin by isolates, which binds with the haemolysin receptor present on the surface of RBC, that favour haemolysis and makes the clear zone surrounding the isolates (clear zone-α haemolysis and greenish surround the colony-β haemolysis) **[17]**. Nuclease production was suggested as an indicator of potentially pathogenic staphylococci over a decade ago **[36]**. Thermonuclease activity of clinical isolates may be due to the breakdown of DNA present in the media by production of nuclease enzyme; suggests that these isolates have the ability to break down the DNA **[23]**. Mannitol salt agar is a selective media for *Staphylococcus aureus*. The clinical isolates ferment mannitol and produced yellow colour. Lysostaphin susceptibility of isolates may be due to the inactivation of the lysostaphin due to structural modification by enzymatic action **[25]**. Non-motility, latex agglutination activity, haemolytic activity, thermonuclease activity, mannitol fermentation activity and lysostaphin susceptibility of clinical isolates suggests that these isolates are *Staphylococcus aureus*. All isolated *Staphylococcus aureus* strains were coagulase positive and have haemolytic activity that suggests that all theses isolates were pathogenic. The clinically isolated *Staphyloccus aureus* strains were newly named as VIH (Vidyasagar Institute of Health) from VIH 1 to VIH 10 and cultured in laboratory for antibiotic assay.

In this study, the result of Minimum Inhibitory Concentration **(Figure 1)**, Minimum Bactericidal Concentration **(Figure 2)** and Disc Agar Diffusion **(Figure 3)** suggests that 50% of isolated *Staphylococcus aureus* strains (VIH 2, VIH 4, VIH 5, VIH 8 and VIH 10) were resistant to penicillin, ampicillin and erythromycin; 40% of isolated *Staphylococcus aureus* strains (VIH 3, VIH 6, VIH 7 and VIH 9)

were resistant to cephotaxime, gentamycin, streptomycin, tetracycline, chloramphenicol, norfloxacin, methicillin and vancomycin **(Figure 5)**. From our study, it was observed that VIH 2, VIH 4, VIH 5, VIH 8 and VIH 10 is resistant to penicillin G and ampicillin, and VIH 3, VIH 6, VIH 7 and VIH 9 are resistant to penicillin G, ampicillin, cephotaxime, gentamycin, streptomycin, tetracycline, erythromycin, chloramphenicol, norfloxacin, methicillin and vancomycin. Henceforth 40% isolated *S. aureus* strains (VIH 3, VIH 6, VIH 7 and VIH 9) are multi drug resistant (MDR) **(Figure 5)**. In our study, the result of screening of suspected isolated *Staphylococcus aureus* strain on vancomycin agar **(Figure 4)** clearly indicated that 40% of isolated strains (VIH 3, VIH 6, VIH 7 and VIH 9) are truly resistant to vancomycin.

Clinically isolated *S. aureus* strains from pus sample are resistant to β-lactam antibiotic, aminoglycosides, macrolides, quinolones, tetracycline, chloramphenicol and vancomycin that may be due to (i) inactivation of the antibiotic due to structural modification by enzymatic action, (ii) prevention of access to target by altering the outer membrane permeability, (iii) alteration of the antibiotic target site, (iv) efflux pump which pumps out the antibiotic, (v) target enzyme bypass or over production **[37]**. Our study exposed the emergence of VRSA in Midnapore surroundings. This emergence of inducible VRSA is alarming. This may soon become a large-scale problem, unless antimicrobial agents are used more prudently.

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Conflict of interest

The author reports no conflicts of interest. The author alone is responsible for the content and writing of the paper.

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Table 1: Standard biochemical tests of clinical isolates, collected from pus samples of patient. ND=Tests are not done, + ve = tests are positive, -ve = tests are negative

Sample	Isolates	Gram Staining	Oxidase	Catalase	Coagulase	Motility	Thermo-nuclease activity	Haemolytic activity	Growth on MSA	Lysostaphin susceptibilily
S1	S1a	+ ve	+ ve	+ ve	+ ve	Non motile	+ ve	+ ve	+ ve	+ ve
S2	S2a	+ ve	+ ve	+ ve	+ ve	Non motile	+ ve	+ ve	+ ve	+ ve
	S2b	-ve	ND	ND	ND	ND	ND	ND	ND	ND
S3	S3a	-ve	ND	ND	ND	ND	ND	ND	ND	ND
	S3b	+ ve	+ ve	+ ve	+ ve	Non motile	+ ve	+ ve	+ ve	+ ve
S4	S4a	-ve	ND	ND	ND	ND	ND	ND	ND	ND
	S4b	+ ve	+ ve	+ ve	+ ve	Non motile	+ ve	+ ve	+ ve	+ ve
S5	S5a	+ ve	+ ve	+ ve	+ ve	Non motile	+ ve	+ ve	+ ve	+ ve
S6	S6a	+ ve	+ ve	+ ve	+ ve	Non motile	+ ve	+ ve	+ ve	+ ve
	S6b	-ve	ND	ND	ND	ND	ND	ND	ND	ND
S7	S7a	+ ve	+ ve	+ ve	+ ve	Non motile	+ ve	+ ve	+ ve	+ ve
S8	S8a	+ ve	+ ve	+ ve	+ ve	Non motile	+ ve	+ ve	+ ve	+ ve
S9	S9a	-ve	ND	ND	ND	ND	ND	ND	ND	ND
	S9b	+ ve	+ ve	+ ve	+ ve	Non motile	+ ve	+ ve	+ ve	+ ve
S10	S10a	+ ve	+ ve	+ ve	+ ve	Non motile	+ ve	+ ve	+ ve	+ ve
S11	S11a	-ve	ND	ND	ND	ND	ND	ND	ND	ND
S12	S12a	-ve	ND	ND	ND	ND	ND	ND	ND	ND
S13	S13a	-ve	ND	ND	ND	ND	ND	ND	ND	ND
S14	S14a	-ve	ND	ND	ND	ND	ND	ND	ND	ND
S15	S15a	-ve	ND	ND	ND	ND	ND	ND	ND	ND
S16	S16a	-ve	ND	ND	ND	ND	ND	ND	ND	ND

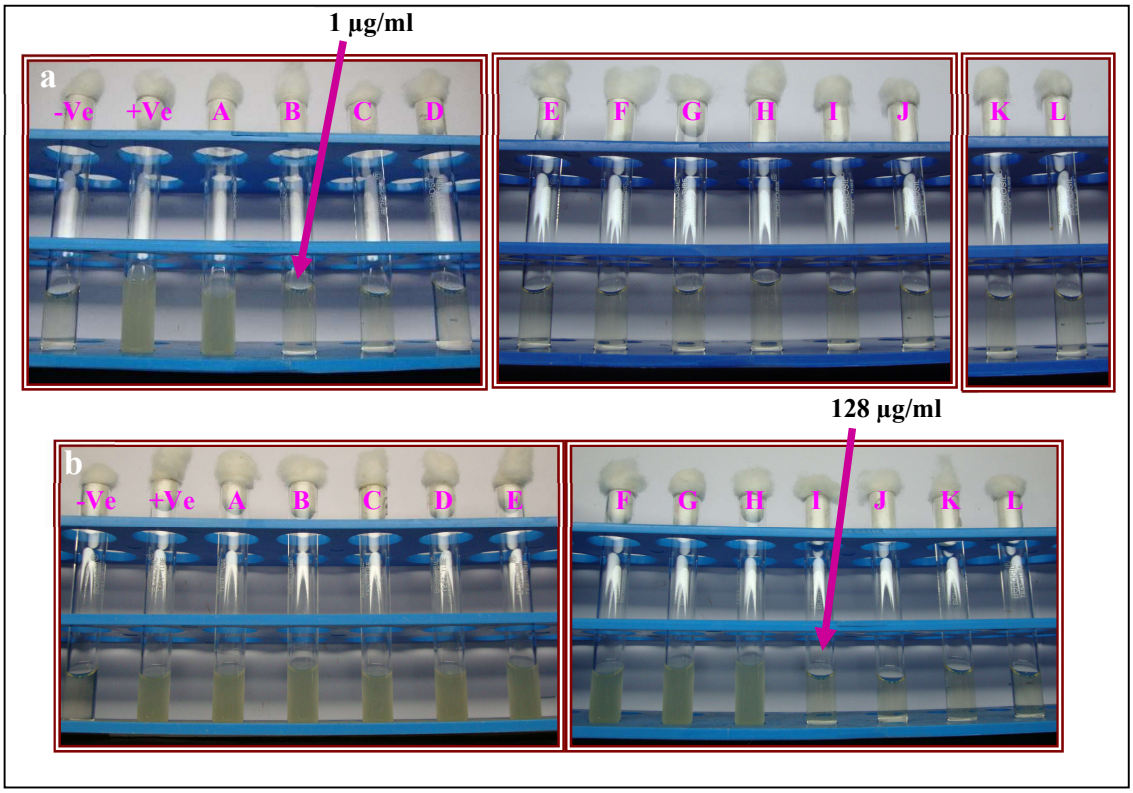

Figure 1: Minimum Inhibitory Concentration determination of antibiotics for *Staphylococcus aureus* isolates. MIC of vancomycin for VIH 1 is 1 µg/ ml (1a) & VIH 3 is 128 µg/ ml (1b)

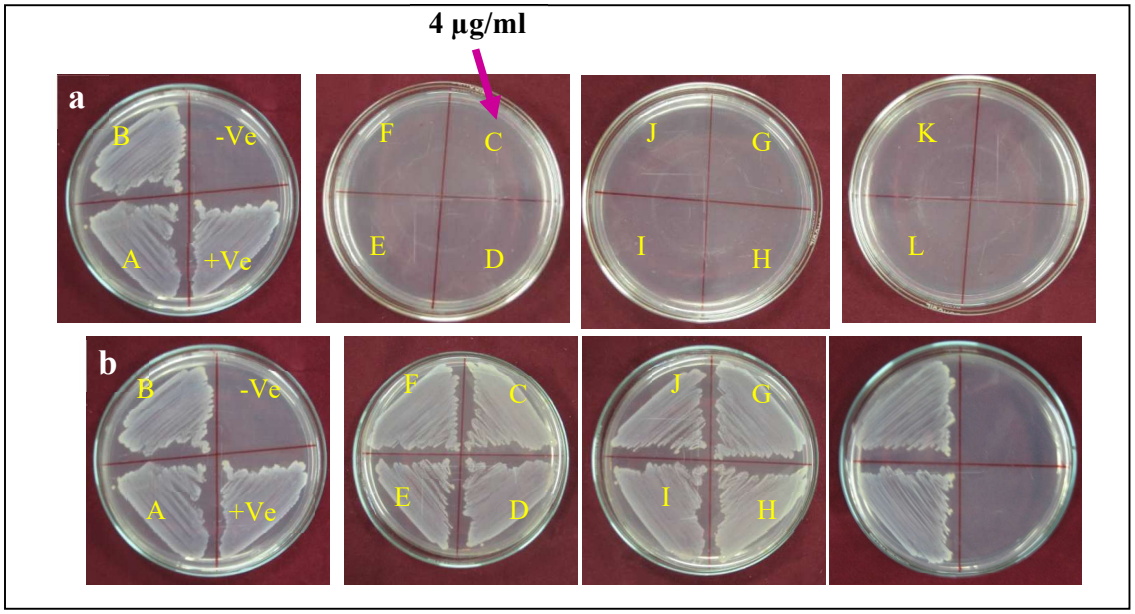

Figure 2: Minimum Bactericidal Concentration determination of antibiotics for *Staphylococcus aureus* isolates. MBC of vancomycin for VIH 1 is 2 µg/ ml (2a) & VIH 3 is > 1024 µg/ ml (2b)

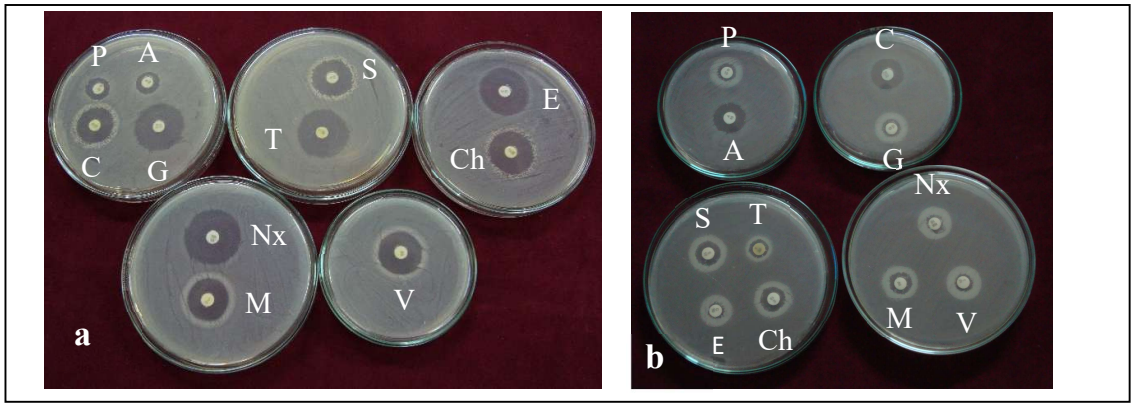

Figure 3: Disc agar diffusion test of VIH 1 (3a) and VIH 3 (3b)

Figure 4: Growth of some *Staphylococcus aureus* isolates on BHI vancomycin screen agar

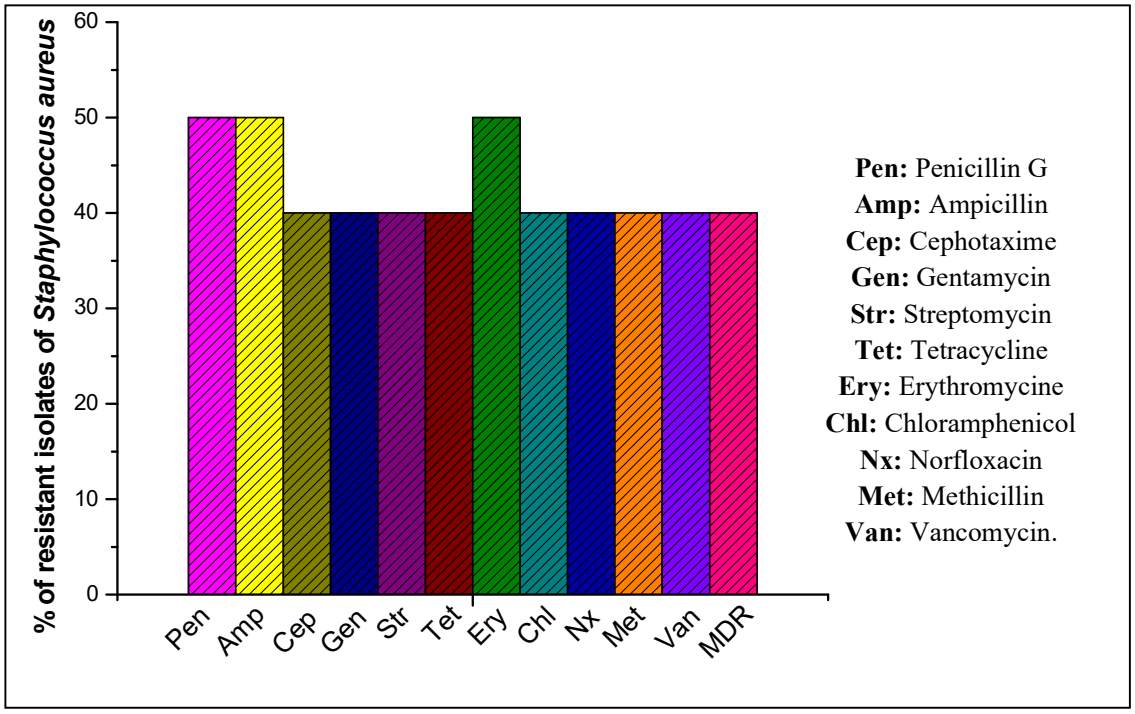

Figure 5: Antibiotic susceptibility profile of isolated *Staphylococcus aureus* strains

Abbreviations:

BHI	**: Brain heart infusion**
CFU	**: Colony formation unit**
DAD	**: Disc agar diffusion**
DNA	**: Deoxyribonucleic acid**
E. coli	**: *Eschrichia coli***
H_2O_2	**: Hydrogen peroxide**
LB	**: Luria broth**
MBC	**: Minimum bactericidal concentration**
MDR	**: Multi-drug resistant**
MHB	**: Mueller-Hinton broth**
MIC	**: Minimum inhibitory concentration**
MRSA	**: Methicillin resistant *Staphylococcus aureus***
MSSA	**: Methicillin sensitive *Staphylococcus aureus***
NA	**: Nutrient agar**
NCCLS	**: National Committee for Clinical Laboratory Standards**
PBP	**: Penicillin Binding Protein**
PG	**: Peptidoglycan**
S. aureus	**: *Staphylococcus aureus***
S. epidermidis	**: *Staphylococcus epidermidis***
TSB	**: Tryptic soy broth**
VRSA	**: Vancomycin resistant *Staphylococcus aureus***