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RESEARCH ARTICLE

OCCURRENCE OF MULTIPLE DRUG RESISTANT *STAPHYLOCOCUS AUREUS* STRAINS ISOLATED FROM HOSPITAL WASTE

***,[1]Warkhade, B. B. and [2]Gupta S. G.**

[1]Department of Biotechnology, Badrinarayan Barwale Mahavidyalaya, Jalna, MS, India 431213
[2]Government Institute of Forensic Science, Aurangabad, MS, India 431004

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ABSTRACT

Staphylococcus aureus has been reported to be a major cause of hospital acquired infections. Indiscriminate use of antibiotics resulted in the development of multi-drug resistant *S. aureus* throughout the world. In present study twenty strains of *S. aureus* were isolated from hospital waste samples like cotton swabs, bandages, and needle. These isolates were identified by several morphological and biochemical test. Antibiotic susceptibility test was performed by Standard disc diffusion method. All the isolates were found to be 100% resistant to Penicillin G (P), Cefoxitin (CX), and Erythromycin (E). The resistance pattern of reference strain *S.aureus* ATCC 25923 was 0%.

Key words: *Staphylococcus aureus,* Antibiotic susceptibility, hospital waste.

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INTRODUCTION

S. aureus is a normal inhabitant of the human skin and the anterior nares. In a healthy person, *S. aureus* is usually not a health concern, but an injury or poor hygiene can cause *S. aureus* infections (Brenda *et al.*, 2008). It is also a serious opportunistic pathogen responsible for a number of infections in immuno-compromised individuals (Burnett *et al.,* 1996). *S.aureus* can cause a range of illness from minor skin infections such as boils, abscesses to life threatening diseases such as pneumonia, meningitis, toxic shock syndrome and sepsis (Lakshmi *et al.,* 2011). *S. aureus* has therefore emerged as one of the main important human pathogens, and has over the past decades, been a leading cause of hospital and community-acquired infections. Attempts to control diseases caused by *S. aureus* through the use of antibiotics have resulted in increased prevalence of resistant strains of the organism (Levy, 2001; Crowder *et al*., 2006). Therefore, in order to effectively treat infections caused by *S. aureus*, culture and antibiotic sensitivity tests must first be determined. Colonization is an important step in the chain of events that leads to *S. aureus* infections. First, individuals are likely to become colonized, invaded and infected from this source (Ako-nai *et al.,* 1991). Thus, colonization with *S. aureus* is a major risk factor for staphylococcal infections (Holtfreter *et al.,* 2007). One of the most frequent bacterial infections in the pediatric population is caused by *S. aureus.*

Corresponding author: **Warkhade B.B.,**
Department of Biotechnology, Badrinarayan Barwale Mahavidyalaya, Jalna, MS, India.431213.

Multi-drug resistance (MDR) is a major health concern in the treatment of staphylococcal infections; more specifically infections of methicillin-resistant *S. aureus* (MRSA) pose a serious challenge to hospital industry. The factors that are responsible for the pathogenicity of the *S. aureus* include enterotoxin, exfoliative toxin, and toxic syndrome toxin (Bukowski *et al.,* 2010). Recently, it has been reported that most of the MRSA strains are becoming resistant and are susceptible only to glycopeptides antibiotics such as Vancomycin (Kaleem *et al.,* 2010). Another literature reported the prevalence of Vancomycin-resistant *S. aureus* and vancomycin-intermediate *S. aureus*, therefore, development of resistance toward Vancomycin warrants the search for a new class of antibiotics (Kaleem *et al.,* 2010, Aligholi *et al.,* 2008, Howden *et al.,* 2010). Prolonged hospital stay and arbitrary use of antibiotics will increase the emergence and spread of MRSA (Tiwari *et al.,* 2009). Hospital acquired Staphylococcal infections are common in newborn babies, surgical patients and hospital staff. Patients develop sepsis in operation wounds, which take place in the theatre during operation, and others post-operations in the ward (Chamber, 2005). *S. aureus* is one of the most important etiological agents of many hospital-acquired infections as well as community-acquired infections and poses a constant therapeutic problem to clinicians (Stobberingh, 2007). In recent years, a strong correlation between isolation of *S. aureus* and occurrence of nosocomial infections became a constant problem to hospitals and clinical centers. Hospital waste contains body parts, organs, tissues, blood and body fluids along with soiled linen, cotton, bandage, and plaster casts from infected and contaminated areas along

with used needles, syringes and other sharps. It contains pathogens in mass, in their invisible forms. Thus data on susceptibility and resistance patterns and characterization are of great importance as these data may be used to devise mechanisms to stem the emergence and subsequent spread of infections and drug resistance by the organism. The aim of the study was to examine antibiotic resistance patterns of *S. aureus* isolated from hospital waste.

MATERIALS AND METHODS

Sample collection

The waste samples like cotton swabs, bandages, needles were collected from the government hospital Aurangabad, Maharashtra, India. All the samples were collected in sterile container and brought to the laboratory.

Isolation and Identification

The collected hospital waste samples then used for the isolation purpose of *S. aureus*. All the samples were enrich in the Nutrient broth for about 24Hours. Enriched culture was then transfer to the Mannitol salt Agar and used for the isolation of *S. aureus*. Representative colony types were sub-cultured on their isolation media until pure cultures were obtained as confirmed by microscopy. The pure cultures were tentatively characterized and identified on the basis of their colonial morphology, Gram's reaction and biochemical tests (Schofield *et al*., 2007; Uppal *et al*., 2007).

Observation of colonial morphology and characteristics

Presumptive morphological identification of the colonies was done by observing their individual appearance on the selective media that were used for the isolation.

Biochemical tests

Catalase test

3 ml of hydrogen peroxide solution was poured into a test tube. With the aid of sterile glass rod, several colonies of the test organism were carefully removed and immersed into 3 ml solution of hydrogen peroxide. Immediate bubbling within few seconds was recorded to be positive test of Staphylococcus species.

Coagulase test

A drop of distilled water was added on each end of a slide. A colony of a suspected organism of 24 h culture from blood agar (previously checked by gram staining) was emulsified in each of the drops of the distilled water and made two different suspensions. A loop of the plasma was then added to one of the suspensions and mixed gently. Clumping or agglutinations of the organisms with the plasma within ten seconds indicated a positive result of *S. aureus*; negative result indicates other *Staphylococcus* species.

McFarland turbidity standard

The turbidity standard of the organisms used was 0.5. One percent (1%) v/v solution of sulphuric acid was prepared by adding 1 ml of concentrated sulphuric acid to 99 ml of distilled water and mixed well. 1% w/v solution of barium chloride was also prepared by dissolving 1 g of the dehydrated salt ($BaCl_2.2H_2O$) in 100 ml of distilled water. Then 0.6 ml of the barium chloride was added to 99.4 ml of the sulphuric acid solution and was mixed well. The small portion of the turbid solution was transferred into a test tube which was used to compare with the inoculated organisms in Mueller Hinton broth (Cheesbrough, 2004).

The standardization of inoculums

The concentration of each of the suspension of the test organisms and the standard isolates were prepared by picking a 24 h colony of the organism using sterile wire loop into sterile test tube containing sterile Mueller Hinton broth to form turbidity that match with 0.5 scale of McFarland's standard (1.5 × 108 cells/ml) (Coyle, 2005). The cell suspensions was inoculated by streaking on prepared Mueller Hinton agar using sterile swab stick, then the antibiotic disc was placed on the inoculated medium aseptically with the help of sterile forceps and incubated at 37°C for 24 h. The zones of inhibition created by each of the antibiotics against the test organisms and the standard strains as positive control were measured and the result was interpreted using guideline from CLSI, 2012. The results were recorded as sensitive, intermediate and resistance.

Antibiotic Susceptibility Test

The Kirby-Bauer disc diffusion method was used for this test. The commercially prepared antibiotics (Himedia) used were Kanamycin (K) 30mcg, Methicillin (MET) 5mcg, Norfloxacin (NX) 10mcg, Penicillin-G (P) 10units, Tobramycin (TOB) 10mcg, Ceftriaxone (CTR) 30mcg, Ofloxacin (OF) 2mcg, Vancomycin (VA) 30mcg, Amikacin (AK) 30mcg, Amoxicillin- clavulanic acid (AMC) 20/10mcg, Cefoxitin (CX) 30mcg, Linezolid (LZ) 30mcg, Tetracycline (TE) 30mcg, Co-Trimoxazole (COT) 25mcg, Ciprofloxacin (CIP) 5mcg, Gentamicin (GEN) 10mcg, Erythromycin (E) 15mcg and Chloramphenicol (C) 30mcg. The various antibiotics discs were carefully placed on the surface of Muller-Hinton agar plates seeded with standardized suspensions of each purified isolate. The standardization of the bacterial suspensions was achieved using 0.5 McFarland solution. Inhibition zone diameters were measured after 18-24 hours of incubation at 37 ^{0}C.

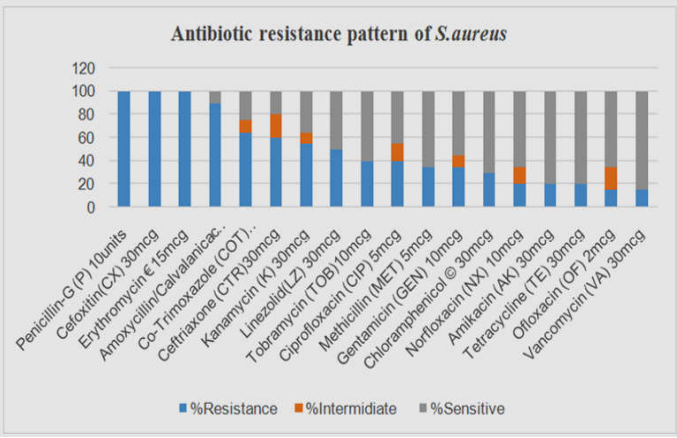
Figure 1. *S. aureus* on mannnitaol salt agar

RESULTS AND DISCUSSION

All 20 isolates has given positive results for the catalase and coagulase test and yellow colour colonies on Mannitol salt agar, (Figure 1) hence these isolates were identified as *Staphylococcus aureus*. While the *S.aureus* ATCC 25923 also shown positive results for the catalase and coagulase test. The antibiotic susceptibility profile of isolates from as in Table 1 shows that *S. aureus* isolates were highly resistant to Penicillin G, Cefoxitin and Erythromycin i.e.100%; 90% were resistant to Amoxicillin- clavulanic acid; 65% were resistant to Co-Trimoxazole; 60% were resistant for Ceftriaxone; 55% were resistant for Kanamycin; 50% are resistant to Linezolid; 40%were resistant for Tobramycin and Ciprofloxacin;35% were resistant for Gentamycin and Methicillin; 30% were resistant to Chloramphenicol;20% were resistant for Tetracyclin and Amikacin and Norfloxacin; 15% were resistant for Ofloxacin and Vancomycin. While, 85% of the isolates of *S. aureus* were susceptible to Vancomycin; 80% are susceptible to Amikacin, Tetracyclin; 70% were susceptible to Chloramphenicol; 65% were susceptible to Norfloxacin, Ofloxacin, and Methicillin; 60% were susceptible to Tobramycin; 55% were susceptible to Gentamycin;50% were susceptible to Linezolid; 45% were susceptible to Ciprofloxacin; 35% were susceptible to Kanamycin; 25% were susceptible to Co-Trimoxazole; 20% were susceptible to Ceftriaxone; 10% were susceptible to Amoxicillin- clavulanic acid. Among the Gram-positive pathogens, *S. aureus*, has become a major nosocomial pathogen causing skin and soft tissue infections in the both community and hospitalized patients (Chambers, 2001). Small colony variants of *Staphylococcus aureus* are sometimes found in antibiotic-refractory infection are sometimes found in antibiotic-refractory infections like osteomyelitis, chronic airway infections in cystic fibrosis and device-related infections (Garcia *et al.*, 2013). They are often resistant to beta-lactam antibiotics due to an exceptionally slow rate of multiplication, and aminoglycoside antibiotics due to auxotrophicity for Vitamin K and Hemin, which are key components of the electron transport chain (Garcia *et al.,* 2013). The present study indicates the prevalence of antibiotic resistant *Staphylococcus aureus* in hospital waste samples like used cotton swab, needles, and bandages.

Table 1. The antibiotic profile of *S.aureus* isolates from Hospital waste samples

Antibiotics	% Resistance	% Intermediate	% Sensitive
Penicillin-G (P) 10units	100	0	0
Cefoxitin(CX) 30mcg	100	0	0
Erythromycin € 15mcg	100	0	0
Amoxycillin/Calvalanicacis (AMC) 20/10mcg	90	0	10
Co-Trimoxazole (COT) 25mcg	65	10	25
Ceftriaxone (CTR)30mcg	60	20	20
Kanamycin (K) 30mcg	55	10	35
Linezolid(LZ) 30mcg	50	0	50
Tobramycin (TOB)10mcg	40	0	60
Ciprofloxacin (CIP) 5mcg	40	15	45
Methicillin (MET) 5mcg	35	0	65
Gentamicin (GEN) 10mcg	35	10	55
Chloramphenicol © 30mcg	30	0	70
Norfloxacin (NX) 10mcg	20	15	65
Amikacin (AK) 30mcg	20	0	80
Tetracycline (TE) 30mcg	20	0	80
Ofloxacin (OF) 2mcg	15	20	65
Vancomycin (VA) 30mcg	15	0	85



Graph 1. Antibiotic resistance pattern of *S.aureus*

Hospital waste contains pathogens in mass, in their invisible forms. Therefore, its proper management is essential to maintain hygienic, aesthetics, cleanliness, and control of environmental pollution. If this substantial amount of waste is not properly managed it can pollute soil, air and water. Further, it can cause deadly diseases, either in endemic, sporadic or epidemic forms. Proper management means proper collection, segregation, storage, transportation and treatment of waste in safer manner to prevent nosocomial or hospital acquired infection. Diseases of modem era like hepatitis-B, AIDS are also drawing attention for proper management of hospital wastes as persons who are in touch of these materials during discharge of their services to mankind are also at the risk. This can be achieved by public awareness about hospital waste hazards and by making mandatory to officials of the institutions to follow the guidelines of Supreme Court and Ministry of Environment Forest, Government of India notification for biomedical (Dwivedi *et al.,*2009).

Conclusion

Resistance to antimicrobial agents is a problem in communities as well as health care facilities, but in hospitals, transmission of bacteria is amplified because of the highly susceptible population. Factors that could be associated with transmission of resistant strains of these microorganisms include poor attention to hygiene, overcrowding, lack of an effective infection control program, and shortage of trained infection control providers.

The highest sensitivity of *S. aureus* isolates was reported to Vancomycin. Vary proportion of *S. aureus* isolates were sensitive to Tetracyclin, Norfloxacin, Ofloxacin, Chloramphenicol, Tobramycin, Gentamycin, Linezolid, Ciprofloxacin, Kanamycin, Methicillin, Co-Trimoxazole, Ceftriaxone and Amoxicillin- clavulanic acid. The *S. aureus* isolated strains were resistant to a large number of antibiotic groups used in the treatment: penicillins (Penicillin Methicillin), β lactam/ β lactamase (Amoxicillin- clavulanic acid), cephems (Ceftriaxone), glycopeptides (Vancomycin), fluroquinolones (Norfloxacin, Ofloxacin, and Ciprofloxacin), oxazolindinones (Linezolid), aminoglycosides (gentamicin, kanamycin, Amikacin, Tobramycin), macrolides (erythromycin) and tetracyclines (tetracycline). The phenomenon of multiple resistance to antibiotics has been noticed in *Staphylococcus aureus* isolates, in varying proportions.

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