

Original Research Article

Pattern of Drug Resistance in Isolates of Staphylococcus Aureus from Hospitalized Patients

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Abstract: *Staphylococcus aureus* is a non motile, non spore- forming gram positive bacterium that causes a wide range of infections such as endocarditis, skin infections, bacteraemia, sepsis and food poisoning. The objective of this study is to evaluate the pattern of drug resistance of staphylococcus aureus in different type of samples that were taken from different wards (emergency and OPD) of the hospital. In this study we analyzed 40 isolates were obtained on blood agar and tryptic soy agar. Identification of the isolates was carried out by catalase test as well as methicillin resistance. Confirmed *staphylococcus aureus* isolates were analyzed for antimicrobial susceptibility testing. In our study the isolates from patients, particularly are frequently resistance to multiple antimicrobial agents more than 90% of *staphylococcus aureus* isolates were resistance to cefalothin, erythromycin and 80% are resistance to vancomycin. Our findings showed that MRSA isolates were coming from the community as indicated by hospital ward types, that even with no prolonged hospital stays the patients coming from the community were carrying MRSA strains.

Keywords: *Staphylococcus aureus*, skin infections, bacteraemia, cefalothin.

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INTRODUCTION

Staphylococcus is a facultative anaerobic bacterium that related to a family staphylococcaceae [1]. *Staphylococcus aureus* is a catalase, coagulase, non- spore forming, non –motile gram-positive bacteria arranged in cluster that is 0.5 to 1µm in diameter [2]. *Staphylococcus aureus* found in both hospital and community environment that causes a high range of infections like skin infections, endocarditis, urinary tract infections, respiratory tract infections, bacteraemia, sepsis and food poisoning [3]. *Staphylococcus aureus* grow well at an optimum temperature ranging from 7 to 48C, pH 4.2 to 9.3 and sodium chloride concentration as high as 15% NaCl [4]. *Staphylococcus aureus* can be transmitted from person to person, contaminated equipment, or by air bubbles [5].

Almost 30% of normal healthy people are affected by *Staphylococcus aureus* because it asymptotically emphasize the skin of human host [6] *Staphylococcus aureus* produces a number of toxins that causes various types of diseases, ranging from mild skin infection to systemic, life threatening diseases.

Toxins that can produce by *Staphylococcus aureus* are enterotoxins, toxic shock syndrome toxin and scalded skin syndrome [7]. Plasmids of *Staphylococcus aureus* carry virulence and resistance genes that can be brutish through *Staphylococcus aureus* population by horizontal gene transfer.

Pathogenic islands are involving in *Staphylococcus aureus*. Pathogenic islands are specific group of genomic islands acquired by microorganism through horizontal gene transfer [8]. *Staphylococcus aureus* pathogenic islands are a group of 15 kb mobile genetic element that can be dwelling in the genome of the wide group of *Staphylococcus aureus* strains [9]. It can occur infrequently in other staphylococcal species but *Staphylococcus aureus* pathogenic islands are common and spread in other gram positive bacteria [10]. Most gene are transferred by *Staphylococcus aureus* pathogenic islands that are involved in pathogenesis and resistance, the staphylococcal enterotoxins like toxins (SEIs) can be located on *Staphylococcus aureus* pathogenic islands that can carry one or more super antigens encoding gene like tst and many SEs gene [11].

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Gram positive bacteria retain crystal violet and turns purple color under the microscope, whereas gram negative bacteria does not retain crystal violet and turns red color under the microscope *Staphylococcus aureus* is gram positive cocci and after gram staining it can appear purple colonies [12]. Two major biochemical tests are used for the identification of *Staphylococcus aureus* is coagulase and catalase test. Catalase test are used for the identification of *Staphylococcus aureus* from enterococci and streptococci [13]. Coagulase test causes plasma to clot by converting the fibrinogen to fibrin. These coagulase tests are used to differentiate *Staphylococcus aureus* from other staphylococcus species [14]. Biofilm can be commonly consider to be occur in four main stages that is attachment, micro colony formation, maturation and dispersal [15].

Staphylococcus aureus biofilm can be a main focus of infection with separation and circulation of aggregates that can lead for developing the infectious diseases such as endocarditis, meningitis, necrotizing pneumonia and septicemia [16]. Distinct agar used for *Staphylococcus aureus* such as nutrient agar, blood agar and Mannitol salt [17]. Genes that introduced virulency in *Staphylococcus aureus* including *sea*, *sed*, *eta*, *hla* and *mecA* gene [18].

Resistance to Penicillin Epidemiology

In 1942, the penicillin resistance *staphylococcus* were introduced in hospitals and then subsequent distinguish in the community. In 1960, 80% community and hospitals required isolates were resistance to penicillin. More than 90% staphylococcal isolates can produce penicillin's.

Methicillin resistant *Staphylococcus aureus* Mechanism of resistance

Methicillin resistance need *mecA* gene, the *mecA* genes are liable for the synthesis of penicillin binding protein 2a. Penicillin binding protein is an enzyme that catalyzes the transpeptidation reaction that can be important for cross linkages of peptidoglycan chains. Methicillin causes resistance to all beta lactam agents such as cephalosporins. Penicillin binding protein 2a differ from other penicillin binding protein the active site of penicillin binding protein 2a stop the binding site of all beta lactamase and can allow the transpeptidation to be persist [19, 23].

Ideometric techniques revealed 74% of total isolates to be beta lactamase producers in this investigation, whereas Nitrocefin sticks revealed 80% isolates to be beta lactamase producers. Although no strains were discovered to be vancomycin resistant, 93.44% were found to be intermediate, and just 65.6% were found be sensitive. A study conducted in Bangladesh that demonstrates the rise in antimicrobial resistance and recommends that antimicrobial medicines with low sensitivity against *Staphylococcus*

aureus be avoided in order to avoid resistance and treatment failure [20]. Penicillin resistance were found to *Staphylococcus aureus* isolates are 82.7% and tetracycline 65.4%, although erythromycin is the most common antibiotic 78.8% sensitive, gentamycin 82.7% resistance with pefloxacin. Methicillin, oxacillin and cefoxitin had sensitive rates of 70%, 80% and 100 %, and specific rates are 76.2%, 69.1% and 78.5% correspondingly, when compare to the *mecA* gene as a gold standard method for MRSA identification [21, 24].

Three isolates tested positive for lincomycin inactivation. 48 of the 225 erythromycin resistance isolates tested positive for *ermA*, 20 for *ErmC*, and 128 for *ErmA-C*. For 15 strains PCR was negative [25]. *LinA* and MRSA were found in one of three isolates with lincomycin inactivation. 334 of the 358 gentamycin resistance isolates tested positive for *acc-aph*, whereas 24 tested negative. 314 of the 350 tetracycline resistance isolates tested positive for *tetM*. Ten of the 36 *tetM* negative isolates were positive for *tetK* [22, 26]. Between 2009 and 2014, 1,116% isolates were produced, Resistance to penicillin, erythromycin, rifampicin, gentamicin and clindamycin were found to be 100%, 18%, 14% and 11% respectively. In 2009, the maximum rate of Methicillin resistance was estimated to be 30% and resistance has reduced in successive years that are 20%, 16% and 21% [27].

During this research period, a total 380% *Staphylococcus aureus* strains were identified from HIV patients. *Staphylococcus aureus* strains were obtained from urine samples (69.5%), and 58.4% of *Staphylococcus aureus* infections were found in hospitalized patients [28]. *Staphylococcus aureus* has a high sensitive to antibiotics, according to the antimicrobial susceptibility testing. Penicillin resistance is the most common 95.2%, followed by cephalixin resistance 84.6%. Methicillin resistance prevalent in 2017, 86%, with MRSA rates progressively increasing from 51.8% in 2012 to 86% in 2017 [29].

MATERIAL AND METHOD

1. Blood agar

Table 1.1: Blood Agar Compositions

Ingredients	Concentration in (g/l)
Beef extract	0.3
Peptone	10.0
Sodium chloride	5.0
Agar	15.0
PH	7.3+-0.2at 25°C

Procedure

In one liter of distilled water, dilute 28g nutritional agar powder. To properly dissolve all components, heat this mixture while stirring. 15 minutes autoclaves, during to the dissolved mixture at

121°C. After autoclaving it is important that agar to be cool but not be solidify.

2. Tryptic Soy agar

Table 1.2: Composition of Tryptic Soy Agar

Components	Concentration in (g/l)
Pancreatic digest of casein	15.0
Peptic digest of soybean meal	5.0
Sodium chloride	5.0
Agar	15.0
Distilled water	1 liter

PH.7.4 +/- 0.2 at 25°C

Procedure: 45g nutritional powder dissolved in 1000 ml distilled water. The medium were completely dissolved by heating. Autoclave at 121°C for 15 minutes, Cool the agar at optimum temperature 45-50°C. Pour into sterile Petri dish after thoroughly mixing.

Biochemical Testing

Catalase Test

The catalase test is used to determine the presence of catalase enzyme; catalase is an enzyme that converts the hydrogen peroxide to oxygen and water. Catalase test is used to determine bacteria that produce the enzyme catalase such as staphylococci to those bacteria that do not produce the catalase enzyme such as streptococci. Catalase test performed into two methods that is slide and tube method. Formation of bubbles shows the presence of catalase, if no bubbles occur, it means the catalase test give negative results.

Composition

Table 1.3: Composition of catalase test

Reagents	Amount
Hydrogen peroxide	3%, hydrogen peroxide
Sterile wooden stick or glass rod	

1) Slide Method

Procedure

Transfer a little amount of well isolated bacterial colony growth to the surface of a clean, dry glass slide using a loop or sterilized wooden stick. Place a drop of 3% hydrogen peroxide solution in the glass slide. Look for immediate bubbling.

2) Tube Method

Procedure

In a test tube pour 1-2 ml of hydrogen peroxide solution. Using a wooden stick, take several colonies of the tested organism and immerse in the hydrogen peroxide solution. Look for immediate bubbling.

Preparation of Blood Agar and Sample Inoculation

The blood agar plates were marked with a sample number. Blood agar was prepared and autoclaved at 121°C for 15 minutes. Aseptically, prepared blood agar was placed over Petri Plates. Using the inoculating loop, the samples (pus, wound) were streaked onto the blood agar.

Preparation of Tryptic Soy Agar and Sample Inoculation

The agar plates were marked with a sample number. Tryptic soy agar was prepared and autoclaved at 121°C for 15 minutes. Aseptically, prepared Tryptic soy agar was placed over Petri plates. Using the inoculating loop, the pus and wound samples were streaked onto the Tryptic soy agar plate. Incubate the plate over night at 37°C for 24 hours. After incubation observe the growth of *Staphylococcus aureus* bacteria.

RESULTS

Blood Agar

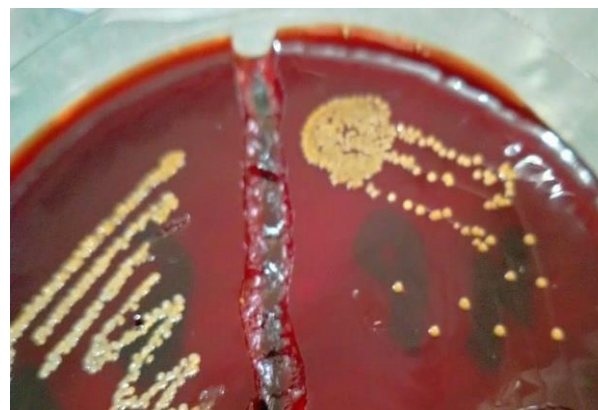


Figure 2.1: *staphylococcus aureus* colonies on blood agar

Tryptic Soy Agar



Figure 2.2: *staphylococcus aureus* colonies on Tryptic soy agar

Biochemical Testing

Catalase Test / Tube Method



Figure 2.3: Catalase tube method for *staph. Aureus*

Slide Method



Figure 2.4: Slide catalase test for *staph. Aureus*

Drug susceptibility testing



Figure 2.5: DST of *Staphylococcus Aureus* show that isolate S1 were resistance to FOX, VA, SXT, DA, CIP, LZD, TEC



Figure 2.6: DST of *Staphylococcus Aureus* show that isolate S2 were resistance to FOX, VA, SXT, DA, CIP, LZD, TE

Drug susceptibility testing

In our study the isolates from patients with nosocomial infection of *Staphylococcus aureus*, particularly are frequently resistance to antimicrobial agents, more than 90% of *staphylococcus aureus* isolates were resistance to cefalothin, erythromycin and 80% are resistance to vancomycin.

Drug zone measurement in mm

Sample	FOX	DA	LZD	VA	CIP	TEC	SXT	E
2265	1.8mm	2mm	2.6mm	0.8mm	3.8mm	0.8mm	1mm	-
7037	0.8mm	2.4mm	2.4mm	1.2mm	-	1.8mm	-	-
5437	1.5mm	-	-	2.6mm	2.2mm	-	-	1.5mm
5462	2.2mm	2.3mm	-	1.3mm	-	-	-	2.3mm
5471	2.6mm	1.7mm	-	0.9mm	2.8mm	1.3mm	-	1.1mm
5485	1.9mm	-	-	1.2mm	2.3mm	2.1mm	-	1.3mm
5487	2.2mm	-	1.3mm	1.9mm	-	-	-	0.8mm
5505	2.5mm	-	-	-	2.8mm	-	2.3mm	1.6mm
5507	2.8mm	-	-	1.2mm	2.2mm	-	-	1.5mm
5516	2.7mm	-	-	1.3mm	-	-	-	-
6006	2.5mm	-	-	1.2mm	-	-	-	1.4mm

DISCUSSION

In this study, we isolated 40 isolates of the *Staphylococcus aureus* from microbiological laboratory of mayo hospital Lahore during 2021. During this study we found that, in addition to *Staphylococcus aureus*

Pseudomonas spp, *E.coli*, *Acetobacter* and *klebsilla* species showed to be highly drug resistance bacteria. Our findings are correlated with past studies that are conducted in India showed that mostly specimens had highly MDR and they are related to emergency and

OPD wards and specimens from pus and wound swab [30].

In this study we assess the isolation and detection of *Staphylococcus aureus* by using blood agar and tryptic soy agar. Blood agar is an enriched media that allow for the growth of different bacteria, it grows fastidious organisms and it is used to differentiate bacteria based on their hemolytic characteristic. Our findings agree with the past study that *Staphylococcus aureus* colonies can be detected by culturing on blood agar. *Staphylococcus aureus* produces golden yellow colonies on blood agar. *Staphylococcus aureus* was identified as to be β hemolytic mainly [31]. Tryptic Soy agar is a non selective enriched media that are used for the isolation and detection of bacteria. Our findings are correlated with past study that *Staphylococcus aureus* produce circular, convex yellow colonies on tryptic soy agar [32].

The catalase test is used to identify those bacteria that produce the enzyme catalase, this enzyme convert hydrogen peroxide to oxygen and water. Production of bubbles indicates catalase positive results. Our findings agree with the past studies in which the catalase test for *Staphylococcus aureus* is positive [33]. Our finding are correlated with past studies that a large number of *staphylococcus aureus* was isolated from pus and wound swab and the isolation rate of bacteria depends on the types of sample of different wards in the hospital. Most of *S. aureus* isolates were recovered from pus samples in emergency ward followed by OPD.

In our study we received 40 isolates of *Staphylococcus aureus* at the Mayo hospital. Lahore was subjected to DST testing using 10 antibiotics. Inhibitory zone were measured for 10 isolates (4.10) for hands on training but the data of resistance and sensitivity was compiled for 40 isolates. The goal of this study was to determine the pattern of antibiotic resistance strains of *Staphylococcus aureus* isolated from patients of different wards in hospital settings. In our study the isolate from patients with nosocomial infections of *Staphylococcus aureus* strains, particularly are frequently resistance to multiple antimicrobial agents more than 90% of *Staphylococcus aureus* isolates were resistance to cefalothin, erythromycin and 80% are resistance to vancomycin, these findings are correlated with the past studies that are conducted in Afghanistan [34].

CONCLUSION

Staphylococcus aureus is a highly drug resistance bacteria. MRSA and VRSA strains are commonly circulated in the community. This study validated this finding by showing widespread resistance against different classes of antibiotic in MRSA. MRSA isolates were coming from the community as indicated by hospital ward types, that even with no prolonged

hospital stays the patients coming from the community were carrying MRSA strains.

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