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Isolation and identification of multidrug resistance *Staphylococcus aureus* from throat infected patients in Tertiary Care Hospital

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Abstract

To investigate the prevalence of Multi Drug Resistance *Staphylococcus aureus* isolated from patients having throat infection . In our study totally 16 samples was collected out of 16 samples 10 samples showing positive results for culture and biochemical testes. Culture and biochemical positive isolates (10 isolates) was subjected to the antimicrobial sensitivity testing. In antimicrobial testing out 11 isolates 9 isolates are resistance to the methicillin and two isolates are sensitive for methicillin.

Keywords: MRSA, Thorat samples, culture and biochemical testes.

Introduction

Staphylococcus is a very well known genus of bacteria. Colonies are “gold”, or yellow on sheep blood agar solid media, the golden appearance is the etymology root of the bacteria’s name :aureus means “golden” in latin .*S.aureus* literally the ‘golden cluster seed’or’the seed gold’ and also known as the golden staphi . A common pathogen, boils, acne, wound infections ,food poisoning are among a host of conditions caused by the organism. The organism is both pathogenic and invasive. It produces leukotoxin which kills the white blood cells and a wide variety of other toxins. *S.aureus* is quite pyogenic and in decades past was named staphylococcus pyogenes. Increasingly ,and especially in hospital, strains of both S.aureus and S.epidermitis. Have become resistant to the antibiotic, methicillin.

There are many species in Staphylococcus such as *S.aureus, S.epidermitis* etc. But the significant species for the production of penicillinase enzymes was found to be the methicillin resistant Staphylococcus aureus (Sutherland and Rolinson, 1964) as it produces higher amount of the enzyme compared to the other species.

Staphylococcus aureus is one of the most frequent bacterial pathogens of humans. It causes skin infections, osteoarthritis, and respiratory tract infections in the community, as well as postoperative and catheter – related infections in hospitals. these infections can lead to life – threatening bacteremia and septic metasteases.

Multi Drug Resistant:

Most Methicillin – Resistant S.aureus (MRSA) also produce penicillinase. Methicillin resistant confers resistants to all the b-lactam antibiotics . Unfortunately, most MRSA nad now also resistant to aminoglycosides, microlides, tetraciclines, fluoroquinolones,and rifampin in many countries. Therefore, MRSA strains are multiple resistant strains with therapeutic options that are largely limited to vancomycin. The Detection of vancomycin resistant in clinical isolates of coagulase – negative Staphylococci raises the worrisome possibility that resistant to vancomycin may yet emerge in S.aureus, emphasizing the need for the therapeutic alternatives to this antibiotic.

Nosocomial Methicillin Resistant Staphylococcus aureus isolates are mostly multi – drug resistant staphylococcus aureus is a community associated pathogen causing a wide range of disease, including endocarditis, osteomyelitis, toxic-stock syndrome, pneumonia, food poisoning and carbuncles [Savitha Nadiy etal .,2006] .

Drug resistant is the reduction in effect (Mesh, 2010). When the drug is not intended to kill (or) inhibit a pathogen . The term is used in the context of resistance acquired by pathogens (Daniel. etal.,2007).

When an organism is resistant to more than one drug it is said to be as multi drug resistant the condition enabling the disease causing organism resist distant drug (or) chemicals wide variety of structure and function targeted at eradicating the organism (Mash 2010).

Bacterial resistant to antibiotic various micro organisms have survived for thousands of years by the one able to adapt to antimicrobial agents (Bennot 2008). Microorganism employs several mechanisms in attaining multi drug resistant ,ex:- no longer relying on a glycoprotein cell wall , enzymetic deactivation of antibiotics etc., (Nikadio 2009).

Many different bacterium exhibit multi – drug resistantce such as staphylococcus aureus infection is penicillin-resistance is extremely common and first line therapy is most commonly a penicillinase – resistant b.lactum antibiotic oxacillin (or) combination therapy with gentamycin may be used to treat serious infections like endocarditis (Korzeniuslci etal.,1982) (Bayer etal.,1998)

Aim and objectives

Aim

To investigate the prevalence of Multi Drug Resistance Staphylococcus aureus isolated from patients having throat infection

Objectives

- Collection of clinical specimen
- Isolation and characterization of Staphylococcus
- Evaluating its percentage of antibiotic resistance

Materials and Methods

Isolation and identification of *staphylococcus aureus*

Collection of throat specimen; The mouth is held wide open and the tongue depressed. Swabs are firmly rubbed over the tonsils and pharyngeal mucosa, an attempt should made to collect any purulent material that is present

Throat swab culture; Throat swab is inoculated on to Blood agar, MacConkey agar, and Mannitol salt agar and DNase agar and incubated at 37^{o}C for 48hours. After incubation the colony morphology was observed.

Presumptive Staphylococcus aureus colonies were then subjected to gram staining and a series biochemical tests such as motility, oxidation and fermentation, catalase, indole production ,methyl red test ,voges proskaur test , and cultures which matched typical reaction of standard Staphylococcus aureus were confirmed as Staphylococcus aureus .

Antibacterial Susceptibility Testing

Disk Diffusion

Preparation of Mueller-Hinton Agar

Mueller-Hinton agar preparation includes the following steps.
Mueller-Hinton agar should be prepared from a commercially available dehydrated base according to the manufacturer's instructions.
Immediately after autoclaving, allow it to cool in a 45 to 50°C water bath.
Pour the freshly prepared and cooled medium into glass or plastic, flat-bottomed Petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm.
The agar medium should be allowed to cool to room temperature and, unless the plate is used the same day, stored in a refrigerator (2 to 8°C).
Plates should be used within seven days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimize drying of the agar.
A representative sample of each batch of plates should be examined for sterility by incubating at 30 to 35°C for 24 hours or longer.

Procedure for Performing the Disc Diffusion Test

Inoculum Preparation

Growth Method

The growth method is performed as follows

At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth.
The broth culture is incubated at 35°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours)

The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 x 10^8 CFU/ml for E.coli ATCC 25922. To perform this step properly, either a photometric device can be used or, if done visually, adequate light is needed to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

Direct Colony Suspension Method

As a convenient alternative to the growth method, the inoculum can be prepared by making a direct broth or saline suspension of isolated colonies selected from a 18- to 24-hour agar plate (a nonselective medium, such as blood agar, should be used). The suspension is adjusted to match the 0.5 McFarland turbidity standard, using saline and a vortex mixer.

This approach is the recommended method for testing the fastidious organisms, Haemophilus spp., N. gonorrhoeae, and Streptococci, and for testing Staphylococci for potential methicillin or oxacillin resistance.

Inoculation of Test Plates

Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.

The dried surface of a Müeller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60^o C, each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.
The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

NOTE: Extremes in inoculum density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inocula for streaking plates.

Application of Discs to Inoculated Agar Plates

The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface. Whether the discs are placed individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24 mm from center to center. Ordinarily, no more than 12 discs should be placed on one 150 mm plate or more than 5 discs on a 100 mm plate. Because some of the drug diffuses almost instantaneously, a disc should not be relocated once it has come into contact with the agar surface. Instead, place a new disc in another location on the agar.

The plates are inverted and placed in an incubator set to 35°C within 15 minutes after the discs are applied. With the exception of Haemophilus spp., Streptococci and N. gonorrhoeae, the plates should not be incubated in an increased CO_2 atmosphere, because the interpretive standards were developed by using ambient air incubation, and CO_2 will significantly alter the size of the inhibitory zones of some agents.

Results and Discussion

A total of 16 throat specimens were analyzed for detection of Staphylococcus aureus via culture on Blood agar, Mannitol salt agar, DNase agar and Biochemical tests. Out of 16 samples 10 samples shows positive results for Staphylococcus aureus .Colony morphology of isolates on selective media as follows Beta haemolytic colonies blood agar golden yellow colonies on mannitol salt agar. (Table1,2).

Table 1: Sample taken and presence of staphylococcus aureus

Sample	Sample	Result
S_1	Patient 1	+
S_2	Patient 2	+
S_3	Patient 3	+
S_4	Patient 4	+
S_5	Patient 5	-
S_6	Patient 6	+
S_7	Patient 7	+
S_8	Patient 8	-
S_9	Patient 9	+
S_{10}	Patient 10	-
S_{11}	Patient 11	+
S_{12}	Patient 12	+
S_{13}	Patient 13	-
S_{14}	Patient 14	
S_{15}	Patient 15	+
S16	Patient 15	-

Identification of the organism:

Table. 2 Preliminary Identification of Staphylococcus aureus

S.No	Test	Staphylococcus aureus
1	Mannitol salt agar	Yellow color colonies
2	Gram staininig	Gram positive ,cocci (spherical shaped)
3	Blood agar	β - heamolysis

Biochemical tests for identification of Staphylococcus aureus

The isolates from the clinical samples showed positive results for Methyl red test, Voges proskaur and Urea's tests .It showed negative results to Indole test and Citrate test .It suggests that the isolates are Staphylococcus aureus.

The results in Triple sugar iron test is the colour change to yellow and yellow in both slant butt region .Sugar fermentation test showed the result from Acid production without gas production .From the positive result of coagulase and catalase test ,it is clearly found that the 10 isolates are Staphylococcus aureus. (Table. 3).

Table: 3 Biochemical test for identification of Staphylococcus aureus

Sample No S.no	Inodole test	MR Test	VP test	TSI		Coagulae test	Citrate test	Urease test	Catalase test	Sugar fermentation
				Acid Production	H_2S/ gas production					
S1	-	+	+	y/y	-	+	-	+	+	A^+G^-
S2	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S3	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S4	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S6	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S7	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S9	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S11	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S12	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S14	-	+	+	Y/Y	-	+	-		+	A^+G^-
S15	_	+	_	Y/Y	-	+	_	+	+	A^+G^-
S16	_	+	_	Y/Y	-	+	_	+	+	A^+G^-

Antibiotic sensitivity tests for staphylococcus aureus

The 10 isolates obtained from clinical sample were made to undergo antibiotic susceptility test using commercially available antibiotics with the concentration.

The antibiotic discs used and the concentration (µg) present in the discs (Table .4) . The results of antibiotic sensitivity test for Staphylococcus aureus isolated from clinical samples (Table .5) .

The percentage of antibiotics, 81 and 100% resistant to Methicillin , Cefpodoxime, Penicilin-G, 72and 81% , sensitive to Gentamycin , Kanamycin and Vancomycin (Table. 6).

The obtained percentage of all 10 antibiotics plotted standard graph (fig .1) 10 positive isolates are subjected to antimicrobial sensitivity testing .Out of 10 positives 9 isolates are resistant to the methicillin only two isolate is sensitive to the methicillin

Table 4: Concentration of Antibiotics

S.No	Antibiotic	Symbol	Disc concentration(µg/disc)
1..	Amoxyclav	AC	30
2.	Ampicillin	A	10
3.	Bacitracin	B	10
4.	Cefpodoxime	CEP	10
5.	Kanamycin	K	30
6.	Gentamycin	GEN	10
7.	Methicillin	M	30
8.	Novobiocin	NV	30
9.	Pefloxacin	PF	5
10.	Penicillin-G	P-G	10
11.	Tobramycin	TB	10
12.	vancomycin	VA	30

Table; 5 Results of Antibiotic sensitivity tests for staphylococcus aureus isolates

sample	AC	A	B	CEP	K	GEN	M	NV	PF	P-G	TB	VA
S_1	R	R	I	R	S	S	R	R	I	R	I	S
S_2	R	R	R	R	S	I	R	R	I	R	R	S
S_3	R	I	R	R	S	S	R	R	R	R	R	S
S_4	R	I	R	R	R	S	R	R	R	R	S	S
S_6	R	R	R	R	I	S	I	R	R	R	R	S
S_7	R	R	R	R	S	S	R	R	I	R	R	I
S_9	R	I	R	R	S	S	R	R	S	R	R	S
S_{11}	I	I	R	R	S	I	R	R	S	R	I	S
S_{12}	R	R	I	R	I	I	R	R	I	R	R	I
S_{14}	R	R	R	R	S	S	I	R	R	R	I	S
S_{15}	I	R	I	R	S	S	R	R	R	R	R	S
S16	I	R	I	R	S	S	R	R	R	R	R	S

R- Resistance; I-Intermediate; S- Sensitive

Discussion

Methicillin resistant Staphylococci are significant pathogens causing both nosocomial and community acquired infections. High prevalence of methicillin Resistant Staphylococcus aureus (MRSA) in hospitals has been reported from many states of India.(Rajaduraipandi.etal .,2006) Methicillin resistance among S. aureus isolates has reached phenomenal proportions in Indian hospitals, with some cities reporting 70% of the strains to be resistant to methicillin. In the last few years sensitive molecular typing techniques are leading the way to track the source and transmission route of bacterial pathogens. They have also helped in establishing epidemiological investigations and comparing strains across continents. Detection of mecA gene by PCR has been shown to be highly discriminatory in analyzing hospital outbreaks and tracking genetic changes which occur in a relatively short time. The aim of this study was to validate the multiplex polymerase chain reaction (mPCR) technique in Indian isolates by which India specific data will be of immense benefit for the optimal application in our patients.(Rallapalli et al . 2008)

Beta-lactam antibiotics are the preferred drugs for serious S.aureus infections.Since the introduction of methicillin into clinical use, the occurrence of MRSA strains has increased steadily and nosocomial infections have become a serious problem worldwide. Indiscriminate use of multiple antibiotics, prolonged hospital stay, intravenous drug abuse and carriage of MRSA in nose are all important risk factors for MRSA acquisition.(Chin etal.2005). In addition, MRSA infected patients require expensive and intensive isolation measures and strict hygiene. To date, the only standardized means of identifying methicillin resistance in the clinical microbiology laboratory are susceptibility tests such as disk diffusion, agar or broth dilution and agar screen methods. The performance ofthese tests has many drawbacks because factors such as inoculums size, incubation time and temperature, pH of the medium, salt concentration of the medium and exposure to beta lactam antibiotics influences the phenotypic expression Of resistance. Rapid and accurate identification of S.aureus and its methicillin susceptibility pattern has important implications for therapy and management of both colonized and infected patients.

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