Isolation, Characterization And Molecular Diagnostics of Satphylococcus From Catheterized Patient

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Abstract- *Staphylococcus species mainly S. aureus is a causative agent of catheterized infection, but sometimes other than S. aureus like CONS (coagulase negative staphylococci), P. aeruginosa, K. pneumoniae and candida spp. Catheterization is the essential modern medical practice which is used for administration fluids, IV medication, injections and in case of blood transfusion blood delivery. S. aureus is normal flora of skin or performed biofilm formation. At the time of insertion S. aureus can enter into human body then cause infection. sample should be taken from peripheral blood, lumen of the catheter and tip of the catheter. Identification tests are culturing , gram staining, bio chemical testing and molecular testing. Drug susceptibility can be check via AST. The prevention is the main factor to reduce morbidity or mortality like antiseptics or antibiotic should be used with the catheter. Now that time the antibiotic lock therapy should be used.*

Keywords- Biofilm, Catheters,S.aureus,iatrogenic infection, Antibiotic lock therapy

I. INTRODUCTION

Intravenous catheters are essential to modern medical care but frequently cause complications, the most important of which is infection, commonly due to Staphylococcus aureus and coagulase negative staphylococci. Staphylococcus aureus is a gram positive cocci present as a normal flora of human body. Intravascular catheters and urinary catheters are the 2 most commonly inserted medical devicesand they cause blood stream and UTI respectively..Unfortunately, indwelling devices significantly increase the risk of iatrogenic (infection transmitted during medical treatment or care) infection. Indwelling medical devices that are prone to staphylococcal biofilm infection include stents, ventilators, intravenous catheters, invasive blood pressure units, infusion pumps, cardiac defibrillators, mechanical heart valves, pacemakers, stitch materials, and orthopedic devices.

Biofilm formation on the surfaces of indwelling catheters is central to the pathogenesis of infection of both types of catheters. The attribute mortality of these bloodstream infections is 12% to 25%.[3] In contrast, the mortality rate of catheter-associated UTI is less than 5%.

On the basis of sites and uses catheters may be of following types:-

Intravenous catheter, Cardiac catheter, Urinary catheter, Indwelling urinary catheter, Intermittent urinary catheter and Central venous catheter.

Antibiotic therapy for catheter-related infection is often initiated empirically. The initial choice of antibiotics will depend on the severity of the patient's clinical disease, the risk factors for infection, and the likely pathogens associated with the specific intravascular device.Vancomycin is recommended for empirical therapy for methicillin resistant *Staphylococcus aureus*; for vancomycin minimum inhibitory concentration values >2 μg/mL, alternative agents, such as daptomycin, should be used. Linezolid should not be used for empirical.
Antibiotic lock therapy should be used for catheter salvage; however, if antibiotic lock therapy cannot be used in this situation, systemic antibiotics should be administered through the colonized catheter.

II. MATERIAL AND METHOD

Required material:-

Nutrient Agar, Blood Agar, Petri Plates, Slide, Cotton, Sprit, Bunsen Burner, Inoculation loop, Gram staining kit, MSA Agar, Antibiotic Disk, Test tubes, 3% Hydrogen Peroxide, Peptone Water.

Specimens:- Peripheral blood , lumen of the catheter, tip of the catheter, insertion part of catheter.

No. of sample:- 8 We collect samples from 8 different catheterized patients from U.H.M. hospital Kanpur, U.P. India Where as 6 is positive and 2 is negative

Sample should be identified through various methods like **Culturing, Gram Staining, Bio chemical Testing and molecular diagnostics.**

First of all we used routinely used media for growth of sample like nutrient agar media , blood agar media and macConkey agar media. On nutrient agar media sample inoculated via streaking method and incubated for 18-24 hours at least over night. Next day I see colony characters.

On blood agar 5 ml sheep blood with 95 ml of nutrient agar media, shaking slowly, pour into plate after the solidification sample will be inoculated. Then incubated in incubator for 18-24 hours at least over night at 37^0c. next day shows colony characters. Three types of colonies alpha, beta and gamma should be observed.

Then I perform gram staining and it shows two types of staining property: 1^{st} is gram positive and 2^{nd} is gram negative.

After this I used selective media for specific growth of microorganism, for staphylococcus MSA(mannitol salt agar) media should be used. If the bacteria turn into yellow color means bacteria is s. aureus.

After this I perform bio chemical test, which is a confirmatory test for the identification of sample, biochemical test like catalase, coagulase, oxidase and nitrate.

Then we performed molecular diagnostics best method is PCR to identify sample.Polymerase chain reaction **(**PCR**)** is a common laboratory technique used to make many copies (millions or billions) of a particular region of DNA. PCR is used in many areas of biology and medicine, including molecular biology research, medical diagnostics, and even some branches of ecology

For molecular diagnosis following steps-

To extract genomic DNA from bacterial cells. Demonstration of polymerase chain reaction PCR. and Amplified DNA demonstration in gel electrophoresis.

Antibiotic sensitivity test:- Kirby - Bauer method Small wafers containing antibiotics are placed onto a plate upon which bacteria are growing. If the bacteria are sensitive to the antibiotic, a clear ring, or zone of inhibition, is seen around the wafer indicating poor growth. Mueller - Hinton agar is frequently used in this antibiotic susceptibility test.

III. RESULTS

I had taken 8 sample of catheterized patient .In that 6 samples are isolated ,Staphylococcus aureus was identified in the samples after biochemical testing and for those sample I had done AST.

TABLE 1:- SAMPLES GROWN ON DIFFERENT TYPES OF AGAR

AGAR	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8
NUTREINT AGAR	G	G	NG	G	G	G	NG	G
MANNITOL SALT AGAR	G	G	NG	G	G	G	NG	G

WHERE AS:- S= SAMPLE, G= GROWTH, NG= NO GROWTH

TABLE 2:- ANTIMICROBIAL SUSCEPTIBILITY TESTING ON SAMPLE

SAMPLE	Amoxicillin	Chloramphenicol	Gentamycin	vancomycin	Methicillin
1.	18mm	25mm	26mm	24mm	0mm
2.	27mm	30mm	29mm	30mm	0mm
3.	16mm	21mm	22mm	19mm	0mm
4.	14mm	24mm	17mm	18mm	0mm
5.	17mm	21mm	20mm	18mm	0mm
6.	24mm	14mm	23mm	21mm	0mm

IV. CONCLUSION

The catheter contains primarily those bacterial genera found in the normal skin and those microorganism forms biofilm on the surface of catheter however, staphylococcus aureus, p. aeruginosa, Coagulase negative staphylococci(CONS),E.coli, K. pneumonie and candida spp. In 8 samples out of which six were positive(i.e 80%)which swhows high prevealence of catherised infection from stap. in hospitals. For confirmation and identification culturing of the samples, gram staining, biochemical test and molecular diagnostic(PCR) were performed . For future suggestion of curing several antibiotics were used to check drug sensitivity but the gentamicin, amoxicillin and chloramphenicol is mainly affected.Early diagnosis and treatment are vital to reduce the morbidity and mortality involved. New technologies for prevention of infections, which have been shown to reduce the risk of CRBSI, including catheters and dressings impregnated with antiseptics orantibiotics, new hub models and antibiotic lock solutions, are in use. So at the end this study concludes that gentamicin, amoxicillin and chloramphenicol are the drug of choice.

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