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Prospective Study Of Isolates In Pyogenic Samples

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ABSTRACT

The advancement of specific automated systems in microbiology lab has increased the prognosis of treatment for various microbial diseases that were uncured during past. Various automated systems like VITEK 2, microscan make investigation criteria in simple and easy way such that microorganism specific for there biochemical tests are observed under computer operated device which makes the reporting quick and unbiased. In our medical center, we have diagnosed many patients with pyogenic infection[1]. Our aim was to study the isolates that were isolated from consecutive pyogenic samples coming in the hospital. We performed a prospective review of the records of the 70 unselected patients coming with pyogenic infection. We proceed the samples manually along with automated systems. Initial staining and culturing was done on BA, CA, MA which were later observed twice after 24hrs and 48hrs[2]. There was significant change in positivity of sample in case of difference in age and gender. According to the observations carried out by us that percentage of positivity increase with increase of age and with every male gender. Staphylococcus aureus was the the majorly identified organism in study of consecutive pyogenic samples[3]. Various anti toxins, enzymes, anti immune strategies of this organism were studied that are helpful for this bug to cause disease. The evaluations also included an assessment of risk factors, antimicrobial therapy and manual diagnosis methods. By Universal infection-control measures, the transmission of infections may be limited, patient education, screening and decolonization[4].

Keywords-Bacteuria,Bacteremia,Pyogenic

1. **INTRODUCTION**

The term pyogenic drives from the word pus or abscess.Any infection that produce pus in the tissue or organ is known as pyogenic infection. For infection to takes place we require 3 set of criteria. 1) Micro-organism that is capable of invading inside tissue and can do lysis of tissue or organ. 2) Suseptical host in which micro organism may reproduce and fullfil all its requirements of nutrition. 3) Route of exit from which organism may comes out of body to transmit into other host[5].

Pyogenic infections are generelly non fatal if present in case of superficial surfaces of skin and dermis.Fewer cases like liver abscess, brain abscess are can lead to death of the patient if not being properly treated. These kind of infections are prominently cause by Gram positive organisms mostly *Staphylococcus* spps. Now a days many Gram negative bacteria also lead to pyogenic infection[6]. Data from National Nosocomial Infections Surveilllance system suggests that isolation of Staphylococcus aureus has been increased from 35.9% to 64.4%

from pyogenic samples. These kind of infections are mostly treated well by various antibiotic regimens. Ina general population, pyogenic infection mean rate is 37% but the patient with insulin dependent diabetes mellitus are showing more significantly higher carriage rates[7]. And moreover patients with HIV, in dialysis, intravenous drug users.

Relapse vs. Reinfection

Recurrent UTI may be either a relapse, or a reinfection. Same bacterial strain may leads to relapse UTI. Escherichia coli are the most common infectious organism. S. saprophyticus, K.pneumoniae and P.mirabilis. [8].

However this problem is current and depicts an everlasting hazard even to hospitalized patient in the majority modern hospitals. All organisms can be the causative agents of infection but most often there is involvement of bacteria. Depending on the use of antibiotics and introduction of new diagnostic and therapeutic procedures, the type of microbes change that cause hospital infection [9].

Biofilm formation is associated with *Pseudomonas aeruginosa* in cystic fibrosis pneumonia, *Staphylococcus aureus* in chronic rhinosinusitis. Microbial communities linked with the substrate, surrounded by an extracellular polymeric substance (EPS) layer are known as biofilm formation. This is found primarily in constant diseases. [10]. *Klebsiella pneumoniae* is an important core of infection because of multiple antibiotic resistances. Patients with longer stays and greater experience with antibiotics should be latent target for severe infection [11].

It caused by urinary catheter-related infection. Majorly present bacteuria in symptomatic catheterized patients and most frequent isolate is *E.coli.* Diabetes is also a most common factor that may lead to UTIs in the catheterized patients the ratio is 44% [12].

E.coli was the first and usual common pathogen causing urinary tract infection. According to sex bacteria also vary that may lead to UTI. Majorly E.coli is the one who cause half of the infection of urinary tract. This is just because of their resistance to antibiotics was little when it is evaluate with any other pathogens like *Klebsiella and Pseudomonas spp*. which was implicated in UTI, along with this it also having the lowest percentage of multidrug resistant (MDR) isolates[13].

Bacteuria was the mainly common isolate within all suggestive catheterized patients. Diabetes (44%) was also the most common factor in catheterized patients diagnosed with UTI [14]. Knowledge of these factors and simple preventive measures, such as proper hygiene, can restrict disease occurrence. Our infection management relies on adequate antibiotic therapy [15].

2. METHODOLOGY AND METHODS

Collection and Transport ofspecimen

There are different methods to collect pus on the basis of the diagnostic requirement.

However, the most useful method is swab method. This was the initial step of diagnosis criteria.

Swabmethod

We use cotton swab to collect the pus sample from the site. Sterile cotton swab taken first and then it is gently rubbed on the site. Swab is directly cultured on to media which is later incubated for 48hrs. Direct smear or staining is also done to check the Gram positive or negative character of causative organism.

Aspiration

In case of deep pyogenic infection when there is no site for pus to ooze out of the tissue, then aspiration technique is used. Initially sterilization of site is done by alcohol. Puncture is done in the tissue with the help of fine bore needle. Aspiration is done slowly and sample is taken out. Then, the sample is drawn into the universal container with the help of clean needle andsyringe.

Diagnosis

Microscopy and culturing are the most simplest method that are carried out for the investigation of the micro organism. Samples are collected first then being cultured in the media. Obesrvation is done after 48hrs duration. Growth is examined carefully because every organism growth pattern is different from each other. Staining techniques also give revolution in the diagnosis criteria. Now adays computer operated automation system makes the isolation of microorganism quite easier than before.

Most skin and soft tissue infections are diagnosed clinically.Cardinal signs of an SSTI comprise erythema, edema, tenderness to palpation, and increased warmth. Some signs are dependent on the depth of infection such as fluctuance, crepitus, induration, blisters, Some symptoms like fever chills are sign of high infection.

A careful travel and environmental revelation history should be elucidate, as certain pathogens are associated with specific geographic locales. Examples take in*Pseudomonas aeruginosa* acquired from hot tubs, *Vibrio vulnificus* and *Mycobacterium marinum* from water exposure, and *Pasteurella multocida* and *Capnocytophaga canimorsus* from animal bites. A detailed background will also decide whether the patient has been hospitalized recently, as this may put the patient at risk for multidrug-resistant species. It is important to consider complications of necrotizing soft tissues. Induration, rapid expansion, and crepitus of the tissues affected, fever, hypotension, and pain out of proportion to clinical findings indicate necrotizing fasciitis, which would prompt surgical evaluation.It is generally not helpful to obtain antibody serology for suspected streptococcal infections when diagnosing superficial SSTIs, as this is a localized process and systemic antibodies are not made.

Transport:

The sample must be transported at room temperature within half an hour. Or it should be refrigerated at 4 degree Celsius for up to four hours. The sample should not be proceeding after 4 hours for bacterial culture. Because there may be multiplication of contaminating bacteria can occur and give a false positive result.

Procedure/Method:

The following are the different approaches to diagnosis of pyogenic infection in the laboratory:

Culturetechnique:

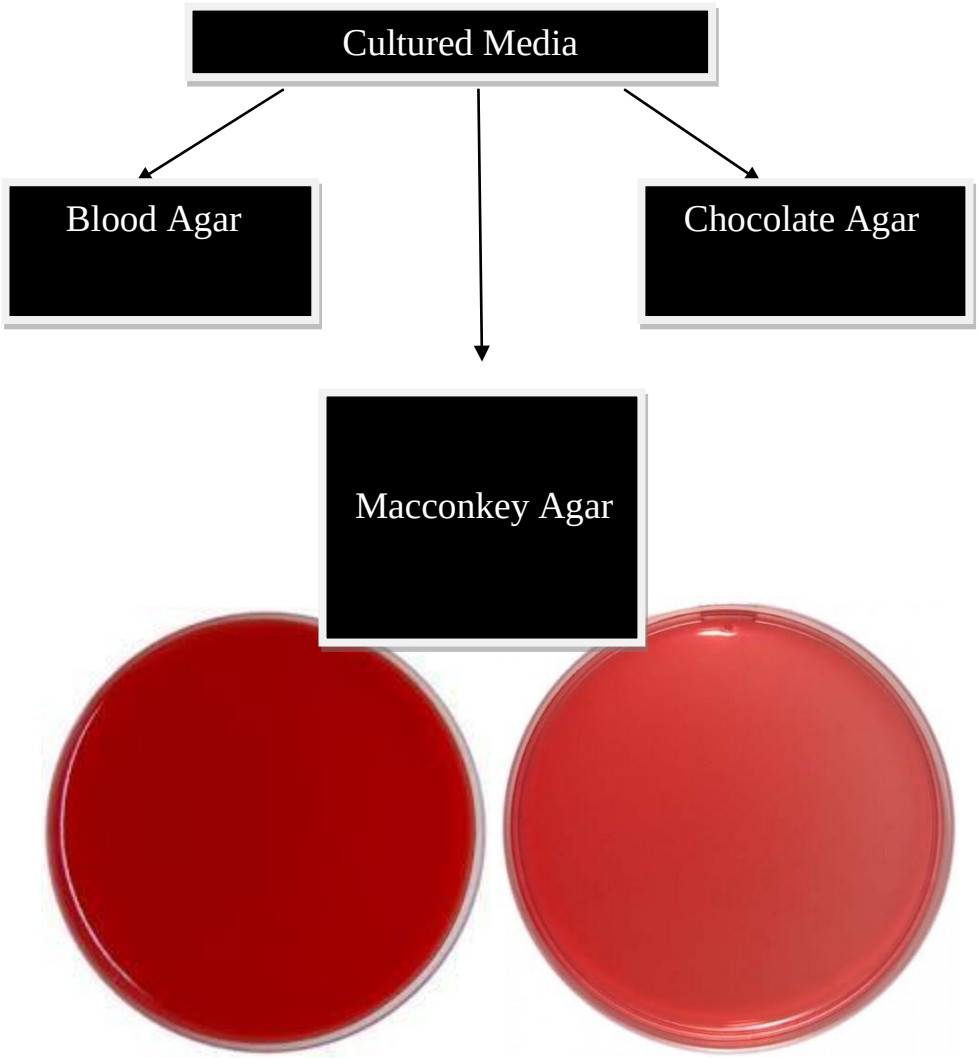
Principle- Pus is generally composed of causative micro organism, dead and decay tissue elements ,huge amount of unsurvived neutrophills and other immunogenic cells. If we provide it same environment and conditions just like in human body, the causative organism will grow on medium within 48hrs.

Procedure-

1. 100 samples of pus were collected randomly from the patients of EscortHospital.
2. Samples were collected in sterile and screw capped containers in case of deepinfections.
3. In case of superficial infection cotton swab were used forcollection.
4. We use three type of culturemedia.

(a) In case of swab, sample is directly rubbed over the media by streakingmethod.

(b) In case of sample in container, sample is inoculated on media with the help ofplastic loop having calibration of 0.5mmdiameter.



Observations

In the training period of four months, about 63 samples are processed. Out of which 45 are found positive. Here is the observations that are carried out in training period. Theses observations are carried out manually and by one of the most advanced automated system in microbiology branch

i.**e VITEK 2**. Detail of the isolations that were observed in pus samples is given below:

Plates are incubated at 37 degree C for 24 hours and colony formation units werecounted for the presence ofbacteria.

Acc. No./ID	Age/sex	Ward	Type of organism	No of sensitivity
0326	62/M	OT	Sterile	00
0677	71/M	IPD	S. aureus	01
0690	31/F	OT	E. faecalis	01
0685	52/F	CC	S. aureus	01
0995	73/M	ICU	A.baumanii	01
1255	50/M	OT	E. coli	01
1304	59/M	IPD	E. coli	01
1324	23/M	CC	S. aureus	01
1730	70/F	OT	K. pnemonie P. aeroginosa	02
2086	68/F	OT	E. coli E. facalis	02
2397	68/F	OT	E. coli	01
2449	68/F	ICU	Sterile	00
2813	58/M	OT	Sterile	00
3066	65/M	ICU	Sterile	00
3088	68/F	ICU	K. pnemonie A. baumanii	02
3089	65/F	ICU	A.baumanii Proteus mirabilis	02
3098	37/M	CC	A.baumanii P. aerogenosa	02
3251	63/M	IPD	E. coli	01
3907	75/M	CC	S. aureus	01
4349	23/F	CC	S. aureus	01
4880	58/M	OPD	Sterile	00
4992	60/F	OPD	S. aureus	01
5872	41/M	CC	S. aureus	01
6080	55/F	CC	Sterile	00
6092	55/F	ICU	Sterile	00
6818	73/M	OPD	S. aureus	01
6948	58/M	OPD	Sterile	00
6834	68/M	ICU	Sterile	00
7017	49/F	IPD	P. aeroginosa	01
7516	62/F	IPD	K. pnemonie P. aeroginosa	02
7634	70/F	OT	S. aureus	01
Acc. No./ ID	Age/ sex	Ward	Type of organism	No. of sensitivity

0419	-	OT	K. pnemonie A. baumanii	02
0448	-	OPD	S. aureus	01
0677	-	IPD	Sterile	00
1358	-	ICU	Sterile	00
1438	-	OPD	Proteus	01
1413	-	OPD	S. aureus	01
2297	-	ICU	E. coli	01
2332	-	OT	K. pnemonie A. baumanii	02
2966	-	OT	Sterile	00
3333	-	CC	E. coli	01
3930	-	-	S. viridians	01
3961	-	CC	K. pnemonie	01
4471	-	OPD	S. aureus	01
5032	-	IPD	Proteus	01
5102	-	CC	S. aureus	01
5114	-	OPD	E.coli	01
5653	-	OT	S. aureus	01
6071	-	OPD	Sterile	00
6552	-	CC	Sterile	00

Staphylococcus aureus :
Colony characters:

- Individual colonies on agar are round, convex, pin head and 1-4 mm in diameter with a sharp border and golden yellow or creamy incolour.
- On blood agar plates, colonies of *Staphylococcus aureus* are frequently surrounded by zones of **clearbeta-hemolysis**.

Biochemical tests:

- Catalasepositive
- Coagulasepositive

Klebsiella pnemonie :

Colony characters:

Lactose fermenting, pink coloured, mucoid colonies due to production of large number of capsular
Biochemical tests:

Indole	Negetive
Methyl red	Negative
VP	Positive
Citrate	Positive
Oxidase	Negative
Urease	Positive

TSI	A/A gas +ve

Klebsiella pnemonie :

Colony characters*:*

Lactose fermenting, pink coloured, mucoid colonies due to production of large number of capsular material.
Biochemical tests:

Indole	Negetive
MR	Negative
VP	Positive
Citrate	Positive
Oxidase	Negative
Urease	Positive
TSI	A/A gas +ve

E. coli :

Colony characters:

Circular, small, punctiform, smooth, raised margins, pink coloured, lactose fermenting colonies.

Biochemical test:

Indole	Positive
Methyl red	Positive
VP	Negative
Citrate	Negative
Urease	Negative
TSI	A/A gas +ve

Pseudomonas :

Colony characters:

Small, rough colony, surface is wrinkled, translucent, pigmented, non lactose fermented

Pyocyanin

Bluish green phenazinepigment

Pyoverdin(fluorescin)

It is a greenish yellowpigment

Pyorubin

Reddish brownpigment

pyomelanin
Brown to black pigment

Biochemicaltest-

Indole	Negative
Methyl red	Negative
VP	Negative
Citrate	Positive
Urease	Negative
Oxidase	Positive
TSI	K/K gas –ve

Colony characters:

Small, opaque, non lactose fermenting, having typical swarming growth pattern due to presence of multiflagella and produce very distinct fishy smell
Biochemical tests:

Indole	Positive
Methyl red	Positive
VP	Negative
Citrate	Negative
Urease	Positive
Oxidase	Negative
TSI	A/A gas +ve

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Results:

Parameters	January	February	March
Total no. of samples	31	12	20
Sterile samples	09	03	06
Positive samples	22	09	14
Staphylococcus	10	05	05
Klebsiella	03	01	02
E. coli	05	01	03
Pseudomonas	04	05	00
Enterococcus	02	01	00

Total number of samples	63
Total number of positive samples	45
Number of most commonly identified bacteria	20

Total number of positive samples taken from inside the hospital	26
Total number of positive samples coming from outside the hospital	19

Total number of patients above age 50	< 30
Total number of patients above below age 50	>15

3. DISCUSSION:

Staphylococcus aureus has emerged in many areas of Punjab as the most common recognizable cause of skin and soft-tissue infections including deep lesions. As about 50.0 per cent of patients with Staph-related pyogenic infections.In this study received empirical therapy. Rapid and automated technology for isolation of bacteria has become a routine practice in resource limited settings for initial screening.

The objective of this study was to gain prospective knowledge about the pyogenic infections, its causative agents, pathogenesis, treatment and new advances in the diagnosis criteria in field of microbiology. 63 random samples were taken received in Fortis Escorts Hospital Amritsar were evaluated for isolation porposes under manual and automated techniques like VITEK 2. In which 72.0% were found positive under culture method and rest were sterile. All type of organisms including Gram positive and Gram negative were found in the isolation technique but SA was most common etiological agent isolated from samples. Percentage of SA isolation in these samples was around 50.0%. Another important data that was observed during this period was out of 45 positive cases 26 were taken from inside the hospital, this means around 60.0% samples were taken froms patients that were admitted in hospital. This data indicates that there was false positive indication for the presence of nosocomial infection because staphylococcal infections are very common in health care community populations and also has more chance of transmission from person toperson.

Pyogenic infections has also relation with the age of the person. As from the above data more than 30 patients were more than the age of 50 that were found positive, results in 70% of patients were found +50 that were infected. This finding indicates that prevalence of this infection increase as age increase because as age increase immunity will surely decrease. Various risk factors also play role in having pyogenic infection that include compromised immune response in case of HIV, person on antibiotic therapy, person with diabetes have more chance for accumulation of pus because of presence of more glucose in blood which increase the growth of bacteria at site of injury. Although along with superficial infections, deep infections are more prone to morbidity if left untreated. In time of antibiotic era every person should get proper awareness and treatment which can save the life of aperson.

4. CONCLUSION

Infections due to *S.aureus* are very common and SA continues to be a serious and formidable challenge to health care providers as their prevalence is reported to be increasing exponentially. In the past, SA infections were reported mostly from hospitalized patients but now they are encountered in community settings as well. Understanding the types of *S. Aureus* infections, their pattern and distribution, as well as the factors associated with their spread are of paramount importance for its management and control.

Staphylococcus aureus infection is also an significant cause of multisystem involvement in

disseminated infection. This may either be associated with health insurance or obtained from the group. In these cases, early diagnosis and prompt initiation of the reactive antimicrobial agent can prevent mortality & morbidity. It is not clear that simple superficial abscesses are associated with systemic manifestations of infection unless there are other factors such as immune suppression, diabetes or misuse of injected drugs

Empiric therapy with β-lactam drugs may no longer be adequate for treatment now that MRSA strains are being identified more frequently as the causative agents for SSTIs.To avoid care errors and to prevent improper use of antibiotics, it is critical for physicians to have working knowledge of the local antimicrobial susceptibilities.

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