Research Paper

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Study of Processing of Pus for Culture and Sensivity Testing in Bacteriology Laboratory

Medical Science

KEYWORDS : Pus culture, Aerobic, Bacterial profile, GNB, Neutrophils.

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ABSTRACT

Pus often results from an infection and is usually made up of dead white blood cells, debris from other damaged cells and tissue and bacteria. Cells called neutrophils can kill the invaders, engulf and destroy the bacteria and the bacteria resist the immune response by releasing toxins called leukocidins. These are often cocci, which are spherical organisms with thick cell walls, and include various Staphylococcus and Streptococcus varieties. In the above context, study was conducted to understand the pyogenic infection by various species and their sensitivity to antibiotics. The study was done over a period of four months. A total number of 152 randomly selected pus samples received by the bacteriology section of microbiology department, from various wards of PIMS Hospital. Result reveals that S. aureus was reported to be the commonest pathogen. The study concluded that incidence of pus infection is expected to be highest in wards housing post-surgical patients. When empirical treatment was intended, GNB may also be expected as the most probable causative agent.

INTRODUCTION

Pus is an exudate, typically white-yellow, yellow, or yellow-brown, formed at the site of inflammation during infection [1]. An accumulation of pus in an enclosed tissue space is known as an abscess, whereas a visible collection of pus within or beneath the epidermis is known as a pustule or pimple. Pus often results from an infection and is usually made up of dead white blood cells, debris from other damaged cells and tissue, and bacteria. Cells called neutrophils can kill the invaders, but are often killed in this process. There, the neutrophils engulf and destroy the bacteria and the bacteria resist the immune response by releasing toxins called leukocidins[2]. As the neutrophils die off from toxins and old age, they are destroyed by macrophages, forming the viscous pus.

Bacteria in pus are therefore sometimes called pyogenic. These are often cocci, which are spherical organisms with thick cell walls, and include various Staphylococcus and Streptococcus varieties.

The overall incidence of wound sepsis in India is from 10 to 33%. Western studies indicate this range to be between 3 to 10%, with an average of 5%.In almost all cases when there is a collection of pus in the body, the clinician will try to create an opening for it to evacuate.

Pyogenic bacteria causes pus:-

- Staphylococcus aureus
- Staphylococcus epidermidis
- Streptococcus pyogenes
- Escherichia coli
- Streptococcus pneumonia
- Klebsiella pneumoniae (Friedländer's bacillus)
- Salmonella typhi
- Pseudomonas aeruginosa
- Neisseria gonorrhoeae
- Actinomyces
- Burkholderia mallei (Glanders bacillus)
- Mycobacterium tuberculosis (tubercle bacillus)

Commensals:-

- Alpha heamolytic streptococci
- Corynebacterium spp.
- Coagulase negative staphylococcus
- Propionobacterium spp.
- Bacillus spp.

REVIEW OF LITERATURE

Poonam Verma (2012)[3] a study conducted on microbiological analysis of infection in 245 patients was undertaken in the out-door patient departments (OPD) of medical college and a hospital, Raipur (C.G.) in 2005. Identification of bacterial isolates was determined. A total of 116 bacterial isolates were obtained from different cultures. In 86 cases, cultures were monomicrobial, 16 cultures were polymicrobial but no bacterial isolate was obtained in 143 cases. Out of 102 patients both female and male suffered but number was different 46 and 56 respectively. In both male and female *Staphylococcus aureus* was the most frequently isolated microorganism (40%) followed by *Klebsiella* sp. (33%), *Pseudomonas* sp. (18%), *Escherichia coli* (16%) and *Proteus* sp. (7%). The *Staphylococci aureus* were the predominant Gram positive organism (40%) because these are present in worldwide in all over environment and the family *Enterobacteriaceae* constituted 60% of the total number of isolates.

VG Bhat & SD Vasaikar (2010) [4] a study was conducted in South Africa to determine the common aerobic bacterial isolates in burn wounds. The total number of patients in the study was 243, of which 129 (53.1%) were males and 114 (46.9%) females. About 131(53%) of the patients were pediatric (up to 18 years) and the remaining 112 (47%) were adults. A total of 229 patient specimens showed growth on culture. The total number of isolates was 629, out of which 269 were Gram-positive cocci and 360 were Gram-negative bacilli. The commonest organism was *S. aureus* (27.7%), followed by K. pneumoniae (13.4%), Proteus mirabilis (12.4%), Group D *Streptococcus* (9.4%), P. aeruginosa (8.9%) and E. coli (6.2%).

A survey was conducted in logos Nigeria, to determine the prevalence of Pseudomonas aeruginosa in Postoperative wound. Swab samples were collected from patients who had undergone operation, sinks, washbasins, floor and nursing staff. Out of the 60 bacterial isolates found in postoperative wound infection, 20 (33.3%) were Pseudomonas aeruginosa, followed by Staphylococcus aureus13 (21.7%), Klebsiellaspecies 10 (16.7%), Escherichia coli 7 (11.7%), Atypical Coliforms 4 (6.7%), Proteus species 4 (6.7%), Streptococcus pyogenes 1(1.7%) and Enterococcus faecalis1 (1.7%) (Oguntibeju OO., et al. 2004)[5].

MATERIAL & METHODS

OBJECTIVES:

- To isolate the microbial species in pus
- To obtain the sensitivity of antibiotics to microbial species

PROCEDURE

The study was done over a period of four months. A total of randomly selected 152 pus samples received by the bacteriology section of microbiology department, from various wards of PIMS Hospital, were processed. Standard diagnostic procedures were followed, using media and stains approved in the laboratory for

aerobic culture.

Relevant baseline information of the patient was noted. . Internal quality control methods were observed to ensure correctness of the results. The data thus created was documented and analyzed.

Processing of specimen

Collection of pus:

General principles:

Successful collection of specimens will depend on the following:

ü Collection at the appropriate time
ü Use of the correct technique
ü Use of the correct equipment
ü Safe transportation to the laboratory without delay

Specimens of pus from various sites were received on swab from patients in Out Door Department (OPD), and indoor form tertiary care hospital. The swabs were then transferred into suitable sterile test tubes and closed immediately.

Fluids like cerebrospinal, pleural, ascitic, pericardial, and synovial fluid etc were collected under aseptic conditions and transferred into suitable sterile containers and closed tightly by the wards and received in the laboratory. Each sample was accompanied by properly filled laboratory form which had the following details such as name of patient, age/sex, hospital number, site from where sample was taken, date and time of collection of specimen and name of the doctor attending the patient.

- Where there are clinical signs of infection i.e. inflammation, oedema, pyrexia,pain or purulent exudate, it is preferable to obtain a specimen of pus rather than to take a swab.
- Pus or exudate can be drawn up in a syringe and transferred to either auniversal container.
- Taking a Tran swab (blue top), remove the swab and gently but firmly rotation the surface directly where infection is suspected.
- Do not take swabs from slough or necrotic tissue.
- Place swab into transport medium.
- Ensure that the specimen containers are labeled accurately and place, with the completed request form, in the appropriate pockets of the clear mini grip transport bag for transportation to the Department of Medical Microbiology

PROCESSING FOR CULTURE

The processing of pus for culture is done by using various following ways;

1. Specimens were routinely processed by smearing the swab immediately by streaking on Blood and MacConkey agar. Samples of CSF were also inoculated on Chocolate agar. Plates were placed in the incubator at 37 C for 24 hours. A smear was also prepared directly from the swab to check the presence or absence and types of organisms.
2. Morphology of colonies was studied followed by Gram stain.

Details of media used

1. **MacConkey agar-** It is both selective and differential media. It contains bile salts and the dye crystal violet, which inhibit the growth of gram-positive bacteria and selective for *gram-negative bacteria.* **It also contains the carbohydrate lactose, which allows differentiation of gram-negative bacteria based on their ability to ferment lactose.** Lactose fermenter bacteria (Pink coloured colonies) & Non-lactose fermenter Bacteria (Pale or colourless colonies).
2. **Blood agar-**This media is an enriched and differential media used to culture and isolate fastidious **organisms. Blood agar uses a nutritionally rich medium with whole blood cells added. It differentiates the organisms on the basis their** hemolytic activity (breakdown of red blood cells).

- **Alpha hemolysis**:- colony is greenish due to incomplete hemolysis. Streptococcus pneumoniae **and** streptococci (Streptococcus viridians) causes alpha hemolysis where hemoglobin gets converted to green methemoglobin.
- **Beta hemolysis :-** appears transparent due to complete hemolysis of red cells on culture media. Streptococcus pyogenes, or Group A beta-hemolytic Strep (GAS), displays beta hemolysis.
- **Gamma hemolysis:-**organism does not induce hemolysisin agar and remains unchanged (this is also called non-hemolytic). Enterococcus faecalis **(formerly called Group D Strep) displays gamma hemolysis.**

Gram staining- Gram staining was performed to identify the organism obtained on culture media after the incubation. It is a type of staining used to differentiate bacteria of various groups (Gram positive and gram negative).

Method & Preparation.

1. Clean and dry glass slide was taken.
2. Smear is prepared by rubbing swab on the centre of slide.
3. In case of fluid materials one loopful of fluid material was taken with inoculating loop and spread evenly on the centre of the slide about 2-2.5cm in diameter.
4. Then slide was kept on table and allow it to dry.
5. After this smear was heat fixed by gently passing over flame 2-3 times.

Procedure-

1. Slide was placed on rack so that smear was facing upwards.
2. Then crystal violet was poured over the smear for 1 min.
3. Then gently washed with tap water.
4. Then covered with Gram's iodine and allowed to stand for 1 min.
5. Then gently washed with tap water.
6. Alcohol was added drop by drop and slide tilted till violet colour stops coming out.
7. Then gently washed with tap water.
8. Smear was air dried and observed under oil-immulsion objective.

Biochemical tests-

Biochemical tests were performed to differentiate the bacterial species that cannot be differentiated by the morphology and cultural characteristics. Biochemical tests are based on the fact that different bacterial species differ in their capacity to metabolize carbohydrates, proteins and fats.

Most biochemical tests are based on-

a. The presence of specific enzymes such has coagulase, catalase, oxidase, urease, gelatinase and others.
b. The production of metabolic end products of some compounds, like sugar fermentation produces acid by the enzymatic action of some bacteria.

IMViC Tests -

IMViC reactions are a set of four useful reactions that are commonly employed in the identification of members of family enterobacteriaceae. The four reactions are: Indole test, Methyl Red test, VogesProskauer test and Citrate utilization test.

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RESULT & DISCUSSION

In this study a total of 152 pus samples were processed for culture, out of which 115 were positive samples and 37 were negative samples. Majority of the patients in our study belonged to the age group between 41-60 years. It included 40.40% of the total samples from patients. The youngest patient was a few days old and the oldest being 95 years.

Out of 115samples positive for pus culture in which growth of pathogens were obtained, 68 were male and 47 female.

A total of 119 bacterial isolates were obtained from 115 specimens. In 116 cases, specimens were monomicrobial and 5 were polymicrobial. 2 organisms were isolated from each 5 specimens. Details of isolates from positive samples showed38 strains of E.coli which happened to be the commonest isolate. It was followed by Staph. aureus(21), Klebsiella (20), Pseudomonas (18), Proteus (6), Acinetobacter (5), Candida albicans(4),Enterococcus (3), Streptococcus spp. (3) and Citrobacter (1).

The observations of this study very well coincide with the works reported by various authors across the country. The predominance of mono-microbial infections observed in this study has been substantiated by a prospective study done by Basu S et al5, stating that chronic wounds do tend to show mono-microbial infections.

S. aureus was found to be the most commonly occurring pathogen in the study group. 0f PIMS hospital quoted to have demonstrated similar findings.-However, I found it to be second most common pathogen after Pseudomonas spp. and E. coli spp. were the most common Gram Negative Bacilli (GNB) obtained from the pus samples. Such GNB dominance in the aerobic growth in pus culture has been highly seconded by studies reported by PIMS hospital. I too reported Pseudomonas and E.coli spp. to be the most commonly occurring pathogens in wound infections, in that order. 72.59 % of all isolates were resistant to more than one drug. 8.90 % organisms showed resistance to all drugs they were tested against, then I also reported on the multi drug resistance capacity of the pathogens isolated from pus samples. 16 isolates were sensitive to only one drug. E. coli contributed maximum number to this group. Cephotaxime was the drug with 50% isolates sensitivity in this group, followed by Co-trimoxazole and Novobiocin. However, additional tests like identifying MRSA or ESBL production were not performed, thus leaving further scope of evaluation& has reported on high incidence of up to 18.18% of ESBL producing GNB and MRSA as a probable contributors to drug resistance.

In six years surveillance at a pediatric ICU in Taiwan, Lee CY et al observed that Staphylococcus aureus was the most common isolate among the Gram-positive organisms, while Pseudomonas, aeruginosa, Escherichia coli, and Klebsiella pneumoniae were the 3 leading Gram-negative isolates.3

In a prospective study conducted by Basu S et al on 52 wounds, it was reported that in chronic wound infections, the most common organisms were Pseudomonas (2wounds) and Escherichia coli (eight wounds). The high rate of drug resistant Pseudomonas and MRSA strains should discourage antibiotic use in chronic ulcers before obtaining culture results