ORIGINAL RESEARCH PAPER

Microbiology

KEY WORDS: Pus culture, Antibiogram, Staphylococcus aureus, Imipenem

# BACTERIOLOGICAL PROFILE AND ANTIBIOGRAM OF ISOLATES FROM PUS SAMPLES IN A TERTIARY CARE CENTER

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ABSTRACT

**Background:** Pus is one of the major samples received in the Microbiology laboratory for culture and sensitivity.
Objective: Study of bacterial isolates from the pus samples and their sensitivity pattern so that it will help the clinicians to treat patients and help the hospital to have its antibiotic policy.
**Method:** This study was conducted in the Department of Microbiology, Pacific Institute of Medical Science(PIMS),Udaipur (Rajasthan) for a period of 3months.Pus samples from different department of hospital were collected using aseptic technique and were processed in the laboratory immediately using standard microbiological procedures .Identification of bacteria was carried out with motility testing, Gram staining and biochemical reactions. The antibiotic sensitivity testing of all isolates was performed by Kirby Bauer's disc diffusion method on Muller Hinton agar and interpreted as per CLSI guidelines.
**Results :** Analysis of 100 pus samples showed 41.66% culture positivity. Among the 100 culture positive cases 62 (62%) were male and 38 (38%) were female with Surgical wards (34%) being the major contributor. Staphylococcus aureus was the major bacterial isolates (38%) with Pseudomonas aerugionsa being the second predominant one (22%).The prevalence of MRSA was 36.84% .Gram positive cocci were 100% susceptible to Linezolid and vancomycin followed by Doxycycline where as Gram negative bacilli were most susceptible to Colistin ,Imipenem and Amikacin, Gentamicin.
**Conclusion:** Changing antimicrobial resistance pose challenge in treating pyogenic infections .Appropriate and judicious selection of antibiotic would limit the emerging drug resistant strains in the future to treat these clinical conditions successfully.

## INTRODUCTION :

Infectious diseases remain the most common cause of morbidity and mortality worldwide [1]

Pyogenic infections are characterized by local and systemic inflammation usually with pus formation [2].These may be endogenous or exogenous. A break in the skin can provide entry to the surface bacteria which thereby start multiplying locally. The body's defense mechanism includes bringing immune cells into the area to fight against bacteria. Eventually, accumulation of these cells produces pus which is a thick whitish liquid [3].

Staphylococcus aureus (S. aureus), Pseudomonas, Klebsiella spp, Escherichia coli and Streptococci in which S. aureus is the most common bacteria that produces pus [4].Microorganisms are the concealed enemies to the mankind and cause a very profound damage in human body as well as other living organism. The agents, which have the capacity to kill the microbes or arrest the multiplication, are called the antimicrobial agents or drugs. There are a lot of antimicrobial drugs of which some are discovered or established [5].

The inadvertent use of antibiotics leads to emergence of drug resistant pathogens, which in turn acts as a great challenge to the health services. Moreover, highly virulent strains and capacity to adapt quickly to changing environment worsens the situation and draws a matter of concern [6]. Different studies have been conducted across the globe from time to time to assess the bacterial profile and the antibiotic susceptibility pattern in pus samples. This is particularly relevant for the treating physician who needs to start empirical treatment of patient until the lab culture reports are awaited [7].

The microbiology laboratory plays a central role in the decision to choose a particular antimicrobial agent over others. First, causative organism is identified and isolated when the patients' specimens are sent to the microbiology laboratory. Once the microbial species causing the disease have been identified, a rational choice of the class of antibiotics likely to work in on the patient can be made [8].

## AIMS & OBJECTIVE:

- To study the culture positive growth out of total pus samples.
- To study the male female ratio suffering from Pyogenic infection
- To study the department wise distribution as a major contributor of pus samples.
- To identify the type of pathogen from the pus sample and their antibiotic susceptibility pattern. So that it will help the clinicians to treat patients and help the hospital to have its antibiotic policy.

## MATERIAL AND METHODS:

### (1)Processing of Sample:

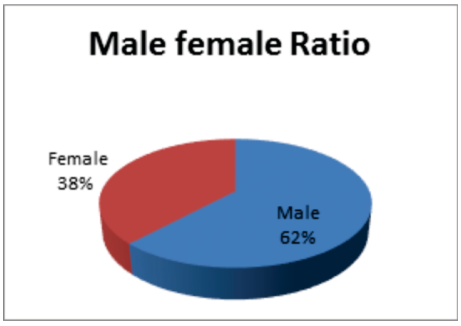
This is a prospective study in which a total number of 240 pus samples obtained for aerobic culture and sensitivity from different IPDs & OPDs of Pacific medical college and hospital during a period from November 2018 to January 2019 (3months) were included in the study. Informed consent was taken from the patient [9]. Pus samples were collected with sterile disposable cotton swabs and aspirates in syringe and were transported and processed in the microbiology laboratory immediately. They were inoculated on to Blood agar (BA), Mac Conkey agar (MA). Culture plates were incubated at 37°C for 24 hrs to 48 hrs in aerobic condition. Observe the growth, if there was no growth on MA & BA but BHI was turbid, and then subculture was done on MA & BA. After incubation, identification of bacterium from positive cultures was done with a standard microbiological technique which includes motility testing , gram staining and biochemical reactions such as catalase, coagulase, Indole, Methyl red, Voges- Proskauer, Citrate, Urease, Phenyl pyruvic acid test and oxidase test [10,11]. Characterization and identification of the isolates was done using the methods of Cowan and Steel (1985) [12], Mathur et al. (2006) [13] and Senthilkumar et al. (2012) [14].

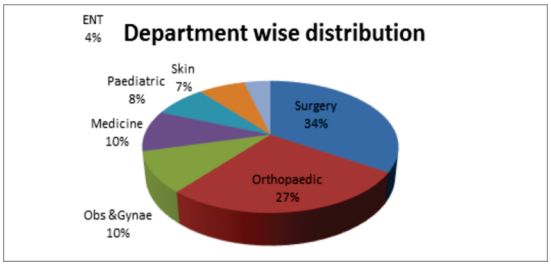
The antibiogram testing was done as per as CLSI guidelines using modified Kirbey-Bauer method. Few colonies from the culture plate were inoculated into 2ml of peptone water. Incubated at 37˚C for 2 hr. Turbidity was compared to that of 0.5 Mc Farland standards. A cotton swab was immersed and rotated in this inoculums, the swab was then pressed to the side of the tube so as to remove excess inoculums. It was then used for carpet streaking on Muller Hinton agar plate. The required antibiotic discs were then placed aseptically on this medium using sterile forcep. The plate was then incubated 24 hr at 37˚C. Next day the zone size was recorded and reported as sensitive or resistant, intermediate by comparing the zone size to the Kirby-bauer chart.

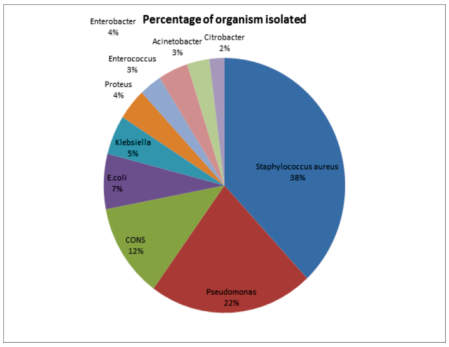
Antimicrobial susceptibility testing of isolates was performed by standard Kirby Bauer disc diffusion methods according to CLSI protocol. Depending on the isolate, antibiotic discs were selected from among the following:

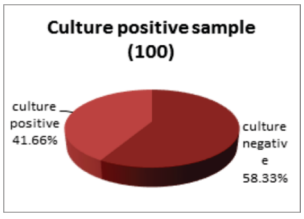
Co-trimaxazole(25µg), Erythromycin(15µg), Gentamicin (10µg),Ciprofloxacin(5µg), Linezolid(30µg), Vancomyci n(30µg), Tetracycline(30µg),Doxycyline (30µg),Clindamycin (2µg),Penicilin-

G(10units),Cefotaxime (30µg),Amikacin(30µg), Ceftazidime(30µg),Imipenem,Meropenem(10µg),PipercillinTazob actam(100µg).Aztreonam (30µg),Tobramycin (10µg), Polymixin (300 units), Colisitin(10 µg) etc. Cefoxitin (30µg) for detection of MRSA and MRCONS. S.aureus ATCC 25923 and E.coli ATCC 25922 were used as quality control [15]. All the culture media, biochemical media and antibiotics used were obtained from Hi Media.




## OBSERVATION & RESULTS:

- Analysis of 240 pus samples showed 41.66% (n=100) culture positivity. Among the 100 culture positive cases 62 (62%) were male and 38 (38%) were female with Surgical wards (34%) being the major contributor.
- Staphylococcus aureus was the major bacterial isolates (38%) with Pseudomonas aerugionsa being the second predominant one (22%). The prevalence of MRSA was 36.84% .
- In gram positive cocci there is prevalence of staphylococcus aureus 38% (n=38) followed by CONS (12%) and methicillin resistant MRSA were 36.84% so it indicated that resistence increases and enterococcus (3%)
- In gram negative bacilli there is Pseudomonas is predominent bacteria (22%) followed by E.coli (7%) , klebsiella (5%),Proteus (4%), Enterobacter (4%), Acinetobacter (3%) and Citrobacter (2%).
- Gram positive cocci were 100% susceptible to Linezolid and Vancomycin followed by Doxycycline where as Gram negative bacilli were most susceptible to Colistin (100%) Meropenem (100%) and Amikacin ,Gentamicin.

**Table 1: Antibiotic susceptibility pattern of Sensitivity pattern of Gram positive isolates**

| Antibiotic. (%) | GEN | CX | P | CIP | LZ | VA | E | CD | TE | MI | DO | C | COT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Staphylococcus aureus (n= 38) | 78.9 | 63.1 | 13.1 | 15.7 | **100** | **100** | 42.1 | 73.6 | 92.1 | 78.9 | 94.7 | 76.3 | 44.7 |
| CONS(n=12) | 83.3 | 50 | 16.6 | 16.6 | **100** | **100** | 33.3 | 75 | 83.3 | **100** | **100** | 41.66 | 16.6 |
| Enterococcus(n=3) | 66.6 | 33.3 | 33.3 | 66.6 | 66.6 | **100** | - | - | 66.6 | - | - | - | 33.3 |

**Table 2: Antibiotic susceptibility pattern of of Pseudomonas & non-fermenter GNB**

| Antibiotic(%) | AK | GEN | MRP | PB | CL | PIT | CAZ | CIPRO | A/S | AT | TOB |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pseudo(n=22) | 90.9 | 36.3 | **95.4** | 86.3 | **100** | 81.8 | 54.5 | 45.4 | 9 | 59.1 | 81.8 |
| Nonferm GNB(n=3) | 66.6 | 66.6 | **100** | - | **100** | 33.3 | 33.3 | 33.3 | 33.3 | 33.3 | 33.3 |

**Table 3: Antibiotic susceptibility pattern of Enterobacteriae**

| | AK | GEN | PIT | MRP | TOB | CL | C | CAZ | AT | CX | COT | CIP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E.coli(n=7) | 85.7 | 85.7 | 71.5 | **100** | 71.5 | **100** | 85.7 | 42.8 | 28.5 | 71.5 | 28.5 | 28.5 |
| Klebsiella(n=5) | 80 | 40 | 80 | **100** | 60 | **100** | 20 | 20 | 40 | 20 | 40 | 60 |
| Proteus(n=4) | 50 | 75 | 75 | **100** | 75 | - | 25 | 25 | 50 | 25 | 50 | 75 |
| Citrobacter(n=2) | 50 | 50 | 50 | **80** | 50 | **100** | 50 | 20 | 0 | 0 | 20 | 25 |
| Enterobacter(n=4) | 25 | 25 | 25 | **75** | 50 | **75** | 50 | 25 | 25 | 0 | 25 | 50 |

## DISCUSSION:

The perio(4%)d of study from November 2018 to January 2019. It was 3 months data. Out of 240 samples , 100 were positive. So growth were 41.66%. where as 140 samples yielded no growth .Out of these culture 62 were male (62%) and 38 were female (38%). In this study highest occurrence were observed in male as compare to female. It was correlated with Sowmya et al (M=66.6%, F=33.33%) and Raghav rao et al (M=58.82%, F=41.18%) study which shows male preponderance. [6, 9]

In this study surgical ward (34%) was major contributor followed by Orthopaedic (27%) this study similar to swati duggal et al (32.43%) and Raghav rao (35.29%)et al [9,16] then other wards percentage were Obs & Gynae (10%),Medicine (10%), Paediatric (8%),Skin (7%) and ENT Staphylococcus aureus were predominant bacteria (38%) followed by Pseudomonas (22%). The present study revealed S. aureus to be the most commonly occurring pathogen in pus samples which is in agreement with the studies by Tiwari P. et al [17] and Lee C.Y. et al [18]; however, Agnihotri N et al [19] found it to be second most common pathogen Pseudomonas spp. Pseudomonas and E. coli spp. were the most common Gram Negative Bacilli (GNB) isolated from the pus samples in our study. Among S.aureus 36.84% of them were found to be methicillin resistant Staphylococcus aureus (MRSA), this study closely corroborates with the study by Swati Duggal (35.90%) N.sowmya et al (37.4%) and Raghav rao et al (24.29%).[6,9,16]

Gram positive cocci were 100% susceptible to Linezolid and vancomycin followed by Doxycycline (94.73%) similar to Saumya kaup et al and Samra et al (100%) [6,20] where as Gram negative bacilli were most susceptible to Colistin(100%), Meropenem which closely corroborates with the study by deepali et al, swati

duggal et al and N.sowmya et al.

The emergence and proliferation of these highly resistant organisms obtained from pus samples is highly threatening given the limited number of antimicrobial agents that are currently available or in the drug development pipelines of the pharmaceutical industry to combat these organisms.

Every effort needs to be made to carefully select antibiotics, balancing the need for a broad spectrum of empiric coverage of potential microorganisms with the need to preserve available antibiotics for when they are absolutely necessary [21]

## CONCLUSION:

The study revealed staphylococcus aureus the predominant pathogen followed by pseudomonas. There is increased methicillin resistence in staphylococcus.Colistin, Meropenem, Gentamicin, Amikacin should be used as Drug of choice in case of Gram negative bacteria.Vancomycin, Linozolid and Doxycycline should be used as drug of choice in case of Gram positive bacteria.

A changing trend in antibiotic sensitivity profile of the isolates need to be monitored as there is limited availability of newer drugs and the emergence of resistant bacteria far exceeds the rate of new drug development.

Changing antimicrobial resistance pose challenge in treating pyogenic infections .Appropriate and judicious selection of antibiotic would limit the emerging drug resistant strains in the future to treat these clinical conditions successfully.

## LIMITATION:

Isolation of anaerobic bacteria could not be carried out in the study.

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