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Detection of Pyogenic Infections among Clinical Samples Received in Department of Microbiology in Integral Institute of Medical Sciences and Research, Lucknow, Uttar Pradesh: A Retrospective Analysis

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Abstract: ***Background: Pyogenic infection is characterized by several local inflammations usually with formation of pus. A break in skin can provide entry to 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. Objective of the study was to evaluate bacteria isolated from pus samples causing pyogenic infections and their antimicrobial susceptibility testing pattern. Material and Methods: A retrospective study was done over a period of 12 months from April 2018- March 2019 in the Department of Microbiology at Integral Institute of Medical Sciences and Research, Hospital Lucknow, India. The study was approved by the Ethical Research Committee (ERC) of the Institute. Data entry and statistical analysis were performed using the Microsoft Excel. Results: Total pus samples were 582, in which pathogenic bacteria were isolated in 429 samples (73.71%) and number of bacterial isolates were 494 (84.87%). In this study males were more affected 228 (53.14%) than female 201 (46.85%). Maximum number of patients belongs to age group 21-30 (20.74%) followed by 11-20 (18.88%). Conclusion: Gram positive bacteria S.aureus (43.72%) is most common. E.coli (17.2%) is the most common gram negative bacterial isolate followed by P.aeruginosa (13.56%) which is second most common gram negative bacterial isolate. S.aureus was susceptible to linezolid (94.90%), Vancomycin (98.61%) and Teicoplanin (99.53%).***

Keywords: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa.

1. Introduction

Pyogenic infection is characterized by several local inflammation responsible for formation of pus. These may be endogenous or exogenous. A break in skin can provide entry to surface bacteria which thereby start multiplying locally. The body's defense mechanism includes bringing immune cells into the area to get rid of bacteria.

Eventually, accumulation of these immune cells produces pus which is a thick whitish liquid [1]**.** The most common pus producing bacteria are S. aureus, Klebsiella, Pseudomonas, Escherichia coli, and Streptococci in which S. aureus is the most common bacteria that produces pus [2]. Impetigo, osteomyelitis, sepsis, septic arthritis, otitis media, spondylitis, cystitis, meningitis are some common diseases processes caused by pyogenic infection. Pyogenic infections destroy neutrophil through release of leukocidins forming abscess which is the typical characterization of *S. aureus* infections [3].

Multidrug resistant gram negative bacterial strains such as Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Gram positive methicillin-resistant staphylococcus aureus (MRSA) were increasingly associated with pus infections under hospital settings due to extensive misprescription and inadequate dose regime of antibiotics [3]**.**

2. Material and Methods

The retrospective study was conducted at Integral Institute of Medical Sciences and Research, Hospital for a period of 12 months from April 2018- March 2019. The study was approved by Ethical Research Committee (ERC).

Inclusion Criteria: Total 582 pus samples obtained for aerobic culture and sensitivity from IPDs & OPDs were sent to the Microbiology laboratory for bacteriological examination.

Specimen Collection: Pus samples were collected from the hospital of Integral Institute of Medical Sciences and Research with sterile disposable cotton swabs and aspirates in syringe and were transported and processed in the microbiology laboratory immediately.

Gram Staining: Spread the smear evenly on a clean grease-free glass slide and then stained by Gram stain technique.

Culture: Pus samples were inoculated on to Blood Agar, MacConkey agar and Nutrient agar. Culture plates were

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inoculated at 37º C for 24-48 hours in aerobic condition. After incubation, identification of bacterium from positive cultures was done with standard microbiological techniques which include colony morphology, motility testing by hanging drop preparation, and biochemical reactions such as Catalase, Coagulase, Oxidase, Indole, Methyl Red, Voges Proskauer, Citrate, Urease, Triple sugar iron, Phenyl Pyruvic Acid test [4].

The antibiotic sensitivity testing of all isolates was performed by Kirby Bauer's disc diffusion method on Muller Hinton agar[5] and interpreted as per CLSI guidelines [6]. Depending on the isolate, antibiotics discs were selected from the following: Penicillin, Cefoxitin , Vancomycin, Teicoplanin, Gentamicin, Amikacin, Tobramycin, Erythromycin, Clindamycin, Tetracycline, Doxycycline, Ciprofloxacin, Ofloxacin, Levofloxacin, Co-Trimoxazole , Linezolid, Ampicillin/Sulbactam, Piperacillin/ Tazobactam, Ceftrixone, Ceftazidime, Imipenem/ Cilastin, Cefepime, Aztreonam, Polymixin-B, Imipenem, Meropenem, Piperacillin, Ticarcillin/ Clavulanic acid, Tigecycline, Ampicillin.

Statistical Analysis

Entry of data and statistical analysis were performed using the Microsoft Excel. The values were represented in number, percentage and bar diagram.

3. Results

During the study period the total number of 582 pus samples were processed of which 429 (73.71%) were positive and number of isolates were 494 (84.87%) and 153 (26.28%) pus samples were negative.

Table 1: Distribution of Patients according to Gender

Male/ Female	Number of Patients	Percentage
Male	228	53.14%
Female	201	46.85%
Total	429	100%

Out of 429 positive patients who were included in the study, 228 (53.14%) were males and 201 (46.85%) were females.

Table 2: Distribution of Patients According to Age Group

Age group	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	>80	Total
No. of patients	60	81	89	69	56	46	16	10	2	429
Percentage	13.98%	18.88%	20.74%	16.08%	13.05%	10.72%	3.72%	2.33%	0.46%	100%

Maximum number of cases falls in the age group 21-30 years (20.74%) while the least number of patients (0.46%) belong to the age group of above 80.

Table 3: Distribution of Bacterial Isolates in Pus Samples

Organisms	*No. Of Isolates*	*Percentage*
S. AUREUS	216	43.72%
CoNS	10	2.02%
STEPTO. PYOGENES	7	1.41%
STEPTO. VIRIDANS	1	0.20%
ENTEROCOCCUS	17	3.44%
MICROCOCCUS	4	0.80%
P. AERUGINOSA	67	13.56%
E.COLI	85	17.20%
KLEBSIELLA	29	5.87%
ACINETOBACTER	25	5.06%
MORGANELLA	3	0.60%
ENTEROBACTER	5	1.01%
PROTEUS MIRABILIS	12	2.42%
PROTEUS VULGARIS	4	0.80%
C. KOSERI	3	0.60%
DIPTHEROIDS	1	0.20%
CANDIDA	5	1.01%
TOTAL	494	100%

Out of 494 positive samples, the most frequent isolates were Staphylococcus aureus (43.72%), Escherichia coli, Pseudomonas aeruginosa, Klebsiella, Acinetobacter, Enterococcus, Proteus mirabilis, Coagulase-negative staphylococci, and the least frequent isolates were Streptococcus pyogenes, Enterobacter, Candida, Micrococcus, Proteus vulgaris, Campylobacter koseri, Streptococcus viridans, Diptheroids (0.20%).

Table 4: This Table Shows Sensitivity Of Antibiotics Against Bacteria

ORGANISMS	*MOST SENSITIVE ANTIBIOTICS*
S. AUREUS	VANCOMYCIN, TEICOPLANIN
CONS	VANCOMYCIN, TEICOPLANIN, LINEZOLID
STEPTO. PYOGENES	VANCOMYCIN, TEICOPLANIN, LINEZOLID, TOBRAMYCIN
STEPTO. VIRIDANS	VANCOMYCIN, TEICOPLANIN, CLINDAMYCIN, LINEZOLID
ENTEROCOCCUS	TEICOPLANIN, LINEZOLID
MICROCOCCUS	VANCOMYCIN, TEICOPLANIN
P. AERUGINOSA	POLYMIXIN-B
E.COLI	TIGECYCLINE
KLEBSIELLA	TIGECYCLINE
ACINETOBACTER	TIGECYCLINE
MORGANELLA	CEFOXITIN, AMIKACIN, AMPICILLIN-SULBACTUM, PIPERACILLIN-TAZOBACTUM, CEFTRIAXONE-SULBACTUM, CEFTAZIDIME, CEFOTAXIME-CLAVULANIC ACID, AZTREONAM, DORIPENEM, MEROPENEM
ENTEROBACTER	AMIKACIN, TETRACYCLINE, AMPICILLIN-SULBACTUM, PIPERACILLIN-TAZOBACTUM, DORIPENEM, MEROPENEM
PROTEUS MIRABILIS	PIPERACILLIN-TAZOBACTUM, DORIPENEM, MEROPENEM
PROTEUS VULGARIS	AMIKACIN, PIPERACILLIN-TAZOBACTUM, DORIPENEM, MEROPENEM, CEFTRIAXONE-SULBACTUM, CEFTAZIDIME, CEFOTAXIME-CLAVULANIC ACID, AZTREONAM
C. KOSERI	TETRACYCLINE, DORIPENEM, MEROPENEM, TIGECYCLINE
DIPTHEROIDS	CEFOXITIN, VANCOMYCIN,

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	TEICOPLANIN, GENTAMYCIN, AMIKACIN, TOBRAMYCIN, ERITHROMYCIN, CLINDAMYCIN, TETRACYCLINE

This table shows approximately 90-100% sensitivity to specific antibiotics. Vancomycin, Teicoplanin, Meropenem, Doripenem was mostly sensitive antibiotics.

4. Discussion

The study is carried out in clinical laboratory, Department of microbiology, IIMS&R, Lucknow. Gram positive bacteria such as Staphylococcus aureus and Gram negative bacteria such as Escherichia coli, Pseudomonas aeruginosa, and Klebsiella spp. are the most common causative agents of pyogenic infection.

In our study total pus samples were 582, in which pathogenic bacteria were isolated in 429 samples with prevalence rate of 73.71% in which 325 (75.75%) samples were from IPD and 104 (24.24%) samples were from OPD, this positivity rate was similar with the study by **Nandkishor Bankar et. al (2018).**

In this study males were more affected 228 (53.14%) than female 201 (46.85%). It was correlated with **Vijeta sharma et.al (2015)** which shows male preponderance 55 (55%) and females 45(45%). Maximum number of patients belongs to age group 21-30 followed by 11-20. Findings were compared to **Vijeta sharma et. al (2015)** showed similar results.

Staphylococcus aureus (43.72%) is the most common gram positive isolate in our study as shown in study of **Swati duggal et.al (2013)** which shows 22.52%. Escherichia coli (17.2%) is the most common gram negative bacterial isolate followed by Pseudomonas aeruginosa (13.56%) which is second most common gram negative bacterial isolate, this differs from the other studies. In our study, candida was also found in 5 pus samples.

There was a preponderance of Gram positive organisms observed in our study, gram positive and gram negative organisms isolated were 51.61% and 48.17% which differs from the study of **Nandkishor Bankar et.al (2018)**

Staphylococcus aureus to be the most prevalent bacteria isolated from the cases of pyogenic infections. Different studies have been performed 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 **(Rameshkhannan S. et. al; 2014).**

In our study the antimicrobial susceptibility testing was done on Mueller-Hinton agar using disk diffusion by Kirby Bauer's method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. We observed that Staphylococcus aureus was susceptible to linezolid (94.90%), Vancomycin (98.61%) and Teicoplanin (99.53%) similar with the result of **Swati Duggal et.al; 2013.** It was also observed that 5.09% isolates were MRSA contrary to the MRSA rate observed by **Nandkishor Bankar et.al; 2018** and **Zulfiqar A Naqvi et.al; 2007.**

We observed that maximum isolates were resistant to penicillin, erythromycin, clindamycin, ofloxacin, levofloxacin, co-trimoxazole.

Gram positive bacteria were sensitive to linezolid, while gram negative bacteria are resistant to linezolid. Antibiotic sensitivity profile of gram negative bacteria showed sensitivity towards doripenem, meropenem, amikacin, ampicillin-sulbactum, piperacillin-tazobactum.

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