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AEROBIC BACTERIAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN PYOGENIC INFECTIONS IN A TERTIARY CARE HOSPITAL IN NORTH INDIA

Microbiology

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ABSTRACT

Background: The aerobic bacterial profile and antibiotic sensitivity pattern of pyogenic infection is very important in view of increasing antimicrobial drug resistance, which renders the treatment of such infections very difficult.

Objective: The aim of the study was to evaluate the bacterial profile and antibiogram of pus isolates in this part of country.

Materials and Methods: Pus samples of 240 patients were evaluated for identification of aerobic bacteria by standard methods and antibiotic sensitivity testing was done by Kirby Bauer method.

Results: Out of 240 pus samples, 143 samples (59.58%) showed growth of organisms. Out of 143 positive samples, 17 samples had growth of two organisms. So, total of 160 isolates obtained from 143 positive samples. *Escherichia coli* was the most common organism (23.1%) followed by *Klebsiella pneumoniae* (16.9%), *Staphylococcus aureus* (16.2%), *Pseudomonas aeruginosa* (13.1%), *Acinetobacter baumannii* (8.1%) among others. Among gram positive bacteria, *Staphylococcus aureus* was very sensitive to most of the antibiotics. On the other hand, gram negative bacteria were found to be very resistant specially *Acinetobacter baumannii* and *Klebsiella pneumoniae* to most of the antibiotics.

Conclusion: Changing pattern of antimicrobial sensitivity is a matter of concern in treating such pyogenic infections. Judicious use of antibiotics will limit the emergence of drug resistance in these organisms.

KEYWORDS

Pus, pyogenic infection, antibiogram, resistance, antimicrobial susceptibility

Introduction:

Pyogenic infections are characterized by local or systemic inflammation along with pus formation, caused by pyogenic bacteria which can lead to accumulation of dead leucocytes and infectious agents. Pyogenic infections may be endogenous or exogenous[1]. Breakage in the skin can provide entry to the pathogenic bacteria, which then starts multiplying at that site. The body's defense mechanism starts bringing immune cells into the area to fight against the bacteria. Accumulation of these cells produce thick whitish liquid which is called as pus[2]. These infections result in delayed wound healing and complications like wound dehiscence[3].

The widespread use of antimicrobial agents locally or systemically has resulted in widespread antibiotic resistance by development of antibiotic resistant genes and transfer the resistance from one bacteria to other[3]. Multidrug resistant bacteria cause serious nosocomial and community acquired infections that are very difficult to treat[4]. The spread of multidrug resistant bacteria from clinical isolates has increased the need for regular updates of antibiotic sensitivity profile of pus samples to avoid unguided empirical treatment [3]. The objective of this study is to characterize the pyogenic bacteria of pus samples and their antibiotic susceptibility to various antibiotics to guide the clinicians in patient care.

Materials and Methods:

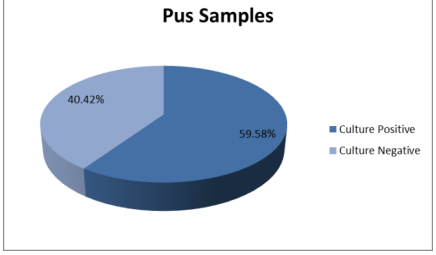
This is a retrospective study conducted in Department of Microbiology, Sarvodaya Hospital and Research Centre, Sector-8, Faridabad, Haryana, India. Pus samples received for bacteriological culture and sensitivity testing from Jan'2015 to Dec'2015 were analyzed.

A total of 240 samples of pus for aerobic culture and sensitivity from IPD were analyzed. Pus samples were received in the form of aspirates in syringe or container and swabs. They were inoculated on to Blood agar (BA) and Mac Conkey agar (MA) media. Culture plates were incubated at 37^{0}C for 24 to 48 hrs in aerobic condition. After incubation, identification of bacteria from positive culture were done with standard microbiological technique which includes Gram staining, motility testing by hanging drop preparation and biochemical reactions such as Catalase, Coagulase, Mannitol fermentation, Indole production, Methyl Red (MR) test, Triple Sugar Iron (TSI) agar, Citrate utilization, Urease test, Phenyl pyruvic acid (PPA) test, Oxidase test and Sugar fermentation tests[5]. The antibiotic sensitivity testing of all isolates were performed by Kirby Bauer's Disk Diffusion method on Mueller Hinton agar and interpreted as per CLSI guidelines as sensitive, intermediate and resistant[6]. All the culture media, biochemical media and antibiotics of standard strength were obtained from HiMedia, Mumbai, India.

Results:

Out of 240 pus samples obtained in Microbiology Lab from various departments of Sarvodaya Hospital and Research Centre for aerobic culture and sensitivity, 143 (59.58%) samples yielded positive culture whereas 97 (40.42%) samples yielded no growth (Figure 1). Out of 143 positive samples, 17 samples had growth of two organisms. So total of 160 isolates obtained from 143 positive samples.

Figure 1: Pie chart showing culture positivity of pus samples



Out of 143 samples, 90 (62.9%) were male patients and 53 (37.1%) were female patients giving male:female ratio of 1.69:1.00 (Table 1). The department wise distribution showed that Surgical department (including General Surgery, Neurosurgery, Cardiothoracic surgery and Urology dept) was the highest contributor of culture positive pus samples (50.3%) followed by Medicine (17.5%), Orthopedics (11.2%), ENT (2.8%), Obs & Gynae (1.4%) and other departments like Nephrology, Neurology, Cardiology, Gastroenterology, Oncology, Pulmonology and Paediatric dept comprises of 16.8% (Table 2). Age group wise distribution showed that 41-60 years of age distribution has highest number of culture positive cases (37.0%) followed by 61-80 years (25.9%), 21-40 years (21.7%), 0-20 years (9.8%) and >80 years (5.6%) (Table 3)

Table 1: Sex wise distribution of culture positive pus samples (n=143)

Sex	Number (%)
Male	90 (62.9%)
Female	53 (37.1%)

Table 2: Department wise distribution of culture positive pus samples (n=143)

Departments	Number (%)
Surgical	72 (50.3 %)
Medicine	25 (17.5 %)
Orthopedics	16 (11.2 %)
ENT	4 (2.8 %)
Obs & Gynae	2 (1.4 %)
Others	24 (16.8 %)

Table 3: Age group wise distribution of culture positive pus samples (n=143)

Age group	Number (%)
0 - 20 years	14 (9.8 %)
21 - 40 years	31 (21.7 %)
41 - 60 years	53 (37.0 %)
61 - 80 years	37 (25.9 %)
> 80 years	8 (5.6 %)

Among the 160 positive isolates the predominant gram negative bacteria was *Escherichia coli* (23.1%) followed by *Klebsiella pneumoniae* (16.9%), *Pseudomonas aeruginosa* (13.1%), *Acinetobacter baumannii* (8.1%), *Proteus spp* (7.5%), *Citrobacter spp* (1.9%), *Enterobacter spp* (1.9%) and *Morganella morganii* (1.9%). The predominant gram positive organism was *Staphylococcus aureus* (16.2%) followed by *Streptococcus pyogenes* (4.4%) and *Enterococcus spp* (2.5%). The percentage of *Candida spp* was 2.5% (Table 4). Two cases of Methicillin Resistant *Staphylococcus aureus* (MRSA) were identified among *Staphylococcus aureus* isolates.

Table 4: Distribution of aerobic bacteria isolated from pus samples (n=160)

Organism	Number (%)
Escherichia coli	37 (23.1 %)
Klebsiella pneumoniae	27 (16.9 %)
Pseudomonas aeruginosa	21 (13.1 %)
Acinetobacter baumannii	13 (8.1 %)
Proteus spp.	12 (7.5 %)
Citrobacter spp.	3 (1.9 %)
Enterobacter spp.	3 (1.9 %)
Morganella morganii	3 (1.9 %)
Staphylococcus aureus	26 (16.2 %)
Streptococcus pyogenes	7 (4.4 %)
Enterococcus spp.	4 (2.5 %)
Candida spp.	4 (2.5 %)

The distribution of *Escherichia coli* showed that it was mostly isolated from male patient (59.4%), mostly found in Surgical department (62.2%) and mostly found in age group of 41-60 years (37.8%). The distribution of *Klebsiella pneumoniae* showed that it was mostly isolated from male patient (74%), mostly found in Medicine department (37%) and mostly found in age group of 21-40 years, 41-60 years and 61-80 years equally distributed (29.6%). The distribution of *Pseudomonas aeruginosa* showed that it was mostly isolated from male patient (57.1%), mostly found in Medicine department and other departments equally distributed (33.3%) and mostly found in age group of 61-80 years (38.1%). The distribution of *Acinetobacter baumannii* showed that it was mostly isolated from male patient (61.5%), mostly found in Surgical department (46.2%) and mostly found in age group of 41-60 years (46.2%). The distribution of *Proteus spp.* showed that it was mostly isolated from male patient (83.3%), mostly found in Surgical department and Orthopedics department equally distributed (25%) and mostly found in age group of 41-60 years (58.3%). The distribution of *Staphylococcus aureus* showed that it was mostly isolated from female patient (53.8%), mostly found in Surgical department (69.2%) and mostly found in age group of 41-60 years (34.6%). The detailed sex wise, age group wise and department wise distribution of all the organisms is displayed in Table 5, Table 6 and Table 7 respectively.

Table 5: Distribution of Pus isolates according to sex (n=160)

Organism	Male (%)	Female (%)
Escherichia coli	59.40%	40.60%
Klebsiella pneumoniae	74%	26%
Pseudomonas aeruginosa	57.10%	42.90%
Acinetobacter baumannii	61.50%	38.50%
Proteus spp.	83.30%	16.70%
Citrobacter spp.	66.70%	33.30%
Enterobacter spp.	66.70%	33.30%
Morganella morganii	66.70%	33.30%
Staphylococcus aureus	46.20%	53.80%
Streptococcus pyogenes	100%	0%
Enterococcus spp.	75%	25%
Candida spp.	75%	25%

Table 6: Distribution of Pus isolates according to age group (n=160)

Organism	0-20 yrs (%)	21-40 yrs (%)	41-60 yrs (%)	61-80 yrs (%)	> 80 yrs (%)
Escherichia coli	8.10%	24.30%	37.80%	27.10%	2.70%
Klebsiella pneumoniae	0%	29.60%	29.60%	29.60%	11.20%
Pseudomonas aeruginosa	0%	9.50%	33.30%	38.10%	19.10%
Acinetobacter baumannii	0%	30.80%	46.20%	23.00%	0%
Proteus spp.	16.70%	8.30%	58.30%	16.70%	0%
Citrobacter spp.	0%	0%	0%	100%	0%
Enterobacter spp.	0%	0%	66.70%	33.30%	0%
Morganella morganii	0%	0%	33.30%	66.70%	0%
Staphylococcus aureus	23.10%	15.40%	34.60%	19.20%	7.70%
Streptococcus pyogenes	42.90%	14.20%	42.90%	0%	0%
Enterococcus spp.	0%	25%	75%	0%	0%
Candida spp.	0%	50%	50%	0%	0%

Table 7: Distribution of Pus isolates according to Departments (n=160)

Organism	Surgical (%)	Medicine (%)	Ortho (%)	ENT (%)	O & G (%)	Others (%)
Escherichia coli	62.20%	13.50%	2.70%	0%	2.70%	18.90%
Klebsiella pneumoniae	26.00%	37.00%	14.80%	0%	0%	22.20%
Pseudomonas aeruginosa	19.00%	33.30%	4.80%	9.60%	0%	33.30%
Acinetobacter baumannii	46.20%	0%	30.80%	0%	0%	23.00%
Proteus spp.	25.00%	16.70%	25%	8.30%	8.30%	16.70%
Citrobacter spp.	100%	0%	0%	0%	0%	0%
Enterobacter spp.	66.70%	33.30%	0%	0%	0%	0%
Morganella morganii	33.33%	33.33%	0%	0%	0%	33.33%
Staphylococcus aureus	69.20%	7.70%	15.40%	0%	0%	7.70%

Streptococcus pyogenes	71.40%	14.30%	0%	14.30%	0%	0%
Enterococcus spp.	75%	0%	0%	0%	0%	25%
Candida spp.	75%	25%	0%	0%	0%	0%

The antibiogram of Enterobacteriaceae showed that the sensitivity in case of *Escherichia coli* was highest in Imipenem

(94.5%) followed by Amikacin and Netilmicin (86.4%), Tobramycin (81%), Meropenem (78.3%), Gentamicin (75.6%), Piperacillin-tazobactam and Chloramphenicol (70.2%). The sensitivity of *Klebsiella pneumoniae* was highest in Imipenem (92.5%) followed by Tetracycline and Doxycycline (62.9%), Chloramphenicol (44.4%), Meropenem (37%), Amikacin, Gentamicin, Tobramicin and Netilmicin (29.6%). The sensitivity of *Proteus spp* was highest in Imipenem and Meropenem (100%) followed by Amikacin, Gentamicin, Tobramicin, Ampicillin-sulbactam and Piperacillin-tazobactam (91.6%), Amoxycillin-clavulinic acid, Cefotaxime, Netilmicin, Ciprofloxacin and Aztreonam (83.3%), Ceftriaxone, Cefoperazone, Ceftazidime, Cefepime and Levofloxacin (75%).

The antibiogram of *Pseudomonas aeruginosa* showed that the sensitivity was highest in Imipenem and Piperacillin-tazobactam (80.9%) followed by Cefepime (76.2%), Amikacin (71.4%), Gentamicin and Tobramicin (66.6%) and Ceftazidime (61.9%). The antibiogram of *Acinetobacter baumannii* showed that the sensitivity was highest in Imipenem (46.1%) followed by Meropenem (38.4%), Piperacillin-tazobactam and Levofloxacin (15.4%).

The antibiogram of *Staphylococcus aureus* showed that the sensitivity was highest in Linezolid, Teicoplanin, Tetracycline, Doxycycline, Netilmicin, Amikacin and Chloramphenicol (100%) followed by Gentamicin, Tobramicin and Moxifloxacin (96.2%), Cefoxitin (92.3%), Clindamycin (88.5%) and Levofloxacin (80.8%) (Table 8)

Table 8: Antibiogram of Pus Isolates (n=160)

Antibiotics	E.coli (%)	Klebsiella (%)	Proteus (%)	Pseudo-monas (%)	Acineto-bacter (%)	S. aureus (%)
Ampicillin-sulbactam	35.10%	18.50%	91.60%	NT	NT	NT
Amoxycillin-clavulinic acid	18.90%	7.40%	83.30%	NT	NT	NT
Piperacillin-tazobactam	70.20%	22.20%	91.60%	80.90%	15.40%	NT
Imipenem	94.50%	92.50%	100%	80.90%	46.10%	NT
Meropenem	78.30%	37%	100%	38.10%	38.40%	NT
Cefuroxime	21.60%	14.80%	16.70%	NT	NT	NT
Cefotaxime	21.60%	18.50%	83.30%	NT	NT	NT
Ceftriaxone	21.60%	18.50%	75%	NT	NT	NT
Cefoperazone	18.90%	18.50%	75%	NT	NT	NT
Ceftazidime	32.40%	14.80%	75%	61.90%	0%	NT
Cefepime	40.50%	18.50%	75%	76.20%	7.70%	NT
Amikacin	86.40%	29.60%	91.60%	71.40%	0%	100%
Gentamicin	75.60%	29.60%	91.60%	66.60%	0%	96.20%
Tobramycin	81%	29.60%	91.60%	66.60%	0%	96.20%
Netilmicin	86.40%	29.60%	83.30%	NT	NT	100%
Ciprofloxacin	10.80%	18.50%	83.30%	38.10%	0%	46.20%
Levofloxacin	27%	25.90%	75%	38.10%	15.40%	80.80%
Ofloxacin	NT	NT	NT	NT	NT	19.20%
Moxifloxacin	NT	NT	NT	NT	NT	96.20%
Tetracycline	37.80%	62.90%	0%	NT	NT	100%
Doxycycline	37.80%	62.90%	0%	NT	NT	100%
Cotrimoxazole	18.90%	14.80%	16.70%	NT	NT	23.10%
Aztreonam	40.50%	14.80%	83.30%	NT	NT	NT
Chloramphenicol	70.20%	44.40%	66.60%	NT	NT	100%
Linezolid	NT	NT	NT	NT	NT	100%
Teicoplanin	NT	NT	NT	NT	NT	100%
Erythromycin	NT	NT	NT	NT	NT	69.30%
Clindamycin	NT	NT	NT	NT	NT	88.50%
Cefoxitin	NT	NT	NT	NT	NT	92.30%

*NT= Not Tested

Discussion: The present study revealed culture positivity rate of 59.58% which correlates with the study conducted by Trojan R et al [7] which showed positivity rate of 60.1% whereas other author like Rao R et al [1] reported higher positivity rate of 89.47% . The sex wise distribution revealed male dominance with male:female ratio of 1.69:1.00 in our study which again correlates with the study conducted by Rao R et al [1] and Duggal S et al [2]. The department wise distribution revealed the dominance of Surgical department (50.3%) in our study which correlates well with the study conducted by Rao R et al [1] and Duggal S et al [2]. The present study revealed dominance of gram negative bacteria. The distribution showed that *Escherichia coli* was the most frequently isolated organism (23.1%) followed by *Klebsiella pneumonie* (16.9%), *Staphylococcus aureus* (16.2%), *Pseudomonas aeruginosa* (13.1%) etc. This finding correlates with the study conducted by Rameshkannan S et al [3]. Study by Trojan R et al [7] also reported *Escherichia coli* as the most frequently isolated organism. Other author like Duggal S et al [2], Sharma V et al [8] and Krishnamurthy S et al [9] also reported dominance of gram negative bacilli in their studies. Whereas other authors like Rao R et al [1], Kumar A R [4] and Mantravadi H B et al [10] reported *Staphylococcus aureus* as the most frequently isolated organism from pus. The dominance of gram negative bacilli in our study is a matter of concern as there is emergence of resistant genes by various mechanisms in such bacteria is very common.

The antibiogram of gram positive organism like *Staphylococcus aureus* showed 100% sensitivity to Linezolid, Teicoplanin, Amikacin, Netilmicin, Tetracycline, Doxycycline and Chloramohenicol, which is better in comparison to other studies conducted by Kumar A R [11] and Yasmeen F et al [12]. The overall antibiotic sensitivity profile of gram negative bacteria showed sensitivity towards Imipenem, Meropenem, Piperacillin- tazobactam, Amikacin, Gentamicin and Tobramicin. Our study is supported by Rao R et al [1] and Duggal S et al [2]. The increased resistance of *Klebsiella pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa* as compared to other gram negative bacteria is a real threat. Present study demonstrates great extent of resistance of these organisms towards cephalosporins, fluoroquinolones and aminoglycosides. Our study closely correlates with the study conducted by Kumar A R [11], Koripella R L et al [13] and Sharma J et al [14] in terms of resistance pattern of these multidrug resistant organisms. The most resistant gram negative bacteria in our study is *Acinetobacter baumannii*, which has also been reported as very resistant in case of other clinical specimen also as shown in a study done by Jaggi N et al [15].

The high level of resistance in case of *Klebsiella pneumoniae* is due to production of β-lactamase enzyme which causes hydrolysis of β-lactam ring resulting in inactivation of β-lactam antibiotics. The resistance to third generation cephalosporins is due to production of extended spectrum β-lactamases (ESBLs). The resistance may also be due to production of metallo-β-lactamases (MBL), which can be chromosomally encoded or plasmid mediated. Another mechanism is by penicillin-binding protein 2a (PBP2a) encoded by $mecA_2$[4]. The mechanism of resistance in case of *Acinetobacter baumannii* is by production of AmpC β-lactamases, class D OXA-type and class B metallo-β-lactamases which allow the organism to resist carbapenems, porin channel alterations, efflux pumps and other genetic changes that may lead to resistance to fluoroquinolones. There are also chromosomally encoded AmpC cephalosporinases known as Acinetobacter-derived cephalosporinases (ADCs). Extended-spectrum β-lactamases (ESBLs) from the Ambler class A group has also been held responsible for resistance in *Acinetobacter baumannii*[16].

The emergence of highly resistant organisms isolated from pus samples is very threatening as the availability of antimicrobial agents are very limited. The spectrum of drug development in pharmaceutical industry is also very narrow imparting a real challenge to the clinician in handling such resistant organisms. Overuse, misuse, improper dosing and lacking of proper antibiotic sensitivity testing has lead to emergence of resistance in these organisms. The selection of antibiotics should be carefully done balancing the need of empiric coverage with the need of preserving the available antibiotics. Updated knowledge of antibiogram profile of pus isolates will help in proper drug and dose selection and will also play role in curbing antibiotic resistance.

Conclusion:

In pyogenic infections the most prevalent organism found to be

Escherichia coli followed by *Klebsiella pneumoniae*, *Staphylococcus aureus, Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Proteus spp*, *Streptococcus pyogenes, Enterococcus spp, Candida spp, Citrobacter spp*, *Enterobacter spp* and *Morganella morganii*. Most of the gram negative bacteria showed moderate to high level of antibiotic resistance to different class of antibiotics as compared to gram positive bacteria. The antibiogram data of this report may be worth consideration while implementing empiric treatment strategies for pyogenic infections.

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Conflict of Interest: None

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