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"A RETROSPECTIVE STUDY OF AEROBIC BACTERIOLOGICAL ISOLATES AND THEIR ANTIBIOGRAM FROM PUS SAMPLES AMONG PATIENTS WHO ATTENDED A TERTIARY CARE HOSPITAL AT LUCKNOW"

Medical Microbiology

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ABSTRACT

Background: When immune cells fight against bacteria the damaged cells form the pus which is usually thick whitish liquid. Correct bacterial isolation with antimicrobial susceptibility testing should be done which can prevent antimicrobial resistance. **Objective:** This is a retrospective study done to find out the aerobic bacteriological isolates and their antibiogram from pus samples. **Materials and methods:** Data of 660 pus samples received for culture and sensitivity during the study period of 1 year from May 2019 to April 2020 were included in the study. Isolates from pus samples were identified according to standard microbiological techniques and antimicrobial susceptibility testing was done by Kirby Bauer Disc Diffusion method. The collected data was analyzed using MS-Excel, percentage was calculated for the numerical data and Chi square test was calculated for categorical variable, $p<0.05$ was considered as significant. **Results:** Out of 660 samples, 485 were positive for culture and 175 showed no growth. Males and females from positive samples were 267 and 218 respectively. Majority of samples belonged to the age group of 16-30 years. The predominant Gram-positive bacteria was Staphylococcus aureus (34.12%),while Pseudomonas spp. (16%) was the predominant Gram-negative bacteria. 46.5% were Methicillin Resistant Staphylococcus aureus (MRSA). Klebsiella spp. (55.8%) followed by Acinetobacter spp. (55.6%) were the most multidrug resistance bacteria. **Conclusion:** There must be awareness about the severity of pyogenic infections and strict prevention and control measures should be taken to prevent nosocomial infection. Treatment must be according to the antibiotic sensitivity testing results to prevent development of resistant organisms.

KEYWORDS

Pus samples, Retrospective study, Antibiogram, MRSA, Polymicrobial.

INTRODUCTION

Pus which is made up of white blood cells, dead tissue and damaged cells is generally formed at the inflammation site at the time of infection.[1] The formation of pus can be caused by either endogenous or exogenous and it can be monomicrobial or polymicrobial.[2] A breach in skin integrity due to injury is called wound. Burns, injuries, trauma etc. are associated with exogenous wounds while endogenous wounds and abscess are associated with cholecystitis, appendicitis etc.[3] Some other factors like abrasion, bites, minor cut, intervention, laceration, crush injury or gunshot injury cause the skin to loss its intact nature which results to produce the wound. Several factors like warmth and moisture in wound attract bacteria from skin surface or normal flora of patients itself which helps the bacteria to multiply and produce various virulence factors causing wound infection.[4]

Pyogenic infections are mostly caused by aerobic bacteria. The most common bacteria causing pyogenic infections are: Gram positive cocci - *Staphylococcus aureus*, CoNS (Coagulase Negative Staphylococcus), *Streptococcus pyogenes*, *Enterococcus* spp. Gram negative bacilli – *Escherichia coli, Klebsiella pneumoniae, Proteus* spp. and *Pseudomonas* spp.[5]

Antibiotics have a great role in therapeutic and prophylactic purpose. But due to their unselective use there have been surge in the antibiotic resistance. Increased mortality and economic costs are linked with antibiotic resistance infection. The rate of resistance in Gram-negative organisms is higher than that of Gram-positive organisms. This condition has become a public health problem globally. The antimicrobial resistance in different organisms varies according to the different geographical area. Therefore, for a better treatment regular observation of the antimicrobial susceptibility test (AST) should be constantly done.[6]

MATERIALS AND METHODS

A retrospective study of pus samples received in the microbiology laboratory for culture and sensitivity was conducted at our Institute over a period of 1 year from May 2019 to April 2020. The data has been collected from records maintained at Department of Microbiology. The demographic profile was noted from the case file. The present study was approved by Institutional Ethical Committee letter number IEC/IIMS&R/2021/09 conducted on 22nd May 2021. Data of 660 pus samples received for culture and sensitivity during the study period were included in the study. Pus samples received in the form of pus swabs or pus within leak proof sterile containers obtained from patients attending outpatient department as well as inpatients were immediately processed. For Direct microscopy, smear was made from pus sample then Gram staining was done and observed under oil immersion. The pus was then inoculated on the Blood agar and MacConkey agar plates which were then incubated overnight (18-24 hours) at 37°C. Any growth of organisms was identified by colony morphology, Gram staining and Biochemical tests such as Catalase, Coagulase, DNA Hydrolysis, Oxidase, Indole, MR (Methyl Red) test, VP (Voges-Proskauer) test, Urease, Citrate utilization, Nitrate reduction test, TSI (Triple Sugar Iron) test, PPA (Phenyl Pyruvic Acid) test, etc. Motility test was done by Hanging drop method wherever required.[7] Antimicrobial Susceptibility Testing (AST) was done on Mueller-Hinton agar plates by Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standard Institute guidelines (CLSI 2018) using the commercially available antibiotic disks from Hi-Media (Mumbai, India). Methicillin-Resistant Staphylococcus aureus (MRSA) was detected using Cefoxitin disc (30µg).[8]

Statistical Analysis

The data was analyzed using MS-Excel, percentage was calculated for the numerical data and Chi square test was calculated for categorical

variable, p<0.05 was considered as significant.

RESULTS

A total of 660 pus samples were received for culture and sensitivity in the Microbiology laboratory of our institute during the study period. Figure 1 shows that out of 660 samples, 485 showed the growth of bacteria, while 175 samples showed no growth.

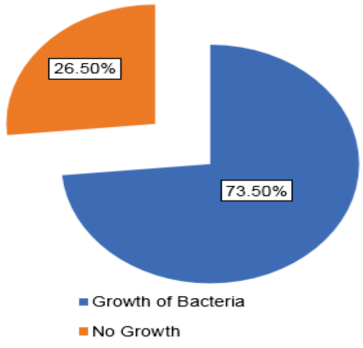

Figure 1: Percentage of pus samples yielding growth of bacteria.

As depicted in Figure 2, out of 485 culture positive samples, 397 were monomicrobial and 88 were polymicrobial, with majority being obtained from inpatients (59.2% of monomicrobials and 63.6% of polymicrobials) as compared to outpatients (40.8% of monomicrobials and 36.4% of polymicrobials).

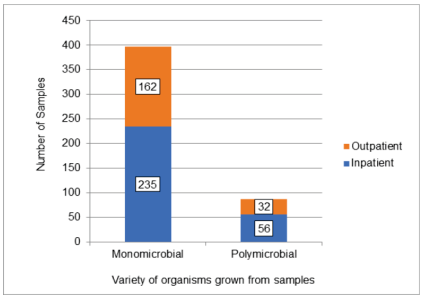

Figure 2: Distribution of culture positive samples according to their monomicrobial and polymicrobial status and the registration status of the patients.

Table 1 shows that amongst the positive cultures majority of samples were obtained from male patients (N = 267) as compared to female patients (N = 218). The ratio of male: female was 1.22 : 1. Also majority were obtained from patients who belonged to age group of 16-30 years and least patients belonged to >60 years old. The difference between positive samples obtained from male and female patients was found to be statistically significant (p = 0.006).

Table 1: Distribution of culture positive patients included in the study according to their age group and gender (N = 485).

Age group (in years)	Gender of patients			Chi-Square ($\chi 2$) & *p value
	Males N = 267 (55.1%)	Females N = 218 (44.9%)	Total N = 485 (100.0%)	
0-15	70 (64.2%)	39 (35.8%)	109 (100.0%)	$\chi 2$ = 14.621 df = 4 p = 0.006
16-30	77 (47.0%)	87 (53.0%)	164 (100.0%)	
31-45	52 (48.1%)	56 (51.9%)	108 (100.0%)	
46-60	40 (65.6%)	21 (34.4%)	61 (100.0%)	
>60	28 (65.1%)	15 (34.9%)	43 (100.0%)	

* p < 0.05 was considered as statistically significant. df = degree of freedom. N = Number of patients.

As depicted in Table 2, the total number of organisms isolated from positive samples was 586. Out of which 300 (51.2%) were Gram-positive bacteria and 286 (48.8%) were Gram-negative bacteria. The most predominant Gram-positive bacteria was *Staphylococcus aureus* (34.1%), whereas, *Pseudomonas* spp. (16.0%) was the commonest isolated Gram-negative bacteria.

Table 2: Distribution of organisms isolated from pus samples (N = 586).

Isolates	Number of Organisms (%)
Staphylococcus aureus	200 (34.1%)
Coagulase negative staphylococci	65 (11.1%)
Enterococcus spp.	29 (5.0%)
Streptococcus pyogenes	6 (1.0%)
Escherichia coli	63 (10.8%)
Klebsiella spp.	43 (7.3%)
Enterobacter spp.	2 (0.3%)
Citrobacter spp.	20 (3.4%)
Proteus spp.	28 (4.8%)
Providencia spp.	4 (0.7%)
Morganella morganii	5 (0.9%)
Pseudomonas spp.	94 (16.0%)
Acinetobacter spp.	27 (4.6%)
Total	586 (100%)

All the bacterial isolates were tested for antimicrobial susceptibility test (AST). As shown in Table 3, the isolates of *Staphylococcus aureus* were found to be most sensitive (100%) to Linezolid and Vancomycin and least sensitive (16.5%) to Ciprofloxacin. Out of 200 isolates 93 were resistant to Cefoxitin, hence, Methicillin resistant *Staphylococcus aureus* (MRSA) were 46.5% isolates. Similarly, isolates of Coagulase Negative Staphylococci (CoNS) were found to be most sensitive (100%) to Linezolid and least sensitive (37%) to Cefoxitin, hence, Methicillin resistant Coagulase Negative Staphylococci (MRCoNS) were 63.1% (41/65 isolates). Isolates of *Streptococcus pyogenes* were sensitive to most of the tested drugs, whereas, isolates of *Enterococcus* spp. showed highest sensitivity (100%) to Vancomycin and Teicoplanin and least sensitivity (62%) to Tetracycline.

Table 3: Antibiotic susceptibility pattern of Gram positive bacteria isolated from pus samples (N = 300).

Antibiotics tested	Staphylococcus aureus (N = 200) S (%)	CoNS (N = 65) S (%)	Streptococcus pyogenes (N = 6) S (%)	Enterococcus spp. (N = 29) S (%)
Vancomycin	200 (100)	64 (98.4)	6 (100)	29 (100)
Teicoplanin	199 (99.5)	64 (98.4)	NT	29 (100)
Cefoxitin	107 (53.5)	24 (37)	NT	NT
Gentamicin	164 (82)	35 (53.8)	NT	NT
Erythromycin	142 (71)	27 (41.5)	6 (100)	NT
Clindamycin	170 (85)	48 (73.8)	6 (100)	NT
Tetracycline	180 (90)	50 (77)	6 (100)	18 (62)
Doxycycline	189 (94.5)	57 (87.7)	NT	20 (69)
Ciprofloxacin	33 (16.5)	29 (44.6)	NT	NT
Levofloxacin	61 (30.5)	34 (52.3)	5 (83.3)	NT
Linezolid	200 (100)	65 (100)	6 (100)	28 (96.5)
Penicillin	NT	NT	5 (83.3)	26 (90)
Amikacin	NT	NT	6 (100)	NT
Ampicillin	NT	NT	NT	26 (90)
High level gentamicin	NT	NT	NT	19 (65.5)
High level streptomycin	NT	NT	NT	19 (65.5)

CoNS = Coagulase Negative Staphylococci, S = Sensitive isolates, NT = Not Tested.

As depicted in Table 4, the isolates of Gram negative bacteria belonging to *Enterobacteriaceae* family showed highest sensitivity (ranging from 81.4% – 100%) to Doripenem and least sensitivity (ranging from 25.4% - 50%) to Ciprofloxacin.

Table 4: Antibiotic susceptibility pattern of Gram negative bacteria belonging to Enterobacteriaceae family that were isolated from pus samples (N = 165).

Antibiotics tested	E. coli (N = 63) S (%)	Klebsiella spp. (N = 43) S (%)	Citrobacter spp. (N = 20) S (%)	Enterobacter spp. (N = 2) S (%)	Proteus spp. (N = 28) S (%)	Morganella morganii (N = 5) S %)	Providencia spp. (N = 4) S (%)
Ampicillin- sulbactam	38 (60.3)	22 (51.1)	11(55)	1(50)	27 (96.4)	5 (100)	4 (100)
Piperacillin-tazobactam	42 (66.7)	26 (60.4)	11(55)	1(50)	27 (96.4)	5(100)	4(100)

Tetracycline	25 (39.7)	31(72.1)	11(55)	2(100)	NT	NT	NT
Amikacin	48 (76.2)	27(62.8)	12(60)	2(100)	27 (96.4)	5(100)	4 (100)
Ceftriaxone	22 (34.9)	13(30.2)	5(25)	1(50)	27 (96.4)	5(100)	4 (100)
Ceftazidime	27 (42.8)	13(30.2)	7(35)	1(50)	27 (96.4)	4(80)	4 (100)
Ceftazidime-clavulanic acid	43 (68.2)	22(51.1)	9(45)	1(50)	27 (96.4)	4(80)	4 (100)
Cefepime	29 (46)	15(34.8)	10(50)	1(50)	NT	NT	NT
Aztreonam	27 (42.8)	17(39.5)	11(55)	1(50)	22 (78.5)	5(100)	4 (100)
Ciprofloxacin	16 (25.4)	17(39.5)	8(40)	1(50)	14 (50)	2(40)	2(50)
Levofloxacin	23 (36.5)	20(46.5)	9(45)	1(50)	16 (57.1)	2(40)	2(50)
Imipenem	49 (77.8)	32(74.4)	13(65)	1(50)	27 (96.2)	5(100)	4 (100)
Doripenem	56 (88.8)	35(81.4)	17(85)	2(100)	28 (100)	5(100)	4 (100)
Meropenem	49 (77.8)	31(72.1)	11(55)	1(50)	28 (100)	5(100)	4 (100)

E. coli = Escherichia coli, S = Sensitive isolates, NT = Not Tested

Table 5 shows that amongst the Non Lactose fermenters (NLF), the isolates of *Pseudomonas* spp. were found to be most sensitive (100%) to Polymixin B, followed by sensitivity to Piperacillin and Piperacillin-tazobactam (93.6% each), whereas, the isolates of *Acinetobacter* spp. were most sensitive to Ampicillin-sulbactam (70.4%) and least sensitive to Ceftazidime (33.3%).

Table 5: Antibiotic susceptibility pattern of Non Lactose Fermenting Gram negative bacteria isolated from pus samples (N = 121).

Antibiotics tested	Pseudomonas spp. (N = 94) S (%)	Acinetobacter spp. (N = 27) S (%)
Piperacillin	88 (93.6)	NT
Piperacillin-tazobactam	88 (93.6)	14 (51.8)
Amikacin	71 (75.5)	12 (44.4)
Aztreonam	82 (87.2)	NT
Ceftazidime	83 (88.3)	9 (33.3)
Cefepime	86 (91.4)	10 (37)
Gentamicin	61 (64.9)	NT
Ciprofloxacin	55 (58.5)	10 (37)
Levofloxacin	60 (63.8)	13 (48.1)
Imipenem	78 (83)	13 (48.1)
Tobramycin	65 (69.1)	NT
Meropenem	73 (77.7)	13 (48.1)
Polymyxin B	94 (100)	NT
Ampicillin-sulbactam	NT	19 (70.4)
Ceftriaxone	NT	10 (37)

S = Sensitive isolates, NT = Not Tested

Table 6 shows the distribution of Extended spectrum beta lactamase (ESBL) producers and Multidrug resistant (MDR) isolates amongst the Gram negative bacteria. ESBL producers were detected by combination disk tests. A ≥5 mm diameter of inhibition zone showed by Ceftazidime-clavulanic acid than Ceftazidime disc only were considered ESBL producers.[8] Isolates which were resistant to ≥ 3 antimicrobial classes were considered multiple drug resistant bacteria.[9] Overall, 13.3% of Gram negative bacterial isolates were found to be ESBL producers, with majority amongst *Proteus* spp. (28.6%) followed by *Escherichia coli* isolates (22.2%), whereas, amongst the 35.3% MDR isolates majority were found to be amongst *Klebsiella* spp. (55.8%) and *Acinetobacter* spp. (55.6%).

Table 6: Distribution of ESBL producers and MDR organisms amongst Gram negative bacteria isolated from pus samples (N = 286).

Gram negative bacteria	Number of isolates	ESBL producing isolates	MDR isolates
Escherichia coli	63	14 (22.2%)	26 (41.3%)
Klebsiella spp.	43	8 (18.6%)	24 (55.8%)
Citrobacter spp.	20	2 (10%)	9 (45%)
Enterobacter spp.	2	0 (0%)	0 (0%)
Proteus spp.	28	8 (28.6%)	5 (17.9%)
Providencia spp.	4	0 (0%)	0 (0%)
Morganella morganii	5	0 (0%)	0 (0%)
Pseudomonas spp.	94	2 (2.12%)	22 (23.4%)
Acinetobacter spp.	27	4 (14.8%)	15 (55.6%)
Total	286	38 (13.2%)	101 (35.3%)

ESBL = Extended spectrum beta lactamase, MDR = Multidrug Resistant, N = Number of isolates.

DISCUSSION

In our study majority of samples showed growth of organisms (73.5%) from pus samples which was more than the study done by Khanam et al. 2018 (61.8%) but less than the study done by Bankar et al. 2018 (78.55%).[10,11]

The highest number of positive cultures were seen among patient belonging to age group of 16-30 (164/485 i.e 33.8%) years which is in concordance with the study done by Mahat et al. 2017, in which majority of patients with positive cultures belonged to the age group of 21-29 (30.45%) years.[12] This is mainly due to outdoor works and infections related to occupation. In our study the least culture positive was seen among patients belonging to age group of >60 (43/485 i.e 8.86%) years old.

Our study showed that 18.14% (88/485) of total growth were polymicrobial infections. In such cases polymicrobial biofilm can be produced which are difficult to treat. Presence of one microorganism provides suitable environment for another known as synergism. In case of wounds the causative agent may be monomicrobial but some commensals may invade and colonize causing opportunistic infection resulting in polymicrobial infection.[13]

In our study we found that pyogenic infection caused by the of Gram-positive bacteria (51.2%) were more than Gram-negative bacteria (44.8%) which was in contrast to finding of the study done by Wadekar et al. 2020 which showed 52.5% were Gram-negative and 33.1% were Gram positive bacteria.[1] Another study done by Mantravadi et al. 2015 showed 59.3% were Gram-negative bacteria and 40.7% were Gram-positive cocci.[14]

Our study showed that amongst the 586 organisms isolated from pus cultures, *Staphylococcus aureus* was the predominant bacteria, N = 200 (34.1%), out of which 46.5% (93/200) were MRSA. This finding is concordant with another study done by Jain et al. 2018 in which the prevalence of MRSA was found to be 42%.[15] Prevalence of MRCoNS in our study was found to be 63.1% (41/65). *Staphylococcus aureus* is one of the commensals of our body. It does contain several virulence factors which promote pyogenic infections like adhesion protein, leukocidins, hemolysins, hyaluronidase, fibrinolysin, membrane damaging toxins. Also, some reports suggest that subcutaneous and intramuscular abscesses may occur after therapeutic injections and *Staphylococcus aureus* shows the main involvement.[16]

In our study it was found that *Staphylococcus aureus* isolates were 100% sensitive to Vancomycin and Linezolid, while isolates of CoNS were 100% sensitive to Linezolid only, this is in concordance with the study done by Jain et al. 2018.[15]

Streptococcus pyogenes are still sensitive to penicillin with 83.3% sensitivity. A similar study done by Abu-Harirah et al. 2020 also showed that isolates of *Streptococcus pyogenes* were not a highly drug resistant.[17] As, most of strains still do not produce penicillinase or β - lactamase enzymes and the efflux pump mechanism for antibiotics is seen to a smaller extent. In case of Penicillin allergic patients with serious infections, Vancomycin can be used for treatment.[18]

Enterococcus spp. isolates were found to be 100% sensitive to Vancomycin and Teicoplanin. These isolates were least sensitive (62%) to Tetracycline, followed by sensitivity (65.5% each) to High Level Gentamicin and High Level Streptomycin.

In our study, amongst the Gram negative bacterial isolates, *Pseudomonas* spp. (16%) was the predominant organism followed by *Escherichia coli* (10.8%). This finding correlates with study by Biradar et al. 2016, which showed that most common isolated bacteria were

Pseudomonas (24.85%) followed by *Escherichia coli* (14.02%).[19] The reason behind *Pseudomonas* overcoming *Escherichia coli* in pyogenic infection is that it is a ubiquitous bacterium which is found in variety of diverse environment including soil, animals, plants, water (mainly swimming pool, whirlpools).[16] The biofilm production and quorum sensing increase its ability to survive and causes difficulties with eradication which is why it is a major nosocomial pathogen than *Escherichia coli*.[18] However, studies done by Rahangdale et al. 2019 and Sudhaharan et al. 2018 reported *Escherichia coli* as the predominant bacterial isolate from pus.[5,20]

Our study found that *Pseudomonas* spp. were 100% sensitive to Polymyxin B, while Ciprofloxacin (41.5%), Levofloxacin (36.2%), Gentamicin (35.1%), Tobramycin (30.9%), Amikacin (24.5%) were the most resistant drugs. Similarly a study done by Deboral et al. 2020, also reported that *Pseudomonas* spp. were highly resistant to Ciprofloxacin (50.8%).[6]

In our study it was found that most of the isolates of *Escherichia coli* (88.8%), *Klebsiella* spp. (81.4%), *Citrobacter* spp. (85%) and *Enterobacter* (100%) were highly sensitive to Doripenem. The isolates of *Escherichia coli* were found to be least sensitive to Ciprofloxacin (25.4%) followed by sensitivity to Ceftriaxone (34.9%). This finding corroborates with another study done by Mukherjee et al. 2020, which reported that isolates of *Escherichia coli* were least sensitive to Ceftriaxone (36%) and Ciprofloxacin (42%).[4]

In our study 101 out of 286 of isolates were MDR (35.3%). *Klebsiella* spp. (55.8%) followed by *Acinetobacter* spp. (55.6%) were the predominant MDR bacteria. This finding is in concordance with another study done by Jamatia et al. 2017, which reported that maximum number of multidrug resistance was shown by the isolates of *Klebsiella* spp. (50%).[21] There are several factors that contribute the bacteria being multidrug resistance. Some of which are: misuse of antibiotics for example using broad spectrum of antibiotics for empirical treatment, using antibiotics without performing antibiotic susceptibility testing (AST). Some people are unaware of how harmful MDR bacteria can be.

Our study found that out of 38 isolates showing ESBL production, majority (28.6%) belonged to *Proteus* spp. followed by isolates of *Escherichia coli* (22.2%). This finding is in contrast to another study done by Rahangdale et al. 2019, who found high incidence of ESBL production among isolates of *Escherichia coli* (2.83%), followed by *Klebsiella pneumoniae* (0.94%).[5]

CONCLUSION

The bacteriological profile of pus showed Gram-positive cocci were predominant than the Gram-negative bacilli. About 1/3 of infection is caused by *Staphylococcus aureus* which is a matter of concern. The hospital environment should be healthy and free of infectious microorganisms. There must be awareness about the severity of pyogenic infections and drugs must be taken after the AST only which will help to prevent further multiple drug resistance in microorganisms. Review of antibiotic sensitivity is extremely essential since there is only presence of limited new drugs.

Conflict of interest: None declared.

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