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RESEARCH ARTICLE

TO STUDY BACTERIOLOGICAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF PUS ISOLATES FROM MATERNAL AND CHILD TERTIARY CARE HOSPITAL AT WESTERN RAJASTHAN

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ABSTRACT

Introduction: Pyogenic wound infections are responsible for causing significant morbidity resulting in longer duration of hospital stay and adding to economic burden for patient as well as hospital. The incidence of wound sepsis in India is reported to be 10-33%. **Objectives:** This study was done to identify bacteriological profile & their antimicrobial susceptibility pattern from various pus sample. **Methods:** This is retrospective study done in a tertiary care hospital from period of 1/4/22 to 31/3/23. In total 665 pus samples received from OBG and Pediatrics department were processed using standard microbiological methods and antibiotic sensitivity test was done as per CLSI 2022guidelines.**Result:**Out of 665 samples, 376 were culture positive (56.55%).Amongst them 350(93%) were females and 26(7%) were males. Maximum 42% cases in 21-30 year age group..Post LSCS wound infection cases were 227(60%) followed by puerperal sepsis (11.9%), post hysterectomy wound infection 30(7.97%).Gram positive cocci were predominant than Gram negative bacilli. 125 (33.2%) were CONS, 111(29.5%) were *staphylococcus aureus*, 57(15%) were *Escherichia coli* followed by *Acinetobactersp* 31(8.2%), *Klebsiellasp* 21(5.5%), *Pseudomonas* 18(4.7%). *Staphylococcus aureus*& Enterococcus species were 100% sensitive to linezolid and vancomycin. MRSA isolates were 62.16%. *86% of Escherichia coli* were sensitive to gentamicin. *Klebsiella pneumonia* showed sensitivity to piperacillintazobactum (66.66%) and gentamicin (66.66%). *Pseudomonas species* & *Acinetobacter* were sensitive to aminoglycoside 88.88% & 70.96% respectively. **Conclusion:** *Staphylococcus aureus* and *Escherichia coli* were the most common causative bacteria isolated in this study with high resistance pattern. Studying the antibiogram of pus isolates in a setup can guide clinician to start appropriate empirical antibiotics and can escalate or deescalate as per culture and sensitivity report.

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INTRODUCTION

Pyogenic infections are the infection that causes pus formation along with several signs and symptoms of inflammation.[1] Pus is a collection of whitish or yellowish fluid made up of dead tissues, white blood cells and damaged cells.[2] The overall incidence of wound sepsis in India is from 10% to 33%.[3] The most common causes of pyogenic infections are – surgical intervention, burn, bites, abrasion, minor cut, laceration, crush injury or gunshot injury resulting in loss of intact skin and produce wound.[4] Surgical site infection (SSI) is one of the major causes of pyogenic infection; it is defined as the infection that develops within 30 days after a surgical procedure or within 1 year if an implant is placed. It can be superficial, incisional/deep and organ/space infection depending on the depth of infection .[5] Post-operative wound infection is the most common wound infection involving both aerobic and anaerobic micro-organism. The most common causative agent isolated is *Staphylococcus aureus*(20-40%), *Pseudomonas species* infections occurs mainly following surgery and burns (5-15%).Aerobic gram negative bacilli (GNB) like *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* species are commonly associated with pyogenic infections.[6,7] There is emergence of drug resistant pathogen due to inadvertent and excessive use of antibiotics which causes prolong hospital stay and economic burden.[8] This study was done to know the bacteriological profile and antibiotic susceptibility pattern of bacteria isolated from pus samples. The information from this research will help in making local antibiotic policy of hospital based up on most common circulating strain of hospital & there susceptibility pattern. Thus help in improving patient treatment & outcome, will reduce hospital stay time thus reduce economic burden also.

MATERIAL AND METHODS

This is a retrospective study done in a tertiary care hospital of western Rajasthan over a period of 1 year from 1/4/22 to 31/3/23.

Study population: Total 665 pus samples were received in study time period (1/4/22 to 31/3/23) from OBG & Pediatric department.

Sampling procedure and processing-All pus samples collected with aseptic techniques during sample collection to minimize contamination. Upon arrival in the laboratory, the pus samples were streaked onto Blood agar, MacConkey agar and were incubated aerobically at 37°C for 24 hours. After incubation, identification of bacteria from positive cultures will be done with a standard microbiological technique which includes studying the colonial morphology, Gram stain as well as biochemical reactions as per standard laboratory protocol. The antibiotic susceptibility patterns of the isolates were determined using the Kirby-Bauer disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines 2022[9].

Antibiotics disks (Himedia, India) used were as follows

Erythromycin	(E)	15ug	Ampicillin-sulbactum	AMS	30/10ug
Penicillin	(P)	10 units			
Cefoxitin	(CX)	30ug	Amoxicillin-clavulanate	AMC	20/10ug
Gentamicin	(GEN)	10ug	Piperacillin-tazobactam	PIT	100/10ug
Cefazolin	(CZ)	30ug	Ceftriaxone	CTR	30 ug
Ciprofloxacin	(CIP)	5ug	Cefepime	CPM	30 ug
Linezolid	(LZ)	30ug	Ciprofloxacin	CIP	5 ug
Vancomycin	(VA)	30ug	Imipenem	IPM	10 ug
Ampicillin	(AMP)	10ug	Aztreonam	AT	20 ug
Cotrimoxazole	(COT)	1.25/23.75ug	Amikacin	AK	30 ug
Clindamycin	(CD)	2 ug	Ceftazidime	CAZ	30 ug
Meropenem	(MRP)	10ug	High level Gentamicin	HLG	150ug

RESULTS

Total 665 pus samples were received in study time period (1/4/22 to 31/3/23). Out of 665 samples, 376 (56.55%) were culture positive and 289(43.45%) were culture negative. Among 376 culture positive, 350(93%) were females and 26(7%) were males, urban areas 226 (60%) and 150(40%) were rural area, 334(89%) cases were from inpatient department (IPD) and 42(11%) cases were from outpatient department(OPD).

Table no.1 Age wise distribution of culture positive isolates

AGE	female	male	Total (%)
0-10 yrs.	19	10	29(7.7)
11-20yrs	27	16	43(11.40)
21-30yrs	157	0	157(41.75)
31-40yrs	117	0	117(31.1)
41-50yrs	22	0	22(5.85)
>50yrs	8	0	8(2.12)
Total	350	26	376(100)

In this study maximum cases were observed in the age group 21-30 years i.e. 157 (42%) followed by 117 (31%) in the age group 31-40 years as shown in table 1.

Table 2. Distribution of isolates as per diagnosis

clinical diagnosis	Number of cases	Percentage %
Post LSCS wound infection	227	60.3
Post hysterectomy incision wound	30	7.97
Bulbourethral cyst abscess	15	3.98
Puerperal sepsis	45	11.9
Breast abscess	8	2.12
Umbilical cord sepsis	15	3.98
Injection abscess	15	3.98
Open wound	14	3.72
Empyema	2	0.5
Armpit abscess	5	1.3
Total	376	100

In this study maximum clinical cases were post LSCS wound infection 227(60%) followed by puerperal sepsis (11.9%), post hysterectomy wound infection 30(7.97%), injection abscess (3.98%), umbilical cord abscess (3.98%), bulbourethral cyst (3.98%), open wound infection (3.72%), breast abscess (2.12%), armpit abscess(1.3%) as shown in table 2

Table 3. Distribution of organism isolated from pus samples

GPC (244)	Organisms isolated	Number(n=376)	Percentage (%)
	Coagulase negative Staphylococcus (CONS)	125	33.2
	Staphylococcus aureus	111	29.5
	beta hemolytic streptococci	3	0.79
	Enterococcus species	5	1.3
GNB (132)	*Escherichia coli*	57	15.1
	Klebsiellapneumoniae	21	5.5
	Citrobacter species	3	0.79
	Pseudomonas aeruginosa	18	4.7
	Acinetobacter species	32	8.51
	Burkholderia species	1	0.2
	Total	376	100

Gram positive cocci were predominant 244 (64.89%) than Gram negative bacilli 132(35.10%). Most common microorganisms isolated were CONS 125(33.2%) followed by *Staphylococcus aureus* 111(29.5%), *Escherichia coli*57 (15%), *Acinetobacter species* 32(8.51%), *Klebsiella pneumoniae* 21 (5.5%) and *Pseudomonas aeruginosa*18 (4.7%) as shown in table 3.

Table 4. Antibiotic sensitivity pattern of Gram positive cocci

SN	Antibiotics	*Staphylcoccusaureus* (N=111)	*CONS* (N=125)	*Beta heamolytic streptococci (N=3)*	*Enterococcus species* (N=5)	Total (N=244)
1	E(15ug)	17(15.3%)	15(12%)	1(33.33%)	-	33(13.52%)
2	CD(2ug)	61(54.9%)	63(50.4%)	2(66.67%)	-	126(51.63%)
3	P(10U)	18(16.21%)	6(4.8%)	0(0%)	0(0%)	24(9.83%)
4	CX(30ug)	69(62.16%)	70(56%)	1(33.33%)	0(%)	140(57.37%)
5	CZ(30ug)	46(41.44%)	51(40.8%)	1(33.33%)	0(%)	98(40.16%)
6	LZ(30ug)	111(100%)	125(100%)	3(100%)	5(100%)	244(100%)
7	VA(30ug)	111(100%)	125(125%)	3(100%)	5(100%)	244(100%)
8	GEN(10ug)	94(84.68%)	114(91.2%)	2(66.67%)	-	210(86.06%)
	HLG(150ug)	-	-	-	2(40%)	2/5(40%)
9	CIP(5ug)	94(84.68%)	94(75.2%)	2(66.67%)	3(60%)	193(79.09%)
10	AMP(10ug)	-	-	-	1(20%)	1/5(20%)

Table 5. Antibiotic sensitivity pattern of lactose fermenting GNB

SN	Antibiotics	*Escherichia coli* (N=57)	*Klebsiellapneumoniae (N=21)*	*Citrobacter species (N=3)*	Total (N=81)
1	AMP(10ug)	22(38.59%)	0(0%))	0(0%)	22(27.16%)
2	AMC(20/10ug)	20(35.08%)	3(14.28%)	2(66.66%)	25(30.86%)
3	GEN(10ug)	49(85.96%)	14(66.66%)	3(100%)	66(81.48%)
4	CIP(5ug)	17(29.82%)	8(38.09%)	3(100%)	28(34.56%)
5	CPM(30ug)	13(22.80%)	8(38.09%)	1(33.33%)	22(27.16%)
6	CTX(30ug)	5(8.77%)	1(4.76%)	1(33.33%)	7(8.64%)
7	CAZ(30ug)	5(8.77%)	4(19.04%)	2(66.66%)	11(13.58%)
8	AT(20ug)	20(35.08%)	7(33.33%)	2(66.66%)	29(35.80%)
9	PIT(100/10ug)	38(66.66%)	14(66.66%)	3(100%)	55(67.90%)
10	MRP(10ug)	26(45.61%)	5(23.80%)	3(100%)	34(41.97%)
11	CTR(30ug)	3(5.26%)	6(28.57%)	2(66.66%)	11(13.58%)
12	IMP(10ug)	26(45.61%)	5(23.80%)	3(100%)	34(41.97%)

Table 6. Antibiotic sensitivity pattern of Non Lactose fermenting GNB (NLF)

SN	Antibiotics	*Pseudomonas aeruginosa(N=18)*	*Acinetobacter species* (N=31)	*Burkholderia species* (N=1)	Total (N=50)
1	GEN(10ug)	16(88.88%)	22(70.96%)	1(100%)	39(78%)
2	AK(30ug)	16(88.88%)	22(70.96%)	1(100%)	39(78%)
3	CIP(5ug)	3(16.66%)	16(51.61%)	-	19(38%)
4	CPM(30ug)	13(72.22%)	8(25.80%)	-	21(42%)
5	CTR(30ug)	-	5(16.12%)	-	5(10%)
6	CAZ(30ug)	4(2.22%)	1(3.22%)	1(100%)	6(12%)
7	AT(20ug)	13(72.22%)	5(16.12%)	-	18(36%%)
8	MRP(10ug)	11(61.11%)	11(35.48%)	1(100%)	23(46%%)
9	LE()	7(38.88%)	8(25.80%)	1(100%)	16(32%)
10	PIT(100/10ug)	11(61.11%)	19(61.29%)	1(100%)	31(62%)
11	AMS(30/10)	-	3(9.67%)	-	3(6%)
12	COT(1.25/23.75ug)	0(0%)	-	1(100%)	1(2%)

In this study *Staphylococcus aureus* were 100% sensitive to linezolid and vancomycin followed by gentamicin (84.68%), ciprofloxacin(84.68%). Out of 111 isolates of *Staphylococcus aureus*, 69 were found to be *MRSA* (62.16%). *Enterococcus species* were 100% sensitivity to linezolid and vancomycin followed by ciprofloxacin (60%). As per table no.5, among LF GNB, *Escherichia coli* (N=57) showed maximum sensitivity to gentamicin (86%) followed by piperacillintazobactum (67%), meropenem (46%) and imipenem (46%) *Klebsiella pneumoniae* (N=21) showed maximum sensitivity to piperacillintazobactum (66.66%) and gentamicin (66.66%) followed by ciprofloxacin (38.09%). As showed in table no.6,among NLF, *Acinetobacter species* (N=31) showed maximum sensitivity to gentamicin (70.96%), amikacin (70.96%) and piperacillintazobactum (61.29%)). *Pseudomonas aeruginosa* (N=18) showed maximum sensitivity to gentamicin ,amikacin (88.88%) followed by cefepime (72%) and aztreonam (72%).

DISCUSSION

In study period total of 665 pus samples were received out of which 376(56.55%) came culture positive. This finding was consistent with study done by Rugina et al [10] (60%), Bhatt et al [11] (60%), Gupta et al [12] (62.8%) but lower than study done by Mukherjee et al [13] (65%), Razina et al [14] (66.2%), Shamanna et al [15] (65.86%) and higher than study done by Roy et al [16] (50.73%). Male to female ratio were 1:13.4 in this study is inconsistent with other studies like Mukherjee et al [13] (1.7:1), Razina et al [14] (1.3:1), Banker et al [17] (1.5:1). This inconsistency can be attributed to the fact that present study was conducted in a maternity and child care hospital and maximum number of cases were from post-operative patients from OBG &GYN department. In this study maximum positive cases were observed in the middle age group (21-30 years) and (31-40 years) which is also seen in study like Banker et al [17], Bhalla et al [18] and Biradar et al[19].The most common organism isolated was CONS (33.2%) followed by *Staphylococcus aureus (29.5%) ,Escherichia coli (15%), Acinetobacter species (8.2%), Klebsiellapneumoniae(5.5%), Pseudomonas aeruginosa (4.7%).* This finding is consistent with study done by Bhalla et al [18], Anshu et al [20], Sowmya et al [21] and Kumar et al [22] showed

Staphylococcus aureus was the most common bacteria isolated by all of them .Bhalla et al [18]and Anshuet al[20] reported Escherichia coli as 2nd most common bacteria which is similar in this study too. Bhatt et al[11] and Sharma et al[23] showed *Klebsiella* as most common bacteria isolated in their study. Multidrug resistance *Staphylococcus aureus and Escherichia coli* are mainly hospital acquired so more emphasis should be given on hand hygiene and appropriate hospital infection prevention & control policy. In this study isolation of CONS is highest (33.2%) .This might be due to non-compliance of aseptic technique during sample collection by the staff posted in wards & ICUs. That's why continuous training program should be conducted for all paramedical, nursing & laboratory staff regarding proper aseptic sample collection & transport because adequate sampling is crucial for isolation of pathogenic microorganism which is usually neglected most of the time.In this study *Staphylococcus aureus* were 100 % sensitivity to linezolid and Vancomycin . Linezolid sensitivity was similar to study like Rugina et al [10](100%), Bhalla et al [18] (100%) and Razina et al [14] (100%) but discordant with study done by Mukherjee et at[13] showed 92% sensitivity .In case of *Enterococci* species, all isolates showed 100% sensitivity to linezolid , 60% sensitivity to ciprofloxacin and low sensitivity to aminoglycoside (40%) which resonates with findings of study like Mukherjee et al [13] and Bhalla et al [18]. Among fermenters*, Escherichia coli* showed maximum sensitivity to gentamicin (86%), piperacillin-tazobactum (67%) and meropenem (46%). Klebsiella pneumoniae showed maximum sensitivity to piperacillintazobactum (67%) and gentamicin (67%). In present study amoxiclav, fluoroquinolones and 3rd generation cephalosporin resistance were high in *Escherichia coli* and *Klebsiella pneumoniae,* similar to findings reported by Mukherjee et at [13] and Rugina et al [10] but discordant to the findings reported by Sharma et al[23]and Jamatia et al[24] . High resistance to cephalosporines could be due to over and miss use of these antibiotics in periphery by local quacks & chemists as most of our patients were referred from periphery. Among non-fermenters, *Acinetobacter* (N=31) showed maximum sensitivity to gentamicin (71%) and piperacillintazobactum (61%) whereas *Pseudomonas* (N=18) showed maximum sensitivity to gentamicin (88%) followed by cefepime (72%) and aztreonam (72%).

Overall gram negative organism showed good sensitivity to piperacillintazobactum, aminoglycoside and carbapenem and lower sensitivity to fluoroquinolones, penicillin, amoxicillin, 2nd and 3rd generation cephalosporin, amoxiclave, amoxicillin -sulbactum combination. Similar findings were seen in study like Mukherjee et al[13], Jamatia et al[24], Razina et al [14].Increasing trend of drug resistance pattern observed among isolated bacteria which might get worsen day by day and are matter of concern. As Bacterial strain will differ from hospital to hospital , different patient population ,geographical area.That's why this is necessary to know the current circulating strain of individual hospital or tertiary care center to prevent miss use and over use of antibiotics. Thus will help in proper implementation of antibiotic policy and infection control practice to prevent development of drug resistant superbugs.

CONCLUSION

In present study, *Staphylococcus aureus & Escherichia coli* were the most common pathogenic bacteria isolated from pus samples with high drug resistance pattern. *Methicillin resistant Staphylococcus aureus (MRSA)* isolates were 62.16% . That's why periodic surveillance is required which will guide in making antibiotic policy of hospital to combat multidrug resistance among bacteria.

Conflicts of Interest: There are No conflicts of Interest.

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Abbreviations

CONS: Coagulase Negative Staphylococcus
GNB: Gram NegativeBacteria
MDR: Multi Drug Resistance
MRSA: Methicillin Resistant Staphylococcus aureus
OBG: Obstetrics and Gynecology

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