



A study on aerobic bacteriological profile and antimicrobial susceptibility pattern of isolates from pus samples in a tertiary care hospital

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Abstract: Pyogenic infections are characterised by local and systemic inflammation usually with pus formation which may be either endogenous or exogenous and polymicrobial or monomicrobial⁽³⁾. As definitive antimicrobial is based on the culture of the organisms and their susceptibility pattern, empirical treatment is a must in debilitated and deliberately ill patients⁽⁵⁾. Hence this retrospective study is conducted to investigate the microorganisms and antimicrobial susceptibility pattern from pus samples in a tertiary care hospital. This retrospective study was conducted from October 2016 to December 2016 in the department of Microbiology in a tertiary care hospital. Pus samples received from various departments was subjected to bacteriological culture as per standard protocol. The isolates were then subjected to phenotyping and their antimicrobial susceptibility was done by Kirby Bauer disc diffusion method according to CLSI guidelines 2016. Among the total of 209 samples received, 63.64% (133) showed positivity for microbial growth and 9.02% (12) were polymicrobial and 90.98% (121) showed single growth. 80.45% (107) were males and 19.55% (26) were females. Gram positive cocci accounts for 39.85% (53), Gram negative bacteria 63.15% (84), *Diphtheroids* 2.26% (3) and *Candida* 3.01% (4). *Staphylococcus aureus* 22.56% (30) were the majority among them followed by *Pseudomonas spp.*, 21.05% (28), *Escherichia coli* 14.29% (19), *Enterococci* 12.78% (17), *Klebsiella spp.* 11.28% (15), *Proteus spp.*, 10.53% (14), *Acinetobacter spp.*, 6.02% (8), *Streptococci* 4.76% (5), *Candida* 3.01% (4) each. The antibiogram of *S. aureus* and *Enterococci* showed 100% susceptibility to Linezolid and Vancomycin. *E. coli* was most susceptible to Imipenem and Meropenem 94.74% followed by Cefoperazone – Salbactam and Piperacillin and Tazobactam 89.43%. *Pseudomonas* showed 89.29% susceptibility to Meropenem followed by Imipenem 85.71%, Piperacillin – Tazobactam 85.71% and Amikacin 82.14%. *Klebsiella* showed 100% susceptibility to Imipenem followed by Piperacillin - tazobactam 73.33%. *Proteus* showed 100% susceptibility to Piperacillin – tazobactam and Imipenem, Meropenem 92.86%. *Acinetobacter* showed higher susceptibility to Meropenem 50%. Our study concludes that empirical treatment should be initiated based upon the data obtained from the bacteriological profile and the antimicrobial surveillance in every institution. It is also insisted to perform periodic surveillance on the changing trends in the antimicrobial susceptibility pattern to combat the evolving the resistance in each institution.

Key words: Pus; Antimicrobial susceptibility; Resistance; aerobic culture.

Introduction

The human skin and soft tissue infections caused by microbial pathogens due to trauma, burns and surgical procedures result in the production of pus which comprised of dead WBCs, cellular debris and necrotic tissues^(1,2). Pyogenic infections are characterized by local and systemic inflammation usually with pus formation which may be either endogenous or exogenous and polymicrobial or monomicrobial⁽³⁾. The most common organisms encountered from pus are Gram positive cocci such as *Staphylococcus aureus* followed by Gram negative organisms such as *Escherichia coli*, *Klebsiella spp.*, *Proteus spp.*, *Pseudomonas spp.*, and *Acinetobacter spp.*, respectively⁽⁴⁾.

Inadvertent use of antimicrobials leads to emergence of drug resistant pathogens which is considered as a great threat to the public health. Definitive antimicrobial treatment is based on the culture of the organisms and

their susceptibility pattern, even though empirical treatment is a must in debilitated and deliberately ill patients.⁽⁵⁾ Different studies have been conducted across the world from time to time to assess the bacterial profile and the antimicrobial susceptibility pattern relevant for the treating physician who needs to start empirical treatment of the patient until the lab culture reports are awaited.⁽⁶⁾ Though the bacterial profile from pus samples remain similar in various studies, there is a considerable variation in their antimicrobial susceptibility pattern in these isolates highlighting the increasing threat of emergence of resistant organisms and hence a need for continuous surveillance of such changing trends. Hence this retrospective study is conducted to investigate the microorganisms and their antimicrobial susceptibility pattern from pus in a tertiary care hospital.

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Materials and Methods

A retrospective analysis of 209 consecutive pus samples received at microbiology laboratory in a tertiary care hospital over a period of 3 months from October 2016 to December 2016. Pus samples received from various outpatient departments and inpatient wards were transported to the diagnostic laboratory in sterile leak proof container and swabs were obtained on sterile cotton swabs were processed immediately.

All the specimens were inoculated onto Nutrient agar, Blood agar and MacConkey agar incubated overnight at 37°C. Bacterial isolates were identified based on colony morphology, Grams staining and biochemical tests. Antimicrobial susceptibility testing was done using Muller Hinton agar by modified Kirby-Bauer disc diffusion method according to CLSI guidelines 2016⁽⁷⁾.

Antimicrobial Agents used: Ampicillin (10µg), Amikacin(30µg), Gentamycin(10µg), Ciprofloxacin(5µg), Cefotaxime(30µg), Cefixime(5µg), Cefpodoxime(10µg), Ceftriaxone(30µg), cefepime(30µg), Cefoperazone / Salbactam (30/10µg), Cotrimoxazole (1.25/23.75 µg), Imipenem (10µg), Meropenem (10µg), Piperacillin-tazobactam, (100/10µg), Vancomycin(30µg), Linezolid (30µg).

Results

Among the total of 209 samples received, 63.64% (133) showed positivity for microbial growth and 9.02% (12) were polymicrobial and 90.98% (121) showed single growth (Table 1). 80.45% (107) were males and 19.55% (26) were females (Table 2). Among all the samples received, 40.60% (54) from out patients and 59.40% (79) were from inpatients. Out of 133 showed positivity for growth, Gram positive cocci accounts for 39.85%(53), Gram negative bacteria 63.15%(84), Diphtheroids 2.26%(3) and Candida 3.01% (4) (Table 3).

Staphylococcus aureus 22.56%(30) were the majority among them followed by *Pseudomonas spp.*, 21.05%(28), *Escherichia coli* 14.29%(19), *Enterococci* 12.78% (17), *Klebsiella spp.* 11.28%(15), *Proteus spp.*, 10.53%(14), *Acinetobacter spp.*, 6.02%(8), *Streptococci* 4.76%(5), *Candida* 3.01%(4) each (Table 3).

The antibiogram of Gram positive cocci revealed that Linezolid and Vancomycin 100% were the most susceptible drug of *S. aureus* (Table 4) followed by Imipenem and Amikacin (96.67%), Cefepime 76.67%, Cefoperazone salbactam 90%, Levofloxacin 73.33% and Cefotaxime 60%. Enterococci showed 100% susceptibility to Vancomycin and Linezolid followed by Doxycycline 88.24% (Table 5).

Gram negative bacteria were most susceptible to Imipenem, Meropenem, followed by Piperacillin-Tazobactam, Amikacin. (Table 6). *E. coli* was most susceptible to Imipenem and Meropenem 94.74% followed by Cefoperazone – Salbactam and Piperacillin

Tazobactam 89.43%. *Pseudomonas* showed 89.29% susceptibility to Meropenem followed by Imipenem 85.71%, Piperacillin – Tazobactam 85.71% and Amikacin 82.14%. *Klebsiella* showed 100% susceptibility to Imipenem followed by Piperacillin - Tazobactam 73.33% and Amikacin 80%. *Proteus* showed 100% susceptibility to Piperacillin – Tazobactam followed by Imipenem, Meropenem 92.86%, Amikacin 85.71% and Ciprofloxacin 78.57%. *Acinetobacter* showed higher susceptibility to Meropenem 50%.

Table 1: Growth distribution of Samples (n=209)

Organism	No	%
Growth	133	63.64
No Growth	76	36.36
Monomicrobial	121	90.98
Polymicrobial	12	9.02

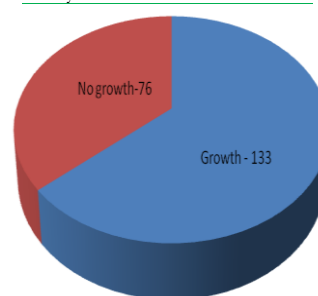


Table 2: Sex – wise distribution of positive cultures obtained from pus samples

Gender	Number	%
Male	107	80.45
Female	26	19.55

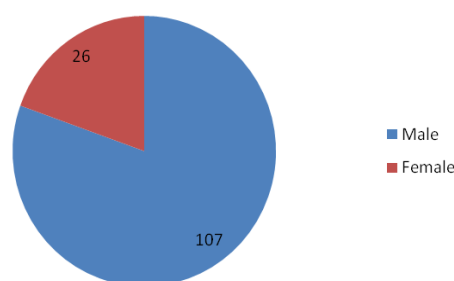


Table 3: Bacterial growth profile of pus culture (n=133)

Gram positive	53	39.85%
Gram negative	75	63.15%
Diphtheroids	3	2.26%
Candida	4	3.01%

S.No.	Organism isolated	Number	Percentage (%)
1	<i>S.aureus</i>	30	22.56
2	CONS	1	0.75
3	<i>Enterococci spp.</i>	17	12.78
4	<i>Diphtheroids</i>	3	2.26
5	<i>Candida spp.</i>	4	3.01
6	<i>Streptococci spp.</i>	5	3.76
7	<i>Klebsiella spp.</i>	15	11.28
8	<i>Escherichia coli</i>	19	14.29
9	<i>Pseudomonas spp.</i>	28	21.05
10	<i>Acinetobacter spp.</i>	8	6.02
11	<i>Proteus spp.</i>	14	10.53

Table 4: Antimicrobial susceptibility pattern of *S. aureus* (n=30)

Antibiotics	Sensitive		Resistant	
	Number	Percentage (%)	Number	Percentage (%)
Ampicillin (10µg)	4	13.33	26	86.67
Amikacin (30µg)	29	96.67	1	3.33
Gentamycin (10µg)	16	53.33	14	46.67
Ciprofloxacin (5µg)	7	23.33	23	76.67
Levofloxacin (5µg)	13	73.33	17	56.67
Cefotaxime(30µg)	18	60	12	40
Cefixime (5µg)	16	53.33	14	46.67
Cefpodoxime (10µg)	11	36.67	19	63.33
Ceftriaxone (30µg)	17	56.67	13	43.33
Cefipime (30µg)	23	76.67	7	23.33
Cotrimoxazole (1.25/23.75µg)	16	53.33	14	46.67
Imipenem (10µg)	29	96.67	1	3.33
Cefoperazone-salbactam (30/10µg)	27	90	3	10
Vancomycin (30µg)	15	100	0	0
Linezolid (30µg)	15	100	0	0

Table 5: Antimicrobial susceptibility pattern of Enterococci (n=17)

Antibiotics	Sensitive		Resistant	
	Number	Percentage (%)	Number	Percentage (%)
Ampicillin (10µg)	11	64.71	6	25.29
Amikacin (30µg)	10	58.82	7	41.18
HLG (120µg)	11	64.71	6	35.29
Ciprofloxacin (5µg)	1	5.88	16	94.12
Levofloxacin (5µg)	3	17.65	14	82.35
Vancomycin (30µg)	17	100	0	0
Linezolid (30µg)	17	100	0	0
Doxycycline (30µg)	15	88.24	2	11.76

Table 6: Antimicrobial susceptibility pattern of Gram negative bacilli (n=84)

Antibiotics	<i>E.coli</i> (No./%)		<i>Klebsiella spp.</i> (No./%)		<i>Proteus spp.</i> (No./%)		<i>Pseudomonas spp.</i> (No./%)		<i>Acinetobacter spp.</i> (No./%)	
	S	R	S	R	S	R	S	R	S	R
Ampicillin (10µg)	1(5.26)	18(94.74)	1(6.67)	14(93.33)	3(21.43)	11(78.57)	0	0(100)	0	8(100)
Amikacin (30µg)	17(89.47)	2(10.53)	12(80)	3(20)	12(85.71)	2(14.29)	23(82.14)	5(17.86)	1(12.5)	7(87.5)
Gentamycin (10µg)	13(68.48)	6(31.58)	10(66.67)	5(33.33)	12(85.71)	2(14.29)	22(78.57)	6(21.43)	1(12.5)	7(87.5)
Ciprofloxacin (5µg)	3(15.79)	16(84.21)	7(46.67)	8(53.33)	11(78.57)	3(21.43)	21(75)	7(25)	2(25)	6(75)
Levofloxacin (5µg)	5(26.32)	14(73.68)	7(46.67)	8(53.33)	12(85.71)	2(14.29)	21(75)	7(25)	5(62.5)	3(37.5)
Cefotaxime (30µg)	8(42.11)	11(57.89)	4(26.67)	11(73.33)	7(50)	7(50)	0	28(100)	1(12.5)	7(87.5)
Cefixime (5µg)	8(42.11)	11(57.89)	4(26.67)	11(73.33)	4(28.57)	10(71.43)	0	28(100)	0	8(100)
Cefpodoxime (10µg)	4(21.05)	15(78.95)	4(26.67)	11(73.33)	5(35.71)	9(64.29)	0	28(100)	1(12.5)	7(87.5)
Ceftriaxone (30µg)	7(36.84)	12(63.16)	4(26.67)	11(73.33)	7(50)	7(50)	1(3.57)	27(96.43)	1(12.5)	7(87.5)
Cefipime (30µg)	8(42.11)	11(57.89)	9(60)	6(40)	11(78.57)	3(21.43)	22(78.57)	6(21.43)	1(12.5)	7(87.5)
Cotrimoxazole (1.25/23.75µg)	10(52.63)	9(47.37)	8(53.33)	7(46.67)	7(50)	7(50)	1(3.57)	27(96.43)	0	8(100)
Imipenem (10µg)	18(94.74)	1(5.26)	15(100)	0	13(92.86)	1(7.14)	24(85.71)	4(14.29)	3(37.5)	5(62.5)
Meropenem (10µg)	18(94.74)	1(5.26)	12(80)	3(20)	13(92.86)	1(7.14)	25(89.29)	3(10.71)	4(50)	4(50)
Cefoperazone-salbactam (30/10µg)	17(89.43)	2(10.53)	10(66.67)	5(33.33)	13(92.86)	1(7.14)	24(85.71)	4(14.29)	1(12.5)	7(87.5)
Piperacillin-tazobactam (100/10µg)	17(89.43)	2(10.53)	11(73.33)	4(26.67)	14(100)	0	24(85.71)	4(14.29)	3(37.5)	5(62.5)

Discussion

Gram negative bacteria such as *Pseudomonas*, *Escherichia coli*, *Klebsiella spp.*, *Proteus spp.*, and Gram positive cocci such as *Staphylococcus aureus* and *Enterococci* are the common causative agents of various pyogenic infections. In our study, a dominance of Gram negative bacteria as the causative agents of various pyogenic infections is seen which is supported by Zubair *et al.*,⁽⁸⁾ *Staphylococcus aureus* is the most common Gram positive isolate in our study and *Pseudomonas spp.* is the most common isolate among Gram negative bacteria as shown in studies of Swati Duggal *et al.*,⁽⁹⁾ Predominant isolates were *S. aureus* followed by *Pseudomonas spp.*, *Klebsiella spp.*, *E. coli*, *Proteus spp.*, which is similar to studies by Rajan, Marton and Nicholas *et al.*,^(10,11) *S. aureus* was susceptible to Vancomycin and Linezolid 100% which is similar to the study by Samra *et al.*,⁽¹²⁾ Both Gram positive isolates were fully susceptible to Vancomycin and Linezolid. *Acinetobacter spp.*, showed multidrug resistance pattern as it was resistant to many antibiotics. Among the Gram-negative bacilli highest resistance was seen with Ampicillin (90-100%). Resistance towards third generation cephalosporins was also high such as Cefotaxime, Ceftriaxone and this may

be because of increasing expression of ESBL resistance among Gram negative bacilli.

This study provides the evidence of high prevalence of multidrug resistant bacteria in pus samples of patients collected from a tertiary care hospital environment. Our findings indicate the predominance of *S. aureus*, *Pseudomonas spp.*, *E. coli*, *Enterococci*, *Klebsiella spp.* and *Acinetobacter spp.* among the bacterial isolates. The prevalence and antibiotic resistance patterns of pyogenic bacterial isolates usually exhibit variability according to geographic areas and climatic conditions. The emergence and proliferation of these highly resistant organisms is highly threatening and is reported from several studies which may be due to negligence on patient part, incomplete treatment, misuse of antibiotics and limited knowledge of emerging drug resistance. Updated knowledge of antimicrobial susceptibility profiles of the isolates assist in designing the most appropriate dose regimen and treatment schedule against wound infections and alarming about drug resistance.

Conclusion

In conclusion, pyogenic wound infection was found prevalent in the tertiary care hospital and *S. aureus* showed highest incidence followed by *Pseudomonas spp.*, *E. coli*, *Enterococci*, *Klebsiella spp.*, *Proteus spp.*, *Acinetobacter spp.*, and *Streptococcus spp.* Bacterial isolates exhibit varying resistance against different classes of antibiotics. A changing trend in antimicrobial susceptibility profile of the isolates needs to be monitored as there is limited availability of newer drugs. Hence continued monitoring of susceptibility pattern needs to be carried out to detect the true burden of antibiotic resistance in the organisms and prevent their further emergence by judicious use of drugs.

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