Aerobic bacteriological profile with antibiogram of pus isolates

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Abstract

Background: Pyogenic wound infection is major cause of morbidity. Difficulty in treatment of such infection is because of emergence of multidrug resistant strains. To avoid such situation, it is very important for right and empirical choice of antibiotics. Hence it is mandatory for every pus samples to undergo culture and sensitivity.

Objectives:

1. To identify and isolate aerobic bacteria from pus samples.
2. To study antibiotic profile of aerobic bacteria.

Methods: A cross sectional study was conducted in Department of Microbiology of a private tertiary care hospital in Vijaypur. Total of 256 pus samples send for aerobic culture and sensitivity from different departments to Department of Microbiology from January 2015 to December 2015 were included in the study. Aerobic bacteria isolated, identified and antibiotic profile was determined from pus samples using standard protocol. Data was analyzed using MS Excel 2010.

Results and interpretation: In our study among 256 pus samples send from various departments, 169(66.01%) showed positive aerobic growth. Gram negative bacteria outnumbered the gram positive isolates. Commonest isolate was Staphylococcus aureus followed by Pseudomonas. 30(39.47%) of MRSA were isolated. All Staphylococcus aureus were sensitive to Vancomycin and linezolid. Among 48 Pseudomonas isolated Polymyxin B and Piperacillin-Tazobactam were effective drugs. In enterobacteriaceae group most effective antibiotic was Meropenam.

Conclusion: It is important for a clinician to send all the pus sample for microbiological analysis and their antibiogram before putting cases on antibiotics so that emergence of drug resistance can be minimized.

Key words: Pus, Antibiogram, Aerobic bacteria, MRSA, Pseudomonas.

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Introduction

Pyogenic infection causes local inflammation, formation of pus, generally caused by one of the pyogenic bacteria, which result in the aggregation of dead leukocytes as well as pyogenic bacteria commonly known as pus.[1] Wounds are result of loss of intact skin due to injury caused by external forces such as surgical wounds, burns, bites, abrasions, minor cuts and more severe traumatic wounds such as lacerations and crush or gunshot injuries such discontinuity in skin is good environment for microbial colonization as there is presence of moisture, warmth and nutrition for their growth. Colonization with proliferation of bacterial flora may lead to wound infection which may be serious even sometimes lead to death.[2] Wound infection can be caused by variety of organisms like bacteria, virus, fungi and protozoa and may co-exist as poly microbial communities especially in wound margins and in chronic wounds.[3] In many cases there is a mixed infection with more than one bacterial species.[4]

The pathogens isolated from infections differ depending on the underlying problem, location and type of surgical procedure. Most common organisms encountered are Staphylococcus aureus, Klebsiella spp., Escherichia coli, Pseudomonas spp., Proteus spp., Enterococci spp.[5] Enterobacter, Proteus spp, Candida and Acinetobacterspp.[6]

S. aureus is a most important pathogen in skin as well as soft tissue infections. Methicillin resistant S. aureus (MRSA) is prevalent in majority of the countries wherever it is sought for. MRSA is one of the important pathogen in hospital acquired infection.[3,7]

The spread of multi-drug resistant bacteria pathogens has added a new angel to the problem of wound infections. This is particularly worse in resource poor countries where sale of antibiotics is under poor control.[8]

The present study made an effort in identifying aerobic bacteria responsible for wound infection as well identify the antibiotic susceptibility pattern of the isolated organism.

Objectives

1. To identify and isolate aerobic bacteria from pus samples.
2. To study antibiotic profile of aerobic bacteria.

Material and Methods

A cross sectional study was conducted in Department of Microbiology of a private tertiary care hospital in Vijaypur. Total of 256 pus samples send for aerobic culture and sensitivity from different departments to Department of Microbiology from January 2015 to December 2015 were included in the study.

Detailed information of each patient was collected. All the pus samples were processed aerobically by inoculating on blood agar(BA), mac conkeys agar(MA), nutrient agar(NA) and incubated at 37^0C for 24 hours aerobically. 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.[4] The antibiotic sensitivity testing of all isolates will be performed by modified Kirby-Bauer's disc diffusion method on Mueller Hinton agar using antibiotics as per CLSI guidelines.[9] The following drugs were tested.

For Gram positive cocci- Penicillin (10units), Erythromycin (15µg), Clindamycin (2µg), Ciprofloxacin (5µg), Cotrimoxazole (25µg), Chloramphenicol (30µg), Gentamicin (10µg), Linezolid 310µg), Vancomycin (30µg), Teicoplanin (30µg), Tetracycline (30µg). High level gentamycin (120 µg) in case of Enterococcus.

For Gram negative bacilli- Ampicillin (10µg), Amoxyclavulanic acid (30 µg), Cotrimoxazole (25 µg), Tetracycline (30 µg), Chloramphenicol (30 µg), Gentamicin (10 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Cefoxitin (30 µg), Cefepime (30 µg), Ceftriaxone(30µg), Cephotaxime(30 µg), Ceftazidime (30 µg), Cefazoline (30 µg), Cefuroxime (30 µg), Aztreonam (30µg), Piperacillin (100 µg), Meropenem (10 µg).

For Non-fermenters- Ampicillin (10 µg), Gentamicin (10 µg), Amoxyclavulanic acid (30 µg), Amikacin (30µg), Ciprofloxacin (5 µg), Cefoxitin (30 µg), Cefepime (30 µg), Ceftriaxone (30 µg), Cephotaxime (30 µg), Ceftazidime (30 µg), Cefazoline (30 µg), Cefuroxime (30 µg), Aztreonam (30 µg), Piperacillin (100 µg), Meropenem (10 µg), Levofloxacin (5 µg), Ticarcillin (75 µg), Tobramycin (10 µg), Piperac-*illin-Tazobactam (100/10 µg), Polymyxin B (30 units).

Whole data was analyzed using MS Excel 2010.

Results

Total of 256 pus samples were send for aerobic culture and sensitivity to department of microbiology, among them 169(66.01%) samples were positive for growth, remaining 87(33.98%) samples were negative for aerobic growth.

Among 169 culture positive pus samples male patients had majority that is 94(55.62%) and 75(44.37%) were females. Male to female ratio was 1.25:1. Maximum cases were positive for culture were in the age group of 21-30 years 45(26.62%) as in Table 1.

Table 1: Age and sex distribution

Age in years	Male		Female		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
0-10	4	4.25	0	0	2	2.33
11-20	10	10.63	12	16	22	13.01
21-30	13	13.82	32	42.66	45	26.62
31-40	10	10.63	5	6.66	15	8.87
41-50	18	19.14	14	18.66	32	18.93
51-60	18	19.14	4	5.33	22	13.01
Above 60	21	22.34	8	10.66	29	17.15
total	94	100	75	100	169	100

Maximum pus samples were received from surgery department 93(55.02%) followed by orthopedic department 31(18.34%) as shown in Table 2.

Table 2: Department wise distribution

Departments	Number of samples received	Percentage(%)
Medicine	2	1.18
Surgery	93	55.02
Orthopedic	31	18.34
OBG	29	17.15
ENT	12	7.01
Pediatric	1	0.59
Dental	1	0.59
Total	169	100

Out of 169 culture positive pus samples 157(92.89%) were pure bacterial isolates remaining 12(7.1%) had more than one bacteria. So total number of isolates from positive pus samples were 181.

Table 3: Different bacterial isolates from positive pus sample

Bacterial isolate	Number	Percentage(%)
Staphylococcus aureus	76	41.98
Enterococcus faecalis	3	1.65
Escherichia coli	30	16.57
Klebsiellapneumoniae	17	9.39
Proteus mirabilis	7	3.86
Pseudomonas aeruginosa	48	26.51
total	181	100

Out of 169 culture positive isolate 41.98% were *Staphylococcus aureus* contributing maximum, followed by *Pseudomonas aeruginosa* 26.51% as shown in Table 3.

Table 4: Antibiotic sensitivity pattern of gram positive cocci (n=76)

Antibiotics	Staphylococcus aureus(76)	Enterococcus faecalis(3)
Penicillin	10(13.15%)	01(33.33%)
Erythromycin	59(77.63%)	02((66.66%)
Clindamycin	64(84.21%)	02(66.66%)
Ciprofloxacin	53(69.73%)	03(100%)
Cotrimoxazole	43(56.57%)	02(66.66%)
Chloramphenicol	52(68.42%)	02(66.66%)
Gentamicin	68(89.47%)	02(66.66%)
Linezolid	76(100%)	03(100%)
Vancomycin	76(100%)	03(100%)
Teicoplanin	66(86.84%)	02(66.66%)
Tetracyclin	71(93.42%)	02(66.66%)

All 76(100%) isolated *Staphylococcus aureus* were sensitive to Vancomycin and linezolid.

Table 5: Antibiotic sensitivity pattern of MSSA and MRSA

Antibiotics	MSSA(46)	MRSA(30)
Penicillin	10(21.73%)	0
Erythromycin	45(97.82%)	14(46.66%)
Clindamycin	46(100%)	18(60%)
Ciprofloxacin	41(89.13%)	12(40%)
Cotrimoxazole	23(50%)	20(66.66%)
Chloramphenicol	38(82.60%)	14(46.66%)
Gentamicin	44(57.89%)	24(80%)
Linezolid	46(100%)	30(100%)
Vancomycin	46(100%)	30(100%)
Teicoplanin	46(100%)	20(66.66%)
Tetracyclin	44(57.89%)	18(60%)

Among the 76(44.97%) *Staphylococcus aureus* 30(39.47%) were MRSA.

Table 6: Antibiotic sensitivity pattern of Enterobacteriaceae

Antibiotics	*Escherichia coli* (30)	*Klebsiellapnuemoniae* (17)	*Proteus mirabilis* (07)
Ampicillin	04(13.33%)	02(11.76%)	03(42.85%)
Amoxyclavulanic acid	07(23.33%)	03(17.64%)	04(57.14%)
Cotrimoxazole.	14(46.66%)	09(52.94%)	05(71.42%)
Tetracycline.	20(66.66%)	12(70.58%)	06(85.71%)
Chloramphenicol.	23(76.66%)	13(76.47%)	05(71.42%)
Gentamicin.	18(60%)	11(64.70%)	04(57.14%)
Amikacin.	22(73.33%)	15(88.23%)	07(100%)
Ciprofloxacin.	05(16.66%)	06(35.29%)	04(57.14%)

Cefoxitin.	13(43.33%)	14(82.35%)	03(42.85%)
Cefepime.	05(16.66%)	03(17.64%)	02(28.57%)
Ceftriaxone.	02(6.66%)	02(11.76%0	02(28.57%)
Cephotaxime.	02(6.66%)	03(17.64%0	03(42.85%)
Ceftazidime.	03(10%)	04(23.52%)	02(28.57%)
Cefazoline.	04(13.33%)	02(11.76%)	03(42.85%)
Cefuroxime.	02(6.66%)	02(11.76%)	02(28.57%)
Aztreonam.	03(10%)	04(23.52%)	03(42.85%)
Piperacillin.	07(23.33%)	02(11.76%)	03(42.85%)
Meropenem.	24(80%)	16(94.11%)	07(100%)

Table 7: Antibiotic sensitivity pattern of Pseudomonas

Antibiotics	Psuedomonas (48)
Ampicillin	02(4.16%)
Gentamicin	12(25%)
Amoxyclavulanic acid	22(45.83%)
Amikacin	25(52.08%)
Ciprofloxacin	18(37.5%)
Cefoxitin	12(25%)
Cefepime	10(20.83%)
Ceftriaxone	08(16.66%)
Cephotaxime	05(10.41%)
Ceftazidime	04(8.33%)
Cefazoline	12(25%)
Cefuroxime	05(10.41%)
Aztreonam	08(16.66%)
Piperacillin	09(18.75%)
Meropenem	31(64.58%)
Levofloxacin	14(29.16%)
Ticarcillin	20(41.66%)
Tobramycin	18(37.5%)
Piperacillin-Tazobactam	29(60.41%)
Polymyxin B	40(83.33%)

Among 48 Psuedomonas isolated in our study 83.33% were sensitive to Polymyxin B and 60.41% were sensitive to Piperacillin-Tazobactam as shown in Table 7.

Discussion

This study was conducted in department of microbiology in a tertiary care hospital of Vijayapur. Pus samples received from different departments of our hospital were 256, of which 169(66.01%) gave a positive aerobic bacterial growth. Rao et al.[1] done a similar study and quoted 89.47% of positive aerobic growth, whereas Hanumanthappa et. al.[10] quoted 56% of positive aerobic growth in their study.

Among 169 samples having positive aerobic growth only 12(7.1%) were polymicrobial, remaining 157(92.89%) were having pure aerobic growth amounting to total of 181 isolates. Prajuli et al.[3] noticed 92% of pure aerobic growth and 8% of mixed growth, Mohanth et. al.[11] quoted in their study that 85.8% has pure growth and 14.2% had mixed aerobic growth.

Among 169 culture positive cases maximum number were males with male to female ratio 1.25:1. Similar observation was done by Raghav et al.[1]

Maximum number of samples were from patients in the age group of 21-30years.

Majority of positive samples belongs to department of Surgery amounting to 93(55.02%), followed by Orthopedics with 31(18.34%). Similar observation was quoted by Vikas Jain et al.[8]

Among 181 isolates most common isolated bacteria was *Staphylococcus aureus* 76(44.97%) followed by Pseudomonas 48(24.85%) and *Esherechia coli* 30(14.20%). Similar studies having same findings like Hanumanthappa et al.[10], Prajuli et al.[3] and Jain et al.[8]

Among the 76(44.97%) *Staphylococcus aureus* 30(39.47%) were MRSA. Almost similar findings were found in other studies like Kshetry et al.[12], Prajuli et al.

In our study more of gram negative bacteria 105(58.01%) isolated compared to gram positive 76(41.98%). Our findings are matching to other studies like Jain et al.[8], Ghosh et al.[13] and Hanumanthappa et al.[10] whereas Prajuli et al.[3] in their study isolated more of gram positive bacteria compared to gram negative.

All 76(100%) isolated *Staphylococcus aureus* were sensitive to Vancomycin and linezolid. Similar findings were found in other studies like Jain et al.[8] Rao et al.[1] and Prajuli et al.[3] whereas Hanumanthappa et al[10]. In their study quoted only 87.3% of Staphylococcus aureus is sensitive to Vancomycin.

Among 48 Pseudomonas isolated in our study 83.33% were sensitive to PolymyxinB and 60.41% were sensitive to Piperacillin-Tazobactam hence making both the drugs effective. Our study is almost similar to Jain et al.[8] whereas Pramila et al. in their study quoted Amikacin as most effective followed by Polymyxin B for pseudomonas.

Among enterobacteriaceae group most common isolate was E.coli 30(14.20%) followed by Klebsiella pnuemoniae 17(10.05%). Our study correlate to study done by Jain et al.[8]

Among isolated enterobacteriaceae group most effective antibiotic was Meropenam. Our findings match with study done by Rao et al.[1]

In the present study most of gram positive as well as gram negative isolate were resistance to penicillin

and cephalosporin group. Similar to study done by Rao et al.[1]

Conclusion

Pyogenic wound infection is most important cause of morbidity. Emerging antibiotic resistance among pyogenic bacteria has a negative impact in treatment of such cases. Staphylococcus aureus is still one of the most important bacteria isolated among pyogenic wound infection. Even though gram negative bacteria outnumber it. Empirical and appropriate use of antibiotics is very crucial in preventing emergence of multidrug resistant bacteria. Our study will definitely will guide clinician for right and appropriate antibiotic choice based on individual isolate. Hence majority of antibiotics which are still sensitive can be prevented from being listed among multidrug resistance.

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