PUS culture isolates and their antibiotic sensitivity at a Tertiary Care Hospital in Hyderabad Karnataka Region

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Abstract

Introduction: Different studies show that aerobic bacteria are the majority of isolates causing pyogenic infection. Even though the bacterial profile of pus samples in many studies remain the same, the antibiotic resistance pattern of these isolates has shown many variations.[2,3] Hence this study was undertaken to detect the microbiological profile and antibiotic sensitivity of pus samples at a tertiary care hospital in North Karnataka.

Materials & Methods: This is a retrospective study in which a total of 293 pus samples were studied. Pus samples were collected with sterile disposable cotton swabs and pus aspirates in syringes and were immediately transported to the microbiology laboratory to be processed. They were inoculated on to Blood agar (BA) and Mac Conkey agar (MA) and plates were incubated at 37°C for 24 to 48 hrs. Identification of isolate from positive cultures was done using standard microbiological techniques. The antibiotic sensitivity testing of all isolates was performed by Kirby Bauer's disc diffusion method on Muller Hinton agar and interpreted as per CLSI guidelines.

Results & Discussion: Out of 293 samples, 177 (60.40%) samples were positive for growth. Out of 177 samples, 11(6.21%) samples showed polymicrobial growth. The total number of isolates was 188 isolates and gram negative bacteria were isolated more compared to gram positive pathogens. The most common pathogen isolated was E.coli (36, 19.14%) and the second most common pathogen isolated was Staphylococcus aureus (31, 16.48). The antibiotic sensitivity of the isolated pathogens displayed that majority of them were resistant to ampicillin and all were sensitive to cefoperazone/sulbactam.

Conclusion: The most common pathogen isolated was E.coli and the antibiotic sensitivity of the isolated pathogens displayed that majority of them were resistant to ampicillin and all were sensitive to cefoperazone/sulbactam.

Keywords: Pus Swabs, Pus Isolates, Pus Culture and Sensitivity.

Introduction

Suppuration or pus is caused mainly because of bacterial infection and such bacteria are said to be pyogenic or pus forming. Suppuration is the common sequel of acute inflammation and pus contains inflammatory exudate consisting of dead or living neutrophils, tissue debris and microorganisms causing the infection. Different studies show that aerobic bacteria are the majority of isolates causing pyogenic infection and the most common pyogenic bacteria include gram positive cocci like Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Enterococci and gram negative bacilli like Escherichia coli, Klebsiella pneumoniae, Proteus and Pseudomonas.[1] These studies have been consistent across the globe and have helped the treating doctor in starting empirical therapy for cases whose bacteriological culture reports are anticipated. Even though the bacterial profile of pus samples in many studies remain the same, the antibiotic resistance pattern of these isolates has shown a lot of variations.[2,3] The emergence of multi-drug resistant strains have resulted in prolonged illness, higher health care expenditures and higher risk of death due to infection. Antimicrobial resistance in addition hampers the control of infectious diseases by reducing the effectiveness of treatment thus patients remain infectious for a long time increasing the risk of spreading resistant microorganisms to others.[4-7] In a developing country like ours, indiscriminate prescription and improper use of antimicrobials has led to emergence of plenty of multidrug resistant strains. The emergence of drug resistance inducing mutations and transmission of genes coding for antibiotic resistance among bacteria has continued to be a major cause for development of resistance among microorganisms.[7] Continuous surveillance of these changing trends has become a necessity and hence this study was undertaken to identify the aerobic bacteriological profile and antibiotic sensitivity of pus samples at a tertiary care hospital in Hyderabad Karnataka region.

Materials & Methods

This is a retrospective study in which a total of 293 pus samples were studied during the period of February 2016 to February 2017. The ethical committee clearance was obtained before conducting the study. Pus samples were collected using Himedia sterile cotton swabs placed in screw capped tubes and pus aspirates were collected by using sterile disposable syringes. Samples were immediately sent to the bacteriology section of microbiology laboratory and were further processed. Samples were inoculated on to

Blood agar (BA) and Mac Conkey agar (MA) and the plates were incubated at 37°C for 24 to 48 hrs. Identification of isolate from positive cultures was done using standard microbiological techniques which include motility testing by hanging drop preparation, Gram staining and biochemical reactions such as catalase, coagulase, indole, methyl red, Voges-Proskauer, citrate, urease, phenyl pyruvic acid test and oxidase test.

Antibiotic sensitivity testing of all isolates was done by Kirby Bauer's disc diffusion method on Muller Hinton agar and results were interpreted as per CLSI guidelines.[8] Standard antibiotics like amikacin (30mcg), amoxyclav (20/10mcg), ampicillin (30mcg), cefipime (30mcg), cefoperazone/sulbactam (75mcg/10mcg), cefoxitin (30mcg), ceftriaxone (30mcg), ciprofloxacin (5mcg), clindamycin (2mcg), colistin (10mcg), cotrimoxazole (25mcg), erythromycin (15mcg), gentamicin (10mcg), imipenem (10mcg), linezolid (30mcg), nalidixic acid (30mcg), piperacillin/tazobactam (100/10mcg), teicoplanin (30mcg), tetracycline (30mcg) and vancomycin (30mcg) were tested. All the culture media, biochemical media and antibiotic discs used were obtained from Hi Media. Analysis of results was done by counts and percentages using MS Excel, 2007 version.

Results

A total of 293 pus samples were collected and sent to the microbiology laboratory for culture and sensitivity testing. This study was conducted for a period of one year from Feb 2016 to Feb 2017. Maximum number of pus samples were sent from surgery department (198, 67.57%) followed by medicine department (46, 15.69%), pediatric department (20, 6.82%), obstetrics and gynaecology department (11, 3.75%), orthopedics department (7, 2.38%), ophthalmology department (3, 1.02%), dermatology department (3, 1.02%), ENT department (2, 0.68%), dental department (2, 0.68%) and chest and TB department (1, 0.34%). The most common age group affected by pyogenic infection in our study was 21-30 years and males were more prone than females as shown in Table 1 and Fig. 1 respectively.

Table 1: Distribution of pus samples in various age groups in our study

Age Group	Number of Samples	Percentage
0-10 years	22	7.50%
11-20 years	32	10.73%
21-30 years	52	27.65%
31-40 years	39	13.31%
41-50 years	46	15.69%
51-60 years	49	16.72%
61-70 years	24	8.19%
71-80 years	23	7.84%
81-90 years	6	2.04%

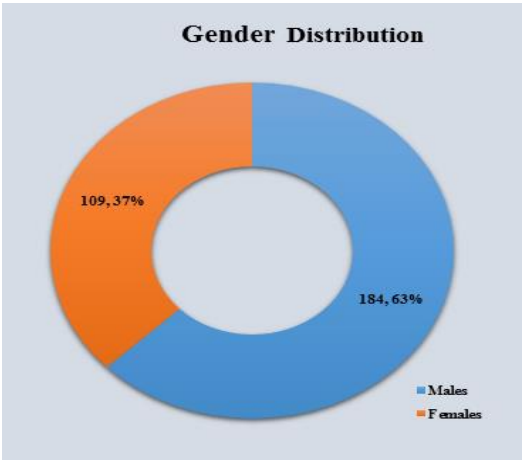

Fig. 1: Gender distribution of pus samples in our study

Out of 293 samples, 177 (60.40%) samples were positive for growth. Out of 177 samples, 11(6.21%) samples showed polymicrobial growth. The total number of isolates was 188 isolates and gram negative bacteria were isolated more compared to gram positive pathogens (Fig. 2). The most common pathogen isolated was E.coli (36, 19.14%) followed by Staphylococcus aureus (31, 16.48%), Klebsiella pnuemoniae (30, 15.95%) and Coagulase negative Staphylococcus (23, 12.23%). All the isolated pathogens have been listed in the order of their prevalence in the Table 2.

Table 2: Prevalence of isolates in pus cultures in our study

Organism isolated	Number of isolates	Percentage
Escherchia coli	36	19.14%
Staphylococcus aureus	31	16.48%
Klebsiella	30	15.95%
Coagulase negative staphylococcus	23	12.23%
Proteus	18	9.57%
Pseudomonas	16	8.51%
Streptococcus pyogenes	14	7.44%
Enterococcus	7	3.72%
Acinetobacter	5	2.65%
Enterobacter	4	2.12%
Edwardsiella	2	1.06%
Citrobacter	1	0.53%
Candida spp	1	0.53%

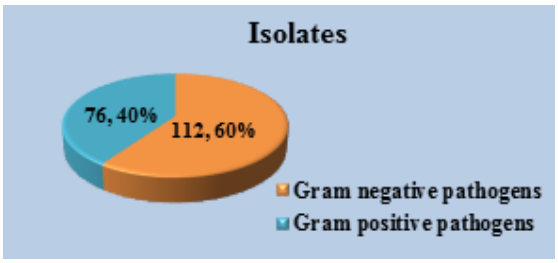

Fig. 2: Percentage of Gram positive and Gram negative pathogens

The antibiotic sensitivity of the isolated pathogens displayed that majority of them were resistant to ampicillin and all were sensitive to cefoperazone/sulbactam. The sensitivity patterns of gram negative pathogens and gram positive pathogens are listed in Table 3 and Table 4 respectively.

Table 3: Antibiotic sensitivity of gram negative isolates in pus cultures in our study

Organism / **Antibiotic**	**E.coli n=36**	**Klebsiella n=30**	**Proteus n=18**	**Pseudomonas n=16**	**Acinetobacter n=5**	**Enterobacter n=4**	**Edwardsiella n=2**	**Citrobacter n=1**
Ampicillin	0	3.33%	0	0	0	0	0	0
Amikacin	77.77%	70%	94.4%	68.75%	60%	50%	100%	100%
Amoxyclav	50%	50%	50%	-	0	0	50%	0
Cefipime	50%	50%	83.33%	87.5%	60%	100%	100%	100%
Cefoperazone/ Sulbactam	100%	100%	100%	100%	100%	100%	100%	100%
Ceftriaxone	50%	50%	83.33%	87.5%	60%	50%	50%	100%
Ciprofloxacin	50%	50%	61.1%	-	0	0	0	0
Colistin	100%	100%	100%	87.5%	100%	100%	100%	100%
Cotrimoxazole	80.55%	80%	50%	12.5%	60%	25%	50%	100%
Gentamicin	77.77%	70%	50%	68.75%	60%	25%	100%	100%
Imipenem	94.44%	93.33%	100%	100%	80%	0	100%	100%
Nalidixic acid	50%	50%	50%	-	0	0	50%	100%
Peperacillin/ Tazobactam	80.55%	80%	83.33%	87.5%	100%	100%	50%	100%

Table 4: Antibiotic sensitivity of gram positive isolates in pus cultures in our study

Organism / Antibiotic	Staphylococcus aureus n= 31	Coagulase negative Staphylococcus n=23	Streptococcus pyogenes n=14	Enterococcus n=7
Ampicillin	51.6%	30.43%	7.14%	14.28%
Amoxyclav	80.6%	34.78%	64.28%	57.14%
Cefoxitin	51.6%	52.17%	-	-
Cefoperazone/Sulbactam	100%	100%	100%	100%
Ceftriaxone	80.6%	65.21%	80.6%	71.4%
Ciprofloxacin	64.5%	43.47%	42.85%	71.4%
Clindamycin	90.32%	69.56%	85.71%	71.4%
Cotrimoxazole	67.7%	39.13%	92.85%	57.14%
Erythromicin	80.6%	56.52%	85.71%	71.4%
Gentamicin	67.7%	39.13%	92.85%	28.57%
Linezolid	100%	82.6%	100%	100%
Teicoplanin	100%	60.86%	100%	100%
Tetracycline	74.19%	65.21%	100%	71.4%
Vancomycin	100%	95.65%	100%	71.4%

Discussion

A total of 293 pus samples were collected in this study and maximum number of pus samples was from surgery department (198, 67.57%) followed by medicine department (46, 15.69%). Higher number of samples from surgery department has been observed in almost all studies done on pus cultures probably because of pus and wound discharge cases presenting to surgery department.[1,2,3,9] The most common age group affected by pyogenic infection in our study was 21-30 years and males were more prone than females comparable to the results in a study done by Biradar A et al, at a tertiary care hospital in North Karnataka.[1] This could be because usually, in our country, young males are involved in outdoor activities due to their occupations, making them prone to injuries and causation of wounds.[10]

Out of 293 samples, 177 (60.40%) samples were positive for growth. This isolation rate correlates with several studies done on pus cultures in developing countries like India and Africa with some variations.[1,2,3,9] The difference in isolation rates in the various studies could be because of the different laboratory facilities used. Out of 177 samples, 11 samples showed polymicrobial growth, very similar to the study done by Biradar A et al.[1] Open wounds can get easily colonized and invaded by numerous bacteria as they provide a warm and moist environment for bacterial colonization and proliferation. This might be the reason for polymicrobial growth in few samples in our study.

The total number of isolates was 188 isolates and gram negative bacteria were isolated more compared to gram positive pathogens. A study done by Basu et al also displays similar results where Pseudomonas and E.coli were the most common pathogens isolated and attributes the isolation of Gram negative pathogens to larger size and duration of the wound.[11] A study done on wound microbiology by Bowler et al implicates that the normal microbial flora of the gut, oral cavity, skin and genitourinary mucous membranes contain many bacteria that can easily colonize wounds especially the ones in close proximity to those sites. A colonized wound becomes infected due to various microbial and host factors which include size of wound, immune status of host and microbial load.[12] In this study, the most common pathogen isolated was E.coli (36, 19.14%), followed by Staphylococcus aureus (31, 16.48%), Klebsiella pnuemoniae (30, 15.95%) and Coagulase negative Staphylococcus (23, 12.23%). Staphylococci and coliform bacteria are frequently isolated pathogens because these colonizers can get established in chronic wounds and cause delayed healing.[12]

The antibiotic sensitivity of the isolated pathogens displayed that majority of them were resistant to ampicillin and all were sensitive to cefoperazone/sulbactam. In this study, gram positive isolates were most susceptible to vancomycin, linezolid, teicoplanin and cefoperazone/sulbactam and least sensitive to penicillins. These findings are correlating with several pus culture studies nationwide.[1,2,3] The majority of gram negative isolates in this study were sensitive to imipenem, piperacillin/tazobactam, cefoperazone/sulbactam and colistin and least sensitive to penicillins and cephalosporins. This is in agreement with the study done by Biradar A et al and several other studies.[1-3] This high incidence of resistance in gram negative isolates is because of indiscriminate use of empirical antibiotics and for inadequate periods of time. The wound type, site and sampling method play a very vital role in assessing the wound isolates and the

microbiology laboratory can provide clinically relevant report to the clinician only when the report can be interpreted in association with clinical information given by the treating doctor.[12] All the studies done on pus culture isolates and their antibiotic sensitivity patterns only emphasize the need for antimicrobial susceptibility testing to be carried out on pus isolates before starting chemotherapy to avoid selection of drug resistant strains.

Conclusion

E.coli was the most common pathogen isolated in our study and the antibiotic sensitivity of the isolated pathogens displayed that majority of them were resistant to ampicillin and all were sensitive to cefoperazone/sulbactam. Such studies need to be done regularly in a hospital setting for continuous surveillance of pathogens causing pus and their antibiotic sensitivity to guide the empirical use of antimicrobials.

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