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Study of bacteria in pus with Antimicrobial Sensitivity Pattern

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Abstract
Aims and Objective: The purpose of the present study was to see the number and distribution of bacteria isolated from pus and sensitivity pattern.
Material and methods: This retrospective study was conducted in the Department of Microbiology at Desh Bhagat Dental College January 2017 to June 2017 for a period of 6 month. The pus samples were collected from the patients who were visited in General hospital of Desh Bhagat University Mandi Gobindgarh Punjab, India, with skin and soft tissue infection. Bacteria were detected by culture and biochemical test and antibiotic susceptibility test done by disc diffusion method.
Result: Among 200 patients majority were in the age group of 20 to 30 years which was 98(49%) cases followed by 30 to 40 years, 40-50 years and less than 20 years which was 52(26%), 34(17%) cases and 16(8%) cases respectively. Among the male and female group 20 to 30 Years was the most common age group which was 52(43.33%) cases and 46(57.5%) cases respectively. Out of 200 cases aerobic culture was positive in majority cases which were 134(67%) cases and the rest of 66(33%) cases were growth negative. *Staphylococcus aureus* was the most common isolated bacteria from pus which was 64(32%) isolates followed by *Escherichia coli*, *Pseudomonas*, *Klebsiella* species and *Acinatobactor* species which were 29(14.5%), 27(13.5%),9(4.5%) and 5(2.5%) isolates respectively. High antibiotic sensitivity was seen by *S. aureus* to 75 % to ampicillin. Macrolides like erythromycin showed approximately 59.37% sensitivity and 40.63% resistance pattern while they were fairly sensitive to Vancomycins like clindamycin. Highest sensitivity was shown by high-end drugs such as linezolid and vancomycin.
Conclusion: the gram positive cocci is less in number than gram negative bacilli in pus .most of the antibiotic show sensitivity for gram positive and gram negative bacteria those was present in pus.

Keywords: Bacteriological profiles, pus, sensitivity pattern, antibiogram.

Introduction
Pyogenic infection is responsible for several local inflammations. It usually presents with pus formation. These are generally caused by one of the pyogenic bacteria [1]. Pyogenic infections may be endogenous or exogenous. The human skin and soft tissue infections (SSTIs) are caused by microbial pathogens during or after trauma, burn injuries, and surgical procedures [2] and these result in the production of pus. [2, 3] Both aerobic and anaerobic bacteria have been implicated in wound infections which commonly occur under hospital environment resulting in significant morbidity, prolonged hospitalization and huge economic burden. [4] Coagulase positive Staphylococcus aureus has been found to be more dominant organism in pus. [5-6] Antibiotic resistance among bacteria is becoming more and more serious problem throughout the world. It is said that evolution of bacteria towards resistance to antimicrobial drugs, including multidrug resistance, is unavoidable because it represents a particular aspect of the general evolution of bacteria that is un-stoppable. [7] Antibiotic resistance emerges commonly when patients are treated with empiric antimicrobial drugs. Monitoring of resistance patterns in the hospital is needed to overcome these difficulties and to improve the outcome of serious infections in hospital settings. [8] The emergence of antibiotic resistance pathogenic bacteria are considered as grave threats to the public health worldwide. [9]
During the last few decades, multidrug-resistant Gram-negative bacterial strains such as *Acinetobacter baumannii, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) were increasingly associated with pus infections under hospital settings due to extensive overuse and

inadequate dose regimen of antibiotics. [9-11] Rapid emergence of multidrug-resistant bacteria poses a serious threat to public health globally due to the limited treatment options and discovery of new classes of antibiotics. [11-12] Therefore, this present study was undertaken to see bacteria in pus with their resistant pattern.

Materials and Methods

A total 200 pus samples were collected by sterile syringe aspiration (n= 35) and by sterile swabs (n=165) from inpatients and outpatients of different wards of Desh Bhagat General Hospital, Mandi Gobindgarh , Punjab (India), over a period of 6 months from January 2017 to June 2017 after taking the approval of the protocol review committee and institutional ethics committee. After taking informed consent detailed history was taken from the patient. The technique, risks, benefits, results and associated complications of the procedure were discussed with all patients. Pus samples were collected from skin (furuncles, pustules, and abrasions), nasal wounds, ears, legs, internal organs (lungs, kidney, and bladder), and catheters. Pus samples were kept in Cary-Blair transport medium and transport to microbiology department of Desh Bhagat Dental College and Hospital, Mandi Gobind Garh Punjab, for Gram staining and culturing. The samples were aseptically inoculated on nutrient agar, blood agar (with 5% sheep blood) and MacConkey agar plates, incubated aerobically at 35 °C–37 °C for 24–48h. Primary identification and characterization of isolates were performed on the basis of Gram staining, microscopic characteristics, colony characteristic, and secondary identification were done with the help of biochemical tests such as tripal sugar iron agar, Hydrogen sulfide test, Carbohydrate fermentation test, Phenylalanine deaminase test, Methyl red test, Nitrate reduction test, Urease test, Voges proskauer, Citrate utilization test, Indole test by using standard microbiological methods.

Antimicrobial Agents: Antibiotics discs containing amikacin (30 µg), amoxicillin-clavulanic acid (30 µg), azithromycin (30 µg),, ceftriaxone (30 µg), cefotaxime (30 µg), cefuroxime (30 µg), cephalexin (30 µg), ciprofloxacin (1 µg), clindamycin (2 µg), cloxacillin (30 µg), erythromycin (15 µg), gentamicin (10 µg), imipenem (10 µg), levofloxacin (5 µg), linezolid (30 µg), meropenem (10 µg), ofloxacin (5 µg), piperacillin-(100/10 µg),, tetracycline (30 µg), and vancomycin (30 µg) were obtained from Himedia Laboratories (Mumbai, India).

Antibiotics Susceptibility Test: Antibiotic susceptibilities of bacterial isolates were determined according to the Disc diffusion methods. Briefly, inoculums were prepared for each bacterial isolate and spread on Muller-Hinton agar plates.

Results

A total number of 200 patients presented with wound infection or pus were recruited for this study. Among 200 patients majority were in the age group of 20 to 30 years which was 98(49%) cases followed by 30 to 40 years, 40-50 years and less than 20 years which was 52(26%), 34(17%) cases and 16(8%) cases respectively.

Table 1: Age and Gender Distribution of Study Population (n=200)

Age Group years	Male	Female	Total
Below 20	9(7.5)	7(8.75)	16(8)
20 to 30	52(43.33)	46(57.5)	98(49)
30 to 40	34(28.33)	18(22.5)	52(26)
40-50	25(20.84)	9(11.25)	34(17)
Total	120(100)	80 (100)	200 (100)

Interestingly male was predominant than female which was 120(60%) cases and 80(40%) cases respectively.

Table 2: Culture Positivity of Study Population (n=200)

Culture	Number	%
No growth	66	33
Growth	134	67
Total	200	100

Among the male and female group 20 to 30 Years was the most common age group which was 52(43.33%) cases and 46(57.5%) cases respectively (Table 1). Out of 200 cases aerobic culture was positive in majority cases which were 134(67%) cases and the rest of 66(33%) cases were growth negative. Therefore culture positive was more than no growth which was shown in this result and reflected the laboratory authenticity. (Table 2).

Table 3: Rate of Isolated Bacteria after Aerobic Culture (n=200)

Bacteria	Number	%
E. coli	29	14.5
Pseudomonas	27	13.5
Staph. aureus	64	32
Klebsiella Spp	9	4.5
Acinatobactor spp	5	2.5
Total	134	67

Staphylococcus aureus was the most common isolated bacteria from pus which was 64(32%) sioaltes followed by *Escherichia coli*, *Pseudomonas*, *Klebsiella* species and *Acinatobactor* species which were 29(14.5%), 27(13.5%), 9(4.5%) and 5(2.5%) isolates respectively (Table3).

Table 4: Sensitivity Pattern of Isolated Bacteria

Antibiotics	*E. coli*	*P. aeruginosa*	*S. aureus*	*K. pneumoniae*	*A. baumanii*
Amikacin	-	-	-	-	99.25
Gentamycin	41.37	37.03	20.31	55.56	20
Cefotaxim	62.06	44.45	25	-	-
Ciprofloxacin	55.17	25.92	20.31	-	100
Imipenem	44.82	29.62	21.87	-	20
Cotrimoxazole	55.17	48.14	34.37	-	40
Azithromycin	72.41	48.14	32.81	-	80
Amoxicillin	89.65	66.67	46.87	88.89	-
Cephalexin	48.27	33.33	43.75	-	60
Vancomycin	79.31	51.85	17.18	100	80
Cephradine	41.37	66.67	56.25	88.89	100
Ceftriaxone	79.31	51.85	48.43	-	80
Nitelmycin	48.27	40.74	37.5	100	20
Ampicillin	72.41	62.96	75	-	80
Erythromycin	62.06	62.96	59.37	-	100
Pefloxacin	75.86	51.85	34.37	55.56	100
Cefuroxime	89.65	88.89	53.12	-	80
Linezolid	79.31	85.18	31.25	44.45	100
Meropenem	48.27	48.14	29.68	-	100
Cloxacillin	48.27	33.33	50	88.89	-
Ceftazidime	68.96	81.48	53.12	-	80
Amoxiclave	72.41	44.45	31.25	-	-
Pipericillin	41.37	11.11	48.43	55.56	-

Discussion

Any wound is at some risk of becoming infected. When a wound fails to heal, the patient suffers from morbidity, treatment costs. Therefore the general wound management practices become more resource demanding. As wound infection is becoming the major hospital acquired infection, hospital environment plays a major role for causing wound infection. In this study out of all samples majority 134(67%) are growth positive. The reason is that the suppurative infection of the skin, ear, and eye are common occurrences in hospitalized patients as well as in the outpatients department. Furthermore wound infection is regarded as the most common nosocomial infection among surgical patients. [8] It has been associated with increased trauma care, prolonged hospitals stay, and treatment. [9]

The most common isolated bacteria is the *Staphylococcus aureus* (32%). Similar to the present study result Mantravadi *et al*. [14] have revealed that *S. aureus* is the most commonly isolated pathogen (37.2%) in pus samples, which is in agreement with the studies by Rao *et al*. [15], Tiwari and Kaur [16], Lee *et al*. [17] and Mahmood. [18] However, Agnihotri *et al* 19 have found *S. aureus* to be the second most common pathogen after *Pseudomonas* species. *E. coli* 29(14.5) followed by *Pseudomonas* 27(13.5) and *Klebsiella* 9(4.5) was the most common Gram negative bacteria isolated from the pus samples in from this present study. Though *S. aureus* was the predominant organism, gram-positive cocci accounted for only 32% of the total isolates, 52.24% being gram negative bacteria .Such Gram negative bacteria dominance in the aerobic growth in pus culture has been highly supported by the studies reported by Ghosh *et al*. [20] and Zubair *et al*. [21] Another study by Basu *et al*. [22] also reported *Pseudomonas* and *E. coli* spp. to be the most commonly occurring pathogens in wound infections which is inconsistent with the present study result. Raza *et al*. [23] found *E. coli* to be the most common pathogen with similar observations by studies conducted in Nigeria.

High antibiotic sensitivity was seen by *S. aureus* to 75 % to ampicillin. Macrolides like erythromycin showed approximately 59.37% sensitivity and 40.63% resistance pattern while they were fairly sensitive to Vancomycins like clindamycin. Highest sensitivity was shown by high-end drugs such as linezolid and vancomycin. Unfortunately, this only shows that *Staphylococcus* has become highly resistant to the first and second lines of treatment. On the other hand, *Streptococcus*, the other gram positive bacteria isolated, still shows fair amount of sensitivity to most of the drugs. These findings are similar to those of Rao *et al*. [15] who also found *S. aureus* to be resistant to penicillin (84.62%), erythromycin (84.62%), and sensitive to clindamycin (65.38%) and vancomycin (100%). Studies by Taiwo *et al*. [24] revealed 99.6% resistance to ampicillin and 33.1% to oxacillin, 72.7% to erythromycin but 100% sensitivity to vancomycin and more than 98% to linezolid. Among the b-lactams, high resistance was seen by gram-negative bacteria to even fourth-generation cephalosporins whereas carbapenems are still sensitive though increasing resistance has been observed to meropenem.

Amikacin among the aminoglycosides showed good sensitivity whereas resistance to gentamicin and tobramycin is on the rise. Resistance was seen by most of the isolates to quinolones. Combination drugs such as piperacillin plus tazobactam and cefoperazone plus sulbactum showed good amount of sensitivity. Similar studies by Taiwo *et al*. [24], Rao *et al*. [15] and Basu *et al*. [22] corroborated our findings. The knowledge of the bacteriology of an infection and the laboratory susceptibility testing of microorganism implicated could make drug selection in antimicrobial chemotherapy more rational.

Conclusion

The gram positive cocci is less in number than gram negative bacilli in pus .most of the antibiotic show sensitivity for gram positive and gram negative bacteria those was present in pus .Among the Gram negative bacilli *E. coli* is the most common bacteria causing wound

infection.

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