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Evaluation of Bacterial Isolates of Pus and Their Antimicrobial Sensitivity Pattern

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Abstract

Background: Antimicrobial resistance leads to increase in morbidity, mortality, prolonged hospitalization and huge economic burden on the society. Therefore we need to know the changing pattern of antimicrobial resistance. This study was done to evaluate the bacteriological profile and their sensitivity pattern of pus culture isolates at Patna Medical College and Hospital Bihar. **Subjects and Methods:** This retrospective study included 213 pus samples collected from patients attending OPD and IPD of different departments of the hospital, presenting pus discharge over a period of 12 months from April 2019 to March 2020. Standard procedure was done for isolation of bacteria from the pus samples and their sensitivity was done using different antibiotics. **Results:** Out of 213 pus samples 134 (62.9%) were found culture positive in which 128 showed single type of isolate and six with two isolates. Rest 76(37.1%) showed no growth. Staph aureus was found the most common isolate 43 (30.71%) followed by E. coli 32(22.86%), Streptococcus pyogenes 21(15%), Klebsiella 18(12.86%), Pseudomonas 15 (10.71%) and Proteus 11(7.86%) in decreasing order. Increased resistance to commonly used antibiotics was seen. **Conclusion:** This study evaluates the major pus isolates and their sensitivity pattern. Most of the isolates has shown resistance to the commonly used antibiotics showing improper or misuse of antibiotics. So culture and sensitivity of each and every pus sample is extremely recommended for proper treatment and prevent resistance based complications.

Keywords: Pus culture, Bacterial isolates, Sensitivity pattern, Resistance.

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Introduction

Break in the skin following burn, injury, bites or any surgical procedures (exogenous) or the organisms reach on site by blood stream (endogenous) allow their multiplication and by the defense mechanism, aggregation of leucocytes, accumulation of tissue debris causes pus formation as thick white liquid. Pyogenic bacteria are the most common cause of pus formation. [1] Most of the pyogenic infections are caused by aerobic bacteria which includes gram positive Staph. Aureus, Streptococcus pyogenes and gram negative like E. coli, Klebsiella, Proteus and Pseudomonas.[2] In most of the cases antimicrobial treatment is started empirically without culture and sensitivity report.[3] Indiscriminate prescription and improper use of antimicrobials are responsible for emergence of resistance strains.[4] These resistant bacterial infections cause significant morbidity, prolonged hospitalization and huge economic burden to the society and grave threat to the public health worldwide.[5] Resistance also causes higher risk of death, hampers the control of infectious diseases by reducing effectiveness of treatment supporting spreading of resistant organisms in the society.[6] Although the bacteriological profile of pus in many studies have similar results, the antimicrobial resistance pattern of isolated organisms show lot of variations.[7] So continued surveillance of the changing sensitivity pattern of microorganisms is the necessity of the time to provide proper treatment This retrospective study was done to evaluate the microorganisms responsible for pus formation and their sensitivity/resistance pattern.

Subjects and Methods

Selection of participants:
Samples were collected from patients attending the OPD and IPD of Patna medical college, Patna during the period of 12 months (April 2019 to march 2020).

Preparation of materials:
All the media in the experiment as nutrient agar (NA), MacConkey agar, blood agar, Chocolate agar and Muller Hinton agar were prepared in sterilized manner supplied by Hi media laboratory. The glassware including petri dishes were sterilized in regular manner by autoclaving during the period.

Collection of samples:
Pus samples were collected using Hi media sterile cotton swabs placed in screw capped tubes and pus aspirates were collected by using sterile disposable syringes.

Isolation of bacteria:
Under the biosafety cabinet after dilution of the specimen in normal saline the specimen was added in the molten agar after being cooled and solidified. The petri dish was incubated at 37°C. After 24 hours of incubation bacterial colonies appear on the plate and counted for detection of significant bacteria. Then a loop full of each sample were smeared on to solid media (MacConkey, chocolate and blood agar plate) by streaking method of culture for isolation of bacteria in the pus sample and inoculated culture plates were incubated at 37°C aerobically overnight in the incubator. On the next day selection of plates with growth were done and subculture of the growth on agar were done for the purpose of identification of bacteria.

Identification of bacteria:
After incubation culture media were taken out of incubator and examined in open light to see the appearance of bacterial colonies (size, shape, consistency, density, color of colony on different culture media and pigment production if any in the media and of any odor). This was followed by Grams staining and motility test under the microscope and lastly a battery of biochemical test as catalase test, oxidase test, indole test, citrate utilization, urease test, triple sugar iron agar test H2S production and others were applied.

Antimicrobial susceptibility test (AST):
Kirby-Bauer's disc diffusion test (1966) was used for antimicrobial sensitivity. The turbidity of the inoculum was adjusted to 0.5 McFarland opacity standard which is equivalent to 1.5*108 CFU/ml of bacteria by inoculating the test organism in broth solution followed by inoculating at 37°C for 2-4 hours, 0.1 ml of broth is inoculated on the surface of culture media by steaking with sterile cotton swab, left for 10 minutes. Then antibiotic impregnated 6mm diameter filter paper disc (Hi media) were dispensed with dispenser on to the media streaked with isolates and the reading were taken after incubating the plate for 24 hours at 37°C aerobically. Next day the diameter of zone of inhibition was measured and compared with the zone diameter interpretation chart provided by Hi media and the result as sensitivity for resistance of the isolated bacteria to antibiotics are determined.

Results

Table 1: Bacterial isolates in pus culture

Culture of Pus Specimen	Frequency	Percentage (%)
Growth (Single growth + mixed)	134 (128 + 6)	62.9
No growth	79	37.1
Total	213	100

Table 2: Type of Bacterial Isolates, Their Number and Percentage

Bacterial isolates	No. of samples	Percentage (%)
Staphylococcus aureus	43	30.71
E. coli	32	22.86
Streptococcus	21	15
Klebsiella	18	12.86
Pseudomonas	15	10.71
Proteus	11	7.86
Total	140	100

Table 3: Sensitivity Pattern of Gram-Negative Isolates (number and percentage)

Antimicrobials	E. Coli (n=32)	Klebsiella(n=18)	Pseudomonas(n=15)	Proteus(n=11)
Amikacin	23(71.8)	13(72.2)	10(66.6)	10(90.9)
Ceftriaxone	15(46.8)	8(44.4)	10(66.6)	8(72.7)
Cefoperazone + sulbactam	27(84.3)	15(83.3)	13(86.6)	10(90.9)
Cefepime	15(46.8)	09(50)	11(73.3)	9(81.8)
Ciprofloxacin	9(28.1)	6(33.3)	8(53.3)	7(63.6)
Cotrimoxazole	11(34.3)	5(27.7)	3(20)	4(36.4)
Gentamicin	23(71.8)	14(77.7)	9(66.6)	7(63.6)
Imipenem	24(75)	12(66.6)	11(73.3)	7(63.6)
Levofloxacin	14(44.8)	7(38.8)	7(46.6)	6(54.4)
Meropenem	22(68.7)	10(55.5)	11(73.3)	7(63.6)
Netilmicin	17(53.7)	11(61.1)	10(66.6)	6(66.6)
Piperacillin + tazobactam	20(62.5)	14(77.7)	11(73.3)	9(81.8)

Table 4: sensitivity pattern of gram-positive isolates

Antimicrobials	Staphylococcus aureus(n=43)	Streptococcus pyrogens (n=21)
Ampicillin	16(37.2)	3(14.2)
Amoxicillin+ Clavulanic Acid	3172.1)	15(71.4)
Azithromycin	30(69.7)	15(71.4)
Cefoperazone + Sulbactam	34(79)	17(80.9)
Ceftriaxone	26(60.5)	13(61.9)
Ciprofloxacin	18(41.8)	9(42.8)
Clindamycin	35(81.4)	18(85.7)
Gentamicin	27(62.7)	12(57.1)
Linezolid	36(83.7)	18(85.7)
Meropenem	35(81.4)	18(85.7)
Teicoplanin	37(86)	18(85.7)
Vancomycin	38(88.3)	18(85.7)

A total number of 213 pus samples were included in the study. Data were collected and presented in the tables. Out if 213 samples 134(62.9%) were found culture positive. Out of which 128 showed single growth and six showed mixed (two organisms) growth. Rest 79(37.1%) samples showed no growth. So total number of isolates became140. Out of which gram negative were 76(54.3%) and rest 64(45.7%) were gram positive.

Discussion

In this study out of 213 samples majority 134(62.9%) were

culture positive whereas rest 79 (37.1%) had shown no growth. The result is very much similar to the report of Trojan R et al (60.1% culture positive and 39.9% negative) Roopa C and Deepali V (60.4% positive and 39.6% negative). Among the positive culture gram negative bacteria were 76 (53%) and gram positive 64 (45.7%) similar to other reports as gram negative dominated over positive.[8]

The most common isolate was Staph. Aureus 30.71% of total. In the report of Mantravadi HB et al, Sudhaharan S et al, Tiwari & Kaur and Kumar R et al [9,10,11,12] also S. aureus was most common isolate. But Agnihotri et al [13] reported S. aureus as 2nd most common isolate. It was highly sensitive to Vancomycin 88.3%; Teicoplanin 86%; Linezolid 83.7 %; Meropenem 81.4%; Clindamycin 81.4% Cefoperazone and Sulbactam 79% and Amoxycillin plus Clavulanic acid 72.1%. but low sensitivity to Ciprofloxacin, Ampicillin and Ceftriaxone.

Second most common isolate was E. coli 22.86%. This was highly sensitive to Cefoperazone plus Sulbactam 84.3%, Imipenem 75%, Amikacin 71.8%, Gentamicin 71.8%, Meropenem 68.7% and low sensitivity to Ceftriaxone, Ciprofloxacin, Levofloxacin and Cotrimoxazole. The result was comparable to study of Kumar R et al. [12]

Percentage of Klebsiella isolates was 12.76%. It was highly sensitive to Cefoperazone plus Sulbactam 83.3%, Piperacillin plus Tazobactam 77.7%, Gentamicin 77.7%, Amikacin 72.2% and low sensitivity to Ceftriaxone, Ciprofloxacin con, Levofloxacin and Cotrimoxazole. The result was comparable to the report of Trojan R et al, Roopa C and Deepali V. [7,8]

Streptococcus pyogenes was isolated in 21 (15%) positive samples. They were highly sensitive to Vancomycin 88.7%, Teicoplanin 85.7%, Meropenem 85.7%, Linezolid 85.7% Clindamycin 83.7% Cefoperazone plus Sulbactam 80.9% and Azithromycin 71.4%, Amoxycillin + Clavulanic acid 71.4% and less sensitive to Ciprofloxacin, Gentamicin and Ampicillin.

Pseudomonas was 10.71% of total isolates. In the study of Trojan et al,[7] Pseudomonas was reported 9% in comparison to 10.71%. It was highly sensitive to Cefoperazone plus Sulbactam 86.6%, Imipenem and Meropenem both 73.3%, Amikacin and Gentamicin both 66.6% and less sensitivity to Ciprofloxacin, Levofloxacin and Cotrimoxazole.

Number of Proteus isolates was 11(7.86%). Roopa C and Deepali V reported Proetus 9.57%. Which is comparable to this study. Proteus was highly sensitive to Amikacin 90.9%, Cefoperazone plus Sulbactam 90.9%, Piperacillin plus Tazobactam 81.8%, Cefepime 81.8% and less sensitive to levofloxacin and Cotrimoxazole.

Almost all the studies show increasing resistance to the antibiotics due to self-medication, improper empirical formula and overuse resulting in increased morbidity, mortality and economic burden on the society. [13]

Conclusion

This study evaluates the bacterial isolates present in the pus samples and their sensitivity pattern which is comparable to other reported studies. Staph. aureus was found the most common isolate in pus samples followed by E. coli, Streptococcus pyogenes, Klebsiella, Pseudomonas and Proteus in decreasing order of frequency. Gram negative bacterial isolates were more in number. E. coli was most common among gram negative bacteria. In this study the isolates have shown different levels of sensitivity to different antimicrobials and mild to moderate level of resistance due to improper and overuse of antibiotics. Sensitivity pattern varies time to time and place to place. So, there is need of periodic surveillance to know the changing pattern of antimicrobial sensitivity of microorganisms to support empirical therapy and culture and sensitivity is must to modify the treatment to cure the patients.

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