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RESEARCH ARTICLE

Characterization of the microorganisms of human pus samples- a tertiary care hospital based study

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ABSTRACT

The term "pyogenic infection" describes an infectious agent-induced localized inflammation and pus production caused by a buildup of dead leukocytes. 120 patients' specimens were gathered and grown on blood agar and MacCokey agar. Biochemical tests were used to identify colonies grown in these conditions, and subsequently their antibiotic susceptibility was examined. When combined with the patient's medical history, these data showed how common pyogenic infections were in BIHS General hospital, Dhaka. Within 86.67% of the Positive pyogenic infection were due to bacterial agents, with *Staphylococcus aureus (23.07%), Escherichia coli (15.39%), Klebsiella (15.39%), Proteus (15.39%), Pseudomonas (7.69%), Enterococci (7.69%), Citrobacter (7.69%), Enterobacter (7.69%)* being the major pathogens. Female was predominant than male which was 72 (60%) cases and 48 (40%) cases respectively. The most common age group for both the male and female groups was 40–60 years old, with 16 (13.33%) and 64 (53.33%) instances, respectively. In conclusion, Staphylococcus aureus is the most often isolated bacterium following aerobic culture of pus. Nonetheless, there are fewer gram-positive cocci than gram-negative bacilli.

Keywords: staphylococcus aureus; escherichia coli; pus sample; pyogenic

1. Introduction

Numerous localized inflammations can be indicative of a pyrogenic infection. Both endogenous and external infections are possible. Typically, it manifests as pus, a thick, opaque substance that is yellowish or greenish that is created from the infected tissue of the lesion. Pus is the outcome of an infection, typically

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brought on by bacteria or fungi, to which the body's natural immune system has responded naturally. They target the microorganisms responsible for infection. One particular function of neutrophils, a subset of leukocytes, is to combat pathogenic bacteria or fungus. Pus therefore contains dead germs as well. Aerobic and anaerobic bacteria are linked to wound infections, causing significant medical costs and prolonged hospital stays. Staphylococcus aureus is more prevalent in pus, indicating coagulase activity. It is possible to list all the pathogens that may be found in pus. Those listed are the more commonly isolated pathogens from wounds and abscesses[1].

Pyogenic infections, caused by microbial pathogens during or after trauma, burn injuries, and surgical procedures, often result in the production of pus, a whitish-yellow, yellow, or brown-yellow protein-rich fluid[2,3]. These infections can be endogenous or exogenous, and often occur under hospital environments, causing significant morbidity, prolonged hospitalization, and a significant economic burden[4]. Staphylococcus aureus is the most common skin colonizer and opportunistic pathogen, causing serious diseases like bacteremia and endocarditis[5]. Its unique ability to rapidly respond and develop resistance to antibiotics makes it a significant concern. The increasing prevalence rate of antibiotic-resistant strains of S. aureus limits treatment options and economic deprivation due to infections, making it crucial to address these infections to prevent further damage to the skin and soft tissue[6].

It accounts for the majority of human infections. Some of them will not respond to treatment with the drug as antibiotics to which it is resistant. In past two decades the rapid emergence of resistance to antimicrobial agents has been one of the mostimportant clinical calamities. Salmonella spp. were the most often reported resistant bacteria, followed by Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, and Streptococcus pneumonia[7]. The way antibiotics are prescribed and used in the world has to alter immediately. Antibiotic resistance will continue to pose a serious concern even in the absence of new medication developments and behavioral changes[8]. Therefore, the present study was undertaken to find out the types of microbiological infection and their antibiotic resistance from the pus sample of the patients who have come to Bangladesh Institute of Health Sciences (BIHS) General Hospital, Bangladesh.

2. Methodology

This study was conducted as a descriptive cross-sectional research. The study was carried out at Bangladesh University of Health Sciences (BUHS), Clinical Microbiology Laboratory and BIHS General Hospital. This study was carried out from January 2019 to December 2019 for the period of 12months. Patients who visited in the BIHS General Hospital's OPD and IPD departments provided pus samples. Variable recorded at the time of enrolment of the study subject includes participantdemographic, personal history. Data relating to critical information were taken fromre designed questionnaire. Sheet of patient kept in the BUHS Patient consent was obtained in questioner sheet by the laboratory person before of sample.

Following a 24-hour aerobic culture at 37 C, bacteria were found. The disc diffusion method was used to test for antibiotic susceptibility and the biochemical test was used to identify the bacterium. Samples of pus were taken from the skin (furuncles, pustules, and abrasions), ears, legs, and nasal wounds. Gram staining and

culture were performed on the pus samples. The samples were aseptically inoculated onto MacConkey's agar and blood agar plates (containing 5% sheep blood), and they were then aerobically incubated for 24 to 48 hours at 35 to 37 degrees Celsius. Using conventional microbiological techniques, isolates were identified and characterized based on biochemical testing, colony characteristics, microscopic features, and Gram staining.

The study used antibiotic discs containing various antibiotics from Himedia Laboratories in Mumbai, India, as per the manufacturer's instructions. The antibiotic susceptibilities of bacterial isolates were determined using the method recommended by the Clinical and Laboratory Standards Institute. The discs included amikacin, amoxicillin-clavulanic acid, azithromycin, ceftriaxone, cefotaxime, cefuroxime, cephalexin, ciprofloxacin, clindamycin, cloxacillin, erythromycin, gentamicin, imipenem, levofloxacin, linezolid, meropenem, ofloxacin, piperacillin-, tetracycline, and vancomycin[13]. To put it briefly, turbidity was adjusted to 0.5 McFarland standard for each bacterial isolate before inocula were generated and distributed out on Muller-Hinton agar plates. The study's numerical data were examined, and statistical techniques were used to determine the significance of the differences. When appropriate, data were presented as percentages.

3. Results

Out of a total 120 pus samples 104 samples were isolated by morphological and biochemical examination. The all-over prevalence of infectious organism in this study was 86.67%. Out of 120 clinical samples.

Table 1. Distribution of study population by age & gender (n=120).

Age Group	Male	Female	Total
< 40 Yrs	0	8 (6.67%)	8 (6.67%)
40-50 Yrs	16 (13.33%)	24 (20%)	40 (33.33%)
50-60 Yrs	0	40 (33.33%)	40 (33.33%)
>60 Yrs	32 (26.67%)	0	32 (26.67%)
Total	**48 (40%)**	**72 (60%)**	**120 (100%)**

Female was predominant than male which was 72 (60%) cases and 48 (40%) cases respectively. The most common age group for both the male and female groups was 40–60 years old, with 16 (13.33%) and 64 (53.33%) instances, respectively.

Table 2. Culture positivity of study population (n=120).

Culture	Frequency	Percent
No growth	8	13.33
Growth	104	86.67
Total	**120**	**100**

Of the 120 cases 104 (86.67%) had aerobic culture positive results, and the remaining 16 (13.33%) had growth negative results. Consequently, culture positive was more than no growth, as demonstrated by this result and reflecting the integrity of the laboratory.

Table 3. Rate of Isolated Bacteria after Aerobic Culture (n=120).

Bacteria	Frequency	Percent
Enterococci	08	7.69%
Citrobacter	08	7.69%
Klebsiella	16	15.39%
Proteus	16	15.39%
E. coli	16	15.39%
S.aureus	24	23.07%
Enterobacter	08	7.69%
Pseudomonus	08	7.69%
Total	**104**	**100%**

Staphylococcus aureus was the most common isolated bacteria from pus which was 3(23.07%) as well as *Klebsiella, Escherichia coli, Proteus, Enterobacter, Enterococci, Pseudomonas* which were 16 (15.39%), 16 (15.39%), 16 (15.39%), 08 (7.69%), 08 (7.69%), 08 (7.69%) isolates respectively.

Table 4. Identification of microorganism through biochemical test.

Test Bacteria	I	N	MR	Co	VP	Ci	PPA	H_2S	U	Cat	M
Staphylococcusaureus	-	+	+	+	+	-	-	-	+	+	+
E.coli	-	+	+	-	-	-	-	-	-	+	+
***Pseudomonas* sp.**	-	+	+	-	-	+	-	-	-	+	+
***Proteus* sp.**	±	-	+	-	-	±	+	+	+	-	+
***Klebsiella* sp.**	±	-	-	-	+	+	-	-	±	-	-

(I)Indole; (N) Nitrate reduction; (MR) Methly red; (Co)Coagulase; (O)Oxidase; (VP) Voges proskauer; (Ci) Citrate;(PPA) Phenylalanine deaminase; (H_2S) Hydrogen sulfide; (U) Urease; (Cat) Catalase; (M)Motility

Figure 1. Antibiotic sensitivity test.

4. Discussion

Any wound has the potential to get infected. The patient becomes sicker and requires more expensive care when a wound refuse to heal. As a result, general wound management procedures become more resource-intensive. Considering that wound infections are now the most frequent infections obtained in hospitals. One of the main causes of wound contamination in hospitals is the environment[9]. In addition to increasing patient trauma, pus infection has been a key cause of worry for medical professionals due to its financial burden and the growing need for cost-effective treatment within the healthcare system[10]. The study found that S. aureus is the leading etiologic agent of infection in health institutions, with it being the most frequently isolated microorganism from pus caused by incisions, fluid collection under the skin surface, and wound types[11,12].

In this study, 86.67% of all samples show growth good results. Of the 120 instances, 104 (86.67%) had aerobic culture positive results, and the remaining 16 (13.33%) had growth negative results. As a result, culture positive growth was greater than no growth, as demonstrated by this outcome and reflecting the validity of the laboratory. There were more female instances than male ones, with 72 (60%) and 48 (40%) cases, respectively. The most common age group for both the male and female groups was 40–60 years old, with 16 (13.33%) and 64 (53.33%) instances, respectively. *Staphylococcus aureus* was the most common isolated bacteria from pus which was 24 (23.07%) as well as *Klebsiella, Escherichia coli*, *Proteus, Enterobacter, Enterococci, Pseudomonas* which were 16 (15 39%), 16 (15.39%), 16 (15.39%), 8 (7.69%), 8 (7.69%), 8 (7.69%) isolates respectively.

In one study conducted in India[13], 86 cases had monomicrobial cultures, 16 had polymicrobial cultures, and 149 cases had no bacterial isolates found. The most common bacteria were Staphylococcus aureus (40%) which was followed by Klebsiella sp. (33%), Pseudomonas sp. (18%), Escherichia coli (16%), and Proteus sp. (2007). This study supports the need of identifying one or more bacterial pathogens from pus cultures due to the diversity of microorganisms and the high occurrence of polymicrobic flora.

A total of 212 individuals who had pus or a wound infection were enrolled in a study conducted in Bangladesh[14]. The majority of the 212 patients (89; 42.0%) belonged to the 20–40 age range. It's interesting to note that there were more male cases than female cases—119 cases, or 56.1%, and 93 cases, or 43.9%, respectively. Out of 131 instances (61.8%), aerobic culture was positive in the majority of cases. The most often isolated bacterium following an aerobic pus culture is Staphylococcus aureus. On the other hand, there are less gram positive cocci than gram negative bacilli. A different study conducted in India[15] included 175 patients and had a 102 (58.28%) bacterial isolation rate. In the samples, the proportion of males to females was greater (M: F ¼ 1.8:1), and the median age was 45 years, with the bulk of the population falling between 40 and 60 years old (41/40.20%). Eleven2 bacterial strains were identified, and 90.1% of the samples had monomicrobial infection, 9.8% had polymicrobial infection. Pseudomonas aeruginosa was the second most common isolate in the current study, after Escherichia coli. The only antibiotic that works against both gram-positive and gram-negative cocci is chloramphenicol. The susceptibility statistic from this research can be

something to take into account when creating empirical treatment plans for pyogenic illnesses.

Singh. A in their study[16], Only 164 (52.73%) of the 311 pus swabs that were collected during their research period revealed bacterial development. Fourteen pus swabs (representing 33 bacterial isolates) and 150 pus swabs (representing 150 bacterial isolates) produced polymicrobial proliferation. Compared to Gram Positive bacteria (39.9%), there were more Gram Negative bacteria (60.1%) present. 36.1% of isolates were S. aureus, followed in frequency by E. Coli (24.0%), Klebsiella (14.2%), Enterobacter (11.5%), Pseudomonas (9.8%), S.pyogenes (3.3%), and Proteus (1.1%). S. aureus had high sensitivity to ceftraixone (72.7%), amikacin (79.5%), doxycycline (90.6%), and chloramphenicol (81.5%). Cefexime, amikacin, chloramphenicol, and azithromycin all showed 100% sensitivity in S. pyogenes, while doxycycline showed 80% sensitivity. Similar to how E. Coli, the most prevalent gram-negative isolate, exhibited higher susceptibilities to Chloramphenicol (71.4%) and Amikacin (66.7%), Klebsiella (92.3%), Gentamicin (87.5%), and Amikacin (81.0%), Enterobacter (90.9%), and Pseudomonas (71.4%) all demonstrated higher levels of sensitivity. Against Proteus species, ceftriaxone, amikacin, gentamicin, ofloxacin, and piperacillin shown 100% sensitivity. Gram negative and gram positive bacterial isolates showed the least sensitivity to amoxyclav, cefepime, and cefexime (except for S. pyogenes). S. aureus was the most often occurring isolate. While doxycycline plus amikacin were found to be successful in treating gram positive isolates, amikacin alone proved to be beneficial for empirical therapy of both gram negative and gram positive bacteria. Every bacterial isolate has a shared resistance to cefepime and amoxyclav[17].

5. Conclusion

Staphylococcus aureus is the most often found isolated bacterium in Pus. E. Coli is the most frequent Gram-negative bacteria that causes wound infections. To determine the true situation with relation to wound infection, a thorough investigation should be carried out.

Disclosure of conflict of interest

No competing interests exist by the authors. This manuscript has not submitted to, nor is under review at another journal or other publishing venue.

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