COMPREHENSIVE ANALYSIS OF BACTERIAL ISOLATES FROM PUS SAMPLES AND THEIR ANTIBIOTIC RESISTANCE PROFILE IN ABBOTTABAD

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Abstract

Pyogenic infections, also known as purulent infections or pus-producing infections result in the production of pus. Pyogenic bacteria usually cause these infections. The widespread and inappropriate use of antibiotics has resulted in antibiotic resistance, which has compromised the effectiveness and consistency of these agents. The aim of the current study was to determine the bacteriological profile from pus samples and their antibiotic susceptibility patterns to commonly used antibiotics. This study was carried out from January to June 2023 at a private diagnostic lab in Abbottabad, Pakistan. 136 pus samples collected over a six-month period were included in the study. Blood agar and MacConkey's agar media were used to inoculate the pus. Standard methods were used to confirm the identification of organisms after incubation at 37 °C for 18-24 hours. Antibiotic sensitivity was determined using the Kirby Bauer disc diffusion method. A total of 136 pus samples were analyzed, with 113 (83.08%) being culture positive. The most common age group was 20-45 years, with 57 (41.92%) cases falling into this group of individuals. 57 (41.92%) of the isolated bacteria were Staphylococcus aureus, 25 (18.38%) were Escherichia coli, 13 (9.55%) were Pseudomonas aeruginosa, 10 (7.35%) were Klebsiella Spp., and 8 (5.88%) were Neisseria meningitides. The most common isolated bacteria were Staphylococcus aureus followed by Escherichia coli, Pseudomonas aeruginosa, Klebsiella Spp, Neisseria meningitides, with the resistant to majority of the commonly used antibiotics.

INTRODUCTION

Pyogenic infections, also known as purulent infections or pus-producing infections result in the production of pus. Pyogenic bacteria usually cause these infections [1, 2]. Pus is an opaque, thick, yellowish or greenish fluid that usually forms at the site of infection. This pus is a product of the immune cells fighting against the pyogenic bacteria or other foreign invaders [2, 3].

Pus is formed because of the accumulation of immune cells (dead leucocytes), dead or damaged tissue, and dead bacteria or fungi [4, 5]. Staphylococcus aureus is the most common causative agent, accounting for 20-40% of all cases [6, 7]. Infection with Pseudomonas aeruginosa is most common after surgery and burns, accounting for 5-15% of cases [2].

Pyogenic infections are frequently associated with Escherichia coli, Klebsiella sp., Proteus sp., and Enterococci sp., [4].

Antibacterial resistance is becoming a serious problem around the globe. However, it has a greater impact in developing countries due to less advanced clinical facilities, poor hygiene and less sanitary conditions as compared to developed countries [8, 9]. Pakistan is one of the developing countries of South Asia where antibiotic resistance is also a major problem [8, 10]. Several studies on clinical isolates from different cities in Pakistan showed that bacterial strains were 93.7% resistant to third generation cephalosporin, 71% resistant to Metallo–lactamase, 40% resistant to extended spectrum -lactamase, and carbapenem and colistin in human samples [10-13].

Regardless with advances in diagnostic techniques and procedures, drug resistance has made it difficult to treat pyogenic infections; especially in developing countries like Pakistan [2, 14, 15]. Antibiotic overuse or misuse has resulted in the development of drug resistance in bacteria (Gram-negative bacteria), contributing to illness and death in humans [2, 16, 17]. Therefore, understanding the etiological agents of pyogenic infections will help the doctors in selecting an appropriate antibiotic therapy to treat such infections [2, 4]. It is best to administer the appropriate antibiotic after the wound swab or pus has been cultured and found to be sensitive [18, 19]. This will help control the rising incidence of drug resistance to widely used medications [19]. Hence, the objective of the current study is to identify the pyogenic bacteria in pus sample and determine their antibiotic susceptibility pattern to commonly used antibiotics in hospitals.

Methodology

This retrospective cross-sectional study was conducted in a private Diagnostic Lab, Abbottabad, Pakistan. The record was collected for a period of six months, from January to June 2023. The biodata of the patients were collected. Pus was cultivated in a standard laboratory setting, and the phenotypic approach was used to determine the antimicrobial sensitivity. The pus culture and sensitivity reports were obtained from the record retrospectively. The study population consisted of pus samples that were taken from the patients' legs, ears, and post-operative wounds as well as from their skin and soft tissue infections (furuncles, pustules, and abrasions). Pus samples were collected using sterile techniques and stored in Cary-Blair transport medium until Gram staining and culturing were performed. These samples were inoculated aseptically onto Blood Agar plates containing 7% sheep blood and MacConkey Agar plates. The plates were then incubated at 37°C for 24 to 48 hours under aerobic conditions. Isolates were identified and characterized using standard microbiological methods such as Gram-staining, microscopic characteristics, colony characteristics, and biochemical tests.

Antibiotic-Susceptibility Test

Antimicrobial susceptibility was tested using Kirby-Bauer's Disc Diffusion method, which was interpreted in accordance with Clinical Laboratory Standard Institution (CLSI) guidelines [20]. Briefly, each bacterium inoculum was prepared through adjusting its turbidity to the 0.5 McFarland standard. The prepared inoculum was then evenly spread onto Muller-Hinton agar plates. The Antibiotic discs with the following contents: amikacin (30μg), amoxicillin-clavulanic acid (30μg), aztreonam (30μg), ampicillin (10μg), azithromycin (30μg), cefepime (30μg), cefoperazone/ Sulbactam (75/30μg), ceftriaxone (30μg), cefuroxime (30μg), ciprofloxacin (1μg), clindamycin (2μg), cloxacillin (30μg), trimethoprim/ sulfamethoxazole (25μg), erythromycin (15μg), imipenem (10μg), levofloxacin (5μg), linezolid (30μg), meropenem (10μg), ofloxacin (5μg), piperacillin-tazobactam (100/10μg), tetracycline (30μg), and vancomycin (30μg), Oxicillin enozabid, sparfloxacin, doxifloxacin, nitrofurontion, nalixidic acid, cephrodine, and cefixime were placed on the M-H agar plates. The plates were then incubated for 24 hours at 37°C. Using CLSI tables and guidelines (CLSI, 2010), the zones of inhibition were measured and the isolates were categorised as sensitive, intermediate, and resistant.

Results

In the current study, 136 pus culture reports were examined. Culture was positive in 113 (83.08%) of the 136 pus samples tested, while it was negative in 23 (16.92%). The bacterial isolates were classified into five species based on biochemical characterization, morphological features, culture characteristics, and Gram staining as depicted in fig-1. The middle age group of 20-45 years was the most commonly affected which was 57 (41.20%). The most commonly affected age group was middle age, 20–45 years, with 57 (41.20%) of them being in this range. Males were affected at a higher rate 83(61.02%) than females 53(38.98%) as presented in the Table-1. The most common isolate was Staph. aureus, with 57 cases (41.92%), followed by E. Coli 25 cases (18.38%), P. aeruginosa 13 cases (09.55%), Klebsiella spp. 10 cases (07.35%), and N. meningitides 8 cases (05.88%) (Table- 2).

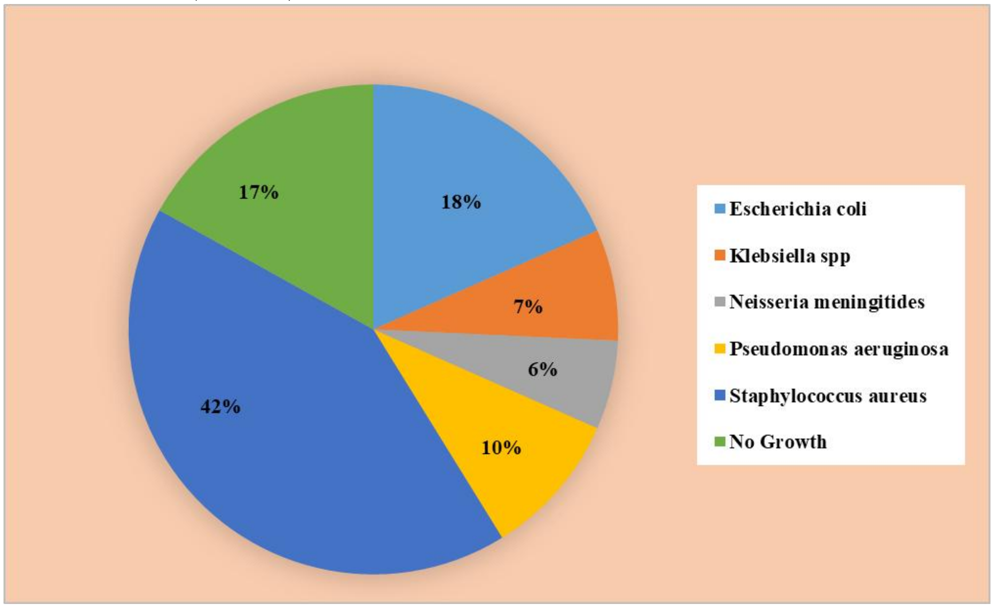

Figure 1. Percentage of isolated bacteria

Table 1: Age and gender wise distribution

Age (Years)	No. of Isolates (%age)	Gender (Sex)	
		Male (%age)	Female (%age)
1 To 19	37 (27.20%)	21 (15.44%)	16 (11.76%)
20 To 45	57 (41.92%)	36 (26.47%)	21 (15.45%)
More than 45	42 (30.89%)	26 (19.12%)	16 (11.77%)
Total	**136 (100%)**	**83 (61.02%)**	**53 (38.98%)**

Table 2: Number of bacteria isolated from pus culture

Bacteria	No. of Bacteria	Percentage (%)
Escherichia coli	25	18.38%
Klebsiella spp	10	07.35%
Neisseria meningitides	08	05.88%
Pseudomonas aeruginosa	13	09.55%
Staphylococcus aureus	57	41.92%
No Growth	23	16.92%
Total	**136**	**100**

The antibiotic sensitivity pattern of all isolated strains were presented in Table-3. This showed that Staph. aureus was 100% sensitive to Fosfomycin and Nitrofurantoin followed by Tobramycin (80%), Gentamycin (58%), Cefoperazone Sulbactam (57%), Lmipenem (57%), Cefixime (50%), and Levofloxacin (50%) and was found highly resistant to Ofloxacin (88%), Amikacin (80%), Amoxicillin (71%), Ciprofloxacin (66%), which is followed by Cephradine (60%). P. aeruginosa was found 100% resistant to Amoxicillin and Levofloxacin (98%) followed by Ofloxacin (80%), Ciprofloxacin (75%), Spraxin (70%) and showed (60%), sensitivity against Tobramycin and Amikacin only, followed by Ciprofloxacin and Nitrofurantoin (50%) each. N. meningitides was highly (97%) sensitive to Cefoperazone Sulbactam and Fosfomycin and showed 97 % resistant to Ciprofloxacin and Lmipenem. Klebsiella spp. found highly resistant to the most of the tested antibiotic including Cefixime, Cefoperazone Sulbactam, Tobramycin (100%) each, followed by Nitrofurantoin (98%), Amoxicillin and Levofloxacin (95%) each, Ciprofloxacin 92% and Spraxin (72%). E. coli showed highest sensitivity to Fosfomycin (98%) and Amikacin (71%) followed by Nitrofurantoin (66%), Gentamycin (54%), Cefoperazone Sulbactam, and Levofloxacin (50%) each and was found 100% resistant to Tobramycin followed by Amoxicillin (96%), Ofloxacin (84%), Aztreonam, and Spraxcin (75%) each.

Table 3: Antibiotic susceptibility pattern of bacterial isolates from pus samples

Antibiotics	**E. coli (n-25)**		**Klebsiella spp. (n-10)**		**N. meningitides (n -08)**		**P. aeruginosa (n-13)**		**Staph. aureus (n-57)**	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
AMC	3.2%	96.8%	5%	95%	–	–	0.0%	100%	28.57%	71.43%
AK	71.42%	28.57%	50%	50%	96.60%	3.40%	60%	40%	20%	80%
ATM	23.70%	76.30%	–	–	–	–	–	–	–	–
CIP	33.33%	66.67%	7.50%	92.50%	–	–	25.34%	74.66%	33.33%	66.67%
CRO	16.66%	83.34%	–	–	–	–	50%	50%	–	–
CFX	–	–	0%	100%	–	–	–	–	50%	50%
CRX	–	–	11.30%	88.70%	–	–	–	–	50%	50%
CPD	–	–	–	–	–	–	–	–	40%	60%
CFS	50%	50%	0%	100%	97.50%	2.50%	–	–	57.14%	42.86%
FO	98%	2.0%	80%	20%	97.35%	2.65%	–	–	100%	0.0%
GTM	54%	46%	29%	71%	10%	90%	32%	68%	58.10%	41.90%
LIP	50%	50%	50.14%	49.86%	2.30%	97.70%	–	–	57.14%	42.86%
LEV	50%	50%	4.30%	95.70%	3.20%	96.80%	1.70%	98.30%	50%	50%
NIT	66.67%	33.33%	1.87%	98.13%	–	–	50%	50%	100%	0.0%
OF	15.67%	84.33%	32%	68%	–	–	20%	80%	12%	88%
PPC	30%	70%	–	–	–	–	–	–	50%	50%
SPX	25%	75%	28%	72%	–	–	30%	70%	50%	50%
TBM	0.0%	100%	0.0%	100%	–	–	60%	40%	80%	20%

S- Sensitive, R-Resistance, AMC- Amoxicillin, AK-Am ATM- Aztreonam, CIP- Ciprofloxacin, CRO- Ceftriaxone Cefixime, CRX- Cefuroxime Sodium, CPD- Cephradine Cefoperazone Sulbactam, FO- Fosfomycin, GTM- Genta LIP- Lmipenem, LEV- Levofloxacin, NIT-Nitrofurantoi Ofloxacin, PPC-Pipracillin, TBM- Tobramycin, SPX-Sprax

Discussions

Pyogenic infections are caused by various pyogenic bacteria and are usually accompanied by localized and systemic inflammation, which results in pus formation. These infections can increase overall costs and morbidity, prolong hospital stays, and hinders wound healing [21, 22]. Understanding the bacterial pathogens and choosing the accurate antibiotics are essential for treating purulent infections effectively. The number of different microbes identified in pus samples is rising, and resistance to various antimicrobial agents has been found in both common as well as uncommon strains [2]. As a result, the clinical microbiologist needs to be educated about the most recent techniques for characterizing these microbes in order to choose the appropriate antibiotic and administer it at the right dose and frequency [23].

Inaccurate prescriptions and misuse of antimicrobial agents in both humans and animals husbandry have contributed to the rapid emergence of antibiotic resistance among microbes [24, 25]. However, doctors must start empirical therapy in order to manage the severe sequel of infections. Steady observation of pus isolates with their local antibiotic susceptibility pattern in a particular region is required to determine the occurrence of bacterial pathogens and to apply effective empirical treatment [2, 26]. The purpose of this retrospective study was to identify the patterns of antibiotic sensitivity associated with various wound infections.

A total of 136 pus culture reports were examined in the present study. Positive cultures were found in 113 (83.08%) of the 136 pus samples tested, while negative cultures were found in 23 (16.92%). These findings are comparable to those of Huda et al. (2022), who found 180 cases of aerobic cultures, 140 (77.8%) of which were positive, and the remaining 40 (22.2%) were growth negative [27]. Similar studies were also carried out by others researchers [28, 29]. Males in this study had showed more growth culture rates 83(61.02%) than females 53(38.98%). The reason might be that men work outside for a living more than women, putting them at a higher risk of injury and infection during activities. These findings were confirmed by other similar studies [2, 27, 30]. Since the age-wise group, the age group with the highest percentage of positive culture was 19 to 45 years old [57(41.92%)], followed by more than 45 years old [42(30.89%)], and less than 19 years old [37 (17.9%)], These results were confirmed by other similar studies [4, 27].

In the current study, both gram-positive and gram-negative bacteria were isolated from pus sample. Staph. aureus was found in 57 (42%) of the 136 culture positive pus samples, E. coli in 25 (18.30%), P. aeruginosa in 13 (09.55%), Klebsiella species in 10 (07.3%), and N. meningitides in 08 (05.88%). This study found that the percentage of Gram-Positive bacteria (57%) was higher than the percentage of Gram-Negative bacteria (43%). These results are consistent with those of Jadoon et al., (2019), who found Staph. aureus, E. coli, K. pneumoniae, and P. aeruginosa predominating in pus samples from patients treated in hospitals. A similar study conducted by Maharjan and Mahawal, showed a higher incidence of Gram-positive bacteria (60.60%) than Gram-Negative bacteria [31]. In this study, the most common pathogen among Gram-Positive pathogen was Staph. aureus, as reported in many other studies [2, 31]. However, among the Gram-Negative bacteria the most common pathogen was E.coli compared to P. aeruginosa as reported in studies [2, 8]. It might be due to their antibiotic resistance patterns differ. As seen in our study, E. coli showed more resistance to the majority of the tested antibiotics than P. aeruginosa [27].

As shown in Table 3, Staph. aureus was found to be sensitive to Fosfomycin and Nitrofurantoin while being highly resistant to Ofloxacin, Amikacin, Amoxicillin, and Ciprofloxacin. Among Gram-negative pathogens however, were all found to be highly resistant to Ciprofloxacin and Amoxicillin and moderately sensitive to Amikacin and Fosfomycin. Klebsiella spp. had the highest number of isolates resistant to multiple drugs compared to other Gram-Negative isolates. Patients in the Jadoon et al.2019 study received pus drainage as well as empirical antibiotics. He reported in this study that S. aureus was found to be susceptible to sipraxin and vancomycin but highly resistant to penicillin, ampicillin, amoxicillin, and nalidixic acid. However, among Gram-Negative, they were all relatively sensitive to azaetronam and amikacin, they were all extremely resistant to ciprofloxacin and amoxicillin (Jadoon et al., 2019). Moreover, the bacteriological profile of children's wound infections were identical to that of adults [32]. The pus culture and sensitivity reports were also found in many other studies [2, 5, 32, 33]. The current study demonstrates that despite the region's differences, the bacterial isolates that cause infection and their antibiogram from this area are similar to those from any other area of Pakistan.

Conclusions

Pyogenic wound infections have been found to be prevalent among the District Abbottabad Hospitals. The majority of the patients in this study are of middle-aged. Staphylococcus aureus is the most isolated bacteria, followed by Escherichia coli, Pseudomonas aeruginosa, Klebsiella Spp, and Neisseria Meningitides. High to moderate levels of resistance to various antibiotic classes were shown by bacterial isolates. This study provides insight into the common organisms responsible for wound infection as well as the antibiotic susceptibility pattern, which will help in the implementation of empiric treatment strategies for pyogenic infections. Furthermore, strict health regulations should be implemented concurrently to control antibiotic prescription and purchase, prohibit free use, and provide regular monitoring and reporting of antibiotic resistance. As a result, background knowledge of local antibiogram is required to settle normal drug use and effectively implement the hospital antibiotic policy.

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