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Bacteriological Profile and Antimicrobial Susceptibility Pattern of Pus Isolates from Tertiary Care Hospital

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ABSTRACT

OBJECTIVE: To detect the most frequent bacterial pathogens causing wound infections and monitor their susceptibility profile at a tertiary care hospital in Karachi, Pakistan.
METHODOLOGY: A retrospective cross-sectional study spanning 12 months from March 2020 to February 2021 was carried out at Patel Hospital Karachi. Wound swabs and pus samples were collected by sterile syringe and using a non-probability consecutive sampling technique from both inpatients and outpatients of all age groups and genders and processed by standard microbiological techniques. Antibiotic sensitivity testing was done using the disk diffusion technique per Clinical and Laboratory Standard Institute (CLSI) guidelines.
RESULTS: Among 1000 samples, 725 (72.5%) showed growth, out of which Gram-negative bacteria were 524 (59%), and 359 (41%) were Gram-positive bacteria. Amongst the Gram-positive bacteria, the majority was *Methicillin Resistant Staphylococcus aureus* (MRSA 76%), followed by *Methicillin Sensitive Staphylococcus aureus* (MSSA 24%), *Streptococcus species* (4%) and *Streptococcus pneumonia* (3%). Vancomycin, Linezolid and Chloramphenicol were the most susceptible antibiotics against Gram-positive organisms. The frequent Gram negative organisms were *Pseudomonas aeruginosa* (23%), *Eschericia. coli* (14%), *Klebsiella spp* (11%) and *Acinetobacter baumannii* (8%). Most Gram-negative bacteria were susceptible to Amikacin, Meropenem and Piperacillin-tazobactam and Tigecycline.
CONCLUSION: *S.aureus* and *P.aeruginosa* were the most identified bacteria in pus samples with varying antibiograms. Growing resistance to beta-lactam antibiotics was a severe concern in the current study. This study helps the physicians about the usual microorganisms encountered in pus samples with prudent prescription of antibiotics.

KEYWORDS: Bacteriological Profile, Antimicrobials, Pus, Wound Swabs, Antibiogram, MRSA, CRE

INTRODUCTION

Pyogenic infections caused by various microorganisms present pus formation associated with inflammation. This pus consists of dead leukocytes, cellular debris, infectious agent and dead tissues.[1] The organisms acquire multiple routes to enter the body, such as breaks in the skin or mucous membranes, traumatic wounds or bites, or surgical complications with foreign body implants are all the various modes of entry of microorganisms. Wound infections can pose serious consequences as they can spread to tissues and organs via a hematogenous route[2] and can even lead to fatal sepsis.[3] The frequency of infecting pathogens isolated and their antimicrobial susceptibility varies due to the different geography and the usage of antibiotics causing various infections with multiple bacterial species. Wound infections may be mono-microbial or poly-microbial. *Staph.aureus* and *P.aeruginosa* collectively represent 20-40% of all hospital-acquired, surgical procedure associated and burn infections. Other microorganisms, for instance the members of family *Enterobacteriaciae* and *Enterococci* are also common, particularly after gastrointestinal surgery in immune-suppressed patients.[4] Specifically, Methicillin-resistant *Staph.aureus* (MRSA), accompanied by multidrug-resistant Gram-negative bacteria, is highly associated with pyogenic infections in the present day.[5]

Regardless of the improvements in diagnostic methods and accessibility of antibiotics, managing purulent infections in progressing countries has become difficult owing to the occurrence of multidrug-resistant (MDR) pathogens. The inappropriate practice of antimicrobials has led to the development of antibiotic resistance. Therefore, correctly understanding the local microbial profile and antibiogram of isolates causing wound infection will help clinicians empirically treat wound infection. The continuous surveillance on pus culture isolates will monitor the susceptibility pattern of isolates and resolve the growing incidence of resistance to conventional drugs.[6,7]

Hence, the current study was directed to define the etiology, frequency, and antibiotic susceptibility pattern of positive pus culture isolates, intending to develop an empirical treatment strategy for infected wound patients and on the implementation of infection control policies to minimize the increasing rate of multiple drug resistance (MDR).[8]

METHODOLOGY

This was a retrospective cross-sectional study spanning 12 months from March 2020 to February 2021 conducted at Patel Hospital Karachi. After obtaining ethical approval from the official ethical committee, a total of 1000 pus aspirates and pus swabs were taken using sterile syringes and swabs from both outpatient and inpatients of different hospital wards using a non-probability consecutive sampling technique. Repeated and improperly handled samples from the same patient were excluded from the study. The samples were referred to the laboratory and were inoculated onto Chocolate, Blood and MacConkey agar. They have incubated aerobically for 24-48 hrs at 37°C. Identification of isolates was made based on colony morphology, gram stain, and biochemical tests such as Catalase, Coagulase, Bile esculin, Optochin, Bacitracin, discs for Gram-positive isolates and Oxidase, Urease, Citrate, Indole, Triple sugar iron tests for gram-negative isolates.[9] Negative growth was confirmed after culturing media for two days.[10] Analytical Profile Index (API 20E and 20 NE) was further used to distinguish gram-negative rods. Muller-Hinton agar was used to check antibiotic susceptibility by Disk Diffusion method consistent with Clinical Laboratory Standard Institute (CLSI) protocol.[11]
For Gram-positive bacteria, Ciprofloxacin (CIP) (5µg), Erythromycin (E) (15µg), Penicillin (P) (10U), Ampicillin (30ug), Chloramphenicol (30ug), Vancomycin (VA) (30 µg), Gentamicin (CN) (10 µg), Linezolid (LZD) (30 µg), Oxacillin (1 µg), Trimethoprim/Sulphamethoxazole (SXT) (25 µg), Clindamycin (DA) were used. For Gram-negative bacteria, Ampicillin (AMP) (10µg), Ciprofloxacin (CIP) (5 µg), Ceftriaxone (CRO) (30 µg), Ceftazidime (CAZ)(30 µg), Cefepime (CPM) (30ug), Gentamicin (CN) (10µg), Amikacin (30 µg), Tobramycin (TOB)(10 µg), Trimethoprim/Sulphamethoxazole (SXT) (25 µg), Amoxicillin-Clavulanic acid (AMC) (30/10 µg), Piperacillin-Tazobactum (TZP) (100/10 µg), Meropenem (MEM)(10 µg), Imipenem (IMP)(10ug) and Tigecycline (TGC) were used. The susceptibility breakpoints were interpreted according to Clinical Laboratory Standard Institute (CLSI) guidelines.[12]
E.coli (ATCC25922), Staph .aureus (ATCC25923) and *P.aeruginosa* (ATCC27853) were used as quality control strains for culture and susceptibility testing.
Data were analyzed by using SPSS software version 2. Percentages and frequencies were calculated for variables like microorganisms, sex and antibiotic susceptibility. Stratification was done for culture and sensitivity.

RESULTS

Among 1000 samples, 725 (72.5%) showed positive growth. There were 418 (58%) males and 307 (42%) females. The microbial profile of pus isolates is shown in **Figure I,** which shows *Staph aureus* as the most frequent pathogen encountered. Frequencies of bacteria isolated are shown in **Table I**. Of 725 samples, 536 (74%) showed growth for a single pathogen, while considerable growth was observed in 189 (26%) cases, so total bacterial isolates were 883. Among the positive isolates, 524 (59%) were Gram-negative, and 359 (41%) were Gram-positive organisms. Among the Gram-positive isolates, the principal isolate was *Methicillin Resistant Staph.aureus* (MRSA 76%) followed by *Methicillin Sensitive Staph.aureus* (MSSA 24%), *Enterococcus spp* (7%), *Streptococcus species* (4%) and *Strep.pneumonia* (3%). The frequent Gram-negative organisms were *P.aeruginosa* (23%), *E. coli* (14%), *Klebsiella spp* (11%) and *Acinetobacter baumannii* (8%). **Table II** and **Table III** showed the antimicrobial profile of Gram-negative rods and Gram-positive cocci, respectively. Gram-positive bacteria were mostly sensitive to Chloramphenicol, Vancomycin and Linezolid. Amikacin, Meropenem/Imipenem, Piperacillin-tazobactam, and Tigecycline were the most sensitive antibiotics among Gram-negative bacteria.

FIGURE I: MICROBIAL PROFILE OF PUS ISOLATES

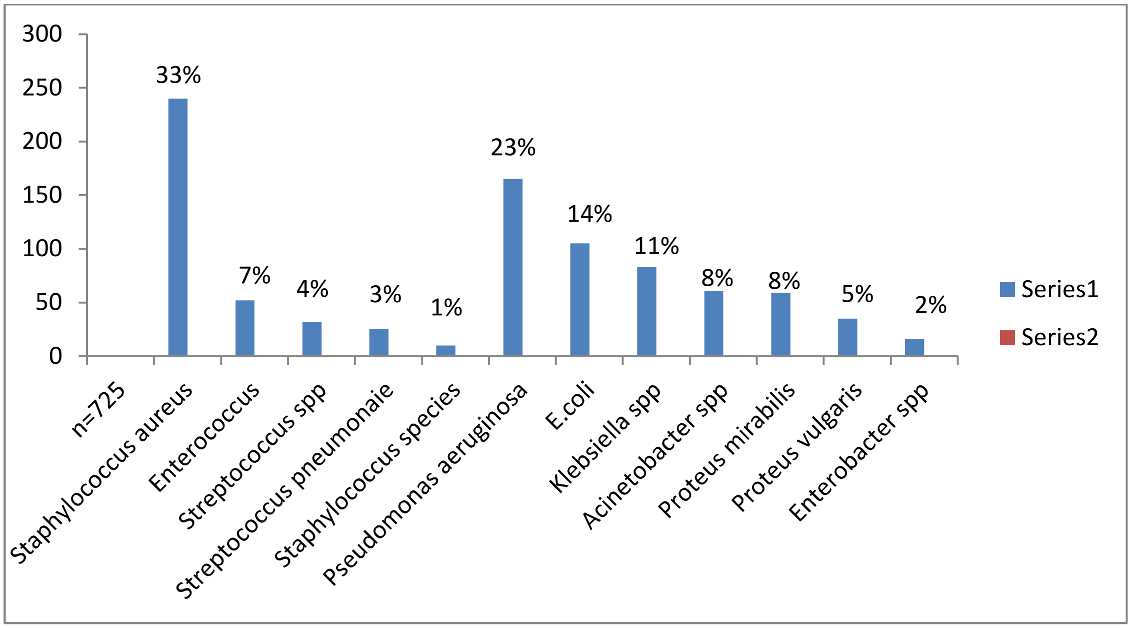

TABLE I: FREQUENCY OF BACTERIA ISOLATED FROM PUS SAMPLES

Organism Isolated	No. of isolates	Percentage
Gram-positive bacteria		
Staphylococcus aureus	240	33%
Enterococcus	52	7%
Streptococcus pneumonaie	25	3%
Streptococcus spp	32	4%
Staphylococcus species	10	1%
Gram-negative bacteria		
Pseudomonas aeruginosa	165	23%
E.coli	105	14%
Klebsiella spp	83	11%
Acinetobacter spp	61	8%
Proteus mirabilis	59	8%
Proteus vulgaris	35	5%
Enterobacter spp	16	2%

TABLE II: ANTIMICROBIAL PROFILE OF GRAM-NEGATIVE RODS

Antibiotic	*E.coli* n=105		*Klebsiella spp* n=83		*Pseudomonas aeruginosa* n=165		*Acinetobacter* n= 61	
	S%	R%	S%	R%	S%	R%	S%	R%
AMP	7	93	NT	NT	NT	NT	NT	NT
AMC	41	59	31	69	NT	NT	NT	NT
TZP	59	41	42	58	59	41	7	93
CRO	21	79	23	77	NT	NT	7	93
CPM	23	77	24	76	50	50	1	99
CAZ	NT	NT	NT	NT	56	44	NT	NT
MEM	80	20	57	43	61	39	20	80
IMI	69	31	52	48	63	37	18	82
AK	86	14	64	36	61	39	28	72
CN	62	38	51	49	55	45	13	87
CIP	19	81	14	86	48	52	7	93
SXT	35	65	28	72	NT	NT	16	84
TGC	90	10	63	37	NT	NT	67	33

**S=Sensitive, *R=Resistant, *NT= Not Tested*

TABLE III: ANTIMICROBIAL PROFILE OF GRAM-POSITIVE COCCI

Antibiotic	*Staph.aureus* n=240		*Staph spp* n=10		*Enterococci* n=52		*Strep pneumo* n= 25		*Strep spp* n=32	
	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%
P	1	99	1	99	40	60	72	68	66	34
AMP	NT	NT	NT	NT	45	55	72	68	72	28
FOX	24	76	10	90	NT	NT	NT	NT	NT	NT
E	4	96	10	90	21	79	40	60	56	44
DA	13	87	50	50	NT	NT	44	56	66	34
SXT	27	73	10	90	NT	NT	48	52	NT	NT
CN	45	55	15	85	NT	NT	NT	NT	NT	NT
C	38	62	60	40	58	42	80	20	75	25
CIP	7	93	10	90	NT	NT	NT	NT	NT	NT
VA	100	0	100	0	63	37	100	0	100	0
LZD	100	0	100	0	100	0	100	0	100	0

**S=Sensitive, *R=Resistant, *NT= Not Tested*

DISCUSSION

Purulent wound infections are illustrated by severe local inflammation, usually with pus formation caused by various purulent bacteria. These infections can extend hospital stays, prevent wound healing, and increase the overall cost and morbidity.[13] Knowledge of bacterial pathogens and the choice of appropriate antibiotics are crucial in effectively treating purulent infections. With the increasing numbers of different organisms being recognized in pus samples and the finding of resistance to multiple antimicrobial agents in both the common and the uncommon isolates, the clinical microbiologist must be familiar with current methods for characterizing these organisms to select the correct antibiotic with proper dosing and frequency.

The rapid development of resistance to various antibiotics has been facilitated by inaccurate prescribing and abuse of these "life-saving" medicines in human and veterinary medicine and animal husbandry. Nevertheless, to control the severe consequences of infection, physicians have to start empirical therapy. To implement effective empirical treatment, periodic surveillance of pus isolates with their local antimicrobial susceptibility pattern in a specific region is needed to assess the prevalence of bacterial pathogens. This research was conducted to detect the causes of different wound infections and their antibiotic sensitivity pattern. In the current study, a total of 1000 pus swabs and pus aspirates were included for one year, out of which 725 came out positive, representing 72.5%, which is consistent with the results reported from Peshawar in 2020 were 80.7% of the cultures were positive.[12] One study in India in 2020 also showed a similar culture positivity rate (65%).[10]

In our present study, the frequency of monomicrobial infection (74%) was more common than polymicrobial infections (26%), which is consistent with the study done in India in 2018.[14] Men showed more excellent positivity rates (58%). The reason may be the greater participation of men in outdoor physical work for a living compared to women and a higher risk of trauma and injuries during activities. Other similar studies supported these findings.[8,15]

In this study, the rate of Gram-negative organisms (59%) was more significant than Gram-positive organisms (41%). The preponderance of Gram Negative bacteria has been highly supported by other studies.[16, 17] However, a similar study conducted in Khatmandu in 2020 showed a more significant occurrence of Gram-positive bacteria (60.6%) than Gram-negative bacteria.[18] *Staph.aureus* was the most frequent pathogen among Gram Positive bacteria, and *P. aeruginosa* was the most frequent pathogen among Gram Negative bacteria, as reported in many studies.[13, 19, 20] Staphylococci are generally found on the skin and mucous membranes of patients and health care practitioners. It can spread via contact with the wound site with the contaminated hands of hospital personnel. Among Gram Negative bacteria, *Pseudomonas aeruginosa* was followed by *E.coli*, *Klebsiella spp* and *Acinetobacter spp*. Gram-negative bacteria are usually found in humans' intestinal tracts, so that they can contaminate wounds during abdominal surgery without many precautions. In contrast, Gram-positive bacteria are commonly present on the skin's surface, where they can infect the wound. A preponderance of mono-microbial growth of the organisms was observed more than polymicrobial growth, which correlates with many studies.[12, 18]

Antibiogram of *Staph.aureus* showed high sensitivity to Linezolid, vancomycin and chloramphenicol. These results agree with the study by Khanam RA et al.[21]. This indicates that Staphylococcus has become highly resistant to the first and second lines of treatment. Methicillin-resistant *Staph.aureus* (MRSA) was found to be 76% in this study, similar to other studies.[14] In contrast, some studies showed the lowest percentage of MRSA.[19, 22] Conversely, *Streptococcus* spp were still sensitive to most antimicrobials. *Enterococcus* spp showed 100 percent sensitivity to Linezolid. Vancomycin-resistant Enterococci (VRE) were found to be 63%in our study.

Among Gram Negative bacteria, Meropenem/Imipenem, Amikacin, Piperacillin-tazobactam and Tigecycline were the most sensitive antibiotics among Gram-Negative isolates. This is following the study done in Rawalpindi in 2017 and India in 2021[23, 24] *Acinetobacter* species were remarkably resistant to multiple antibiotics in our study. Trojan et al. also reported fully bacterial resistance for Imipenem, Amikacin, Ceftriaxone and Ciprofloxacin,[25] Tigecycline showed 67% sensitivity to *Acinetobacter*. MDR *A. baumannii* has become a significant cause of hospital-acquired infections like bacteremia, urinary tract infections, soft tissue infections and surgical site infections because of its considerable ability to survive in both dry and wet environments in hospitals and to acquire resistance to extensive range of antibiotics rapidly. [8] *E.coli* was 86% sensitive to Amikacin and 80% to Meropenem. These findings were nearly identical to Tameezuddin A et al.[23] *P.aeruginosa* showed moderate sensitivity against antibiotics. The most sensitive antibiotics against *P.aeruginosa* were Imipenem, Amikacin, Piperacillin tazobactam and Ceftazidime, as reported by Mudassar S et al. [19] The resistance against Cefepime and Ceftriaxone was high in our study. This determines that bacterial resistance against antibiotics is spreading immensely. The constant exposure of patients to multiple antibiotics, including self-prescribing, is presumed to be the reason for high antimicrobial resistance. Overall, Meropenem/Imipenem, Amikacin, Piperacillin-tazobactam and Tigecycline were found to be the most susceptible antibiotics among Gram Negative bacteria.

CONCLUSION

Gram-negative bacteria were the dominating organisms in our study, with a higher rate of multidrug resistance, indicating a severe problem. These MDR pathogens are resistant to multiple classes of antibiotics. To combat antibiotic resistance, a collaborative and interdisciplinary technique is needed in wound care, continuous surveillance of pus cultures, prudent use of antibiotics and effective infection control policies. Therefore, background knowledge of local antibiograms is required to ensure rational drug use and to implement hospital antibiotic policy.

Ethical Permission: Patel Hospital, Karachi, IRB No: 114, dated 29-04-2021.
Conflict of Interest: The authors have no conflict of interest to declare.
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Data Sharing Statement: The data supporting this study's findings are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

AUTHOR CONTRIBUTIONS

Fatima A:	Concept and design of study
Gohar H:	Drafting and review of manuscript
Dawood K:	Data collection and analysis
Siddiqui HZ:	Interpretation of data
Sajjad M:	Critical review
Naseem S:	Results

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