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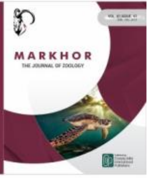

Original Article

Frequency of Micro-organisms in Different Biological Samples of Patients in a Tertiary Care Hospital of Lahore, Pakistan

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ABSTRACT

Bacterial infections are one of the leading causes of illness and death across the world. In this context, the spread of resistant microbes plays a key role. Due to insufficient treatment choices and the sluggish discovery of new antibiotic classes, the rapid emergence of multidrug resistance in bacteria poses a serious threat to global public health. **Objective:** To determine the frequency of clinical isolates in various samples. **Methods:** A cross-sectional investigation was undertaken at the Pathology Department of the Fatima Memorial Hospital in Lahore, Pakistan. The study lasted one year. A total of 1,200 samples (sputum, wound swabs, blood, urine, pus, cerebrospinal fluid, and semen) were collected. Each sample was taken in a sterile container. The sample container was labeled with the source, date, and time of collection within one hour of collection and brought to the laboratory for analysis. Bacteria were identified using traditional culture and biochemical testing. **Results:** A total of 1200 clinical isolates were identified. Positive cultures obtained from 1200 biological samples were mostly from Urine (71.1%). 55.3% of the positive sample were male and 73% of the clinical isolates were gram-negative. Among Gram-negative isolates, the most common pathogen was Klebsiella species (42.9%) and among Gram-Positive isolates, the most common pathogen was Staphylococcus Aureus 68.5%. **Conclusions:** Gram-negative bacterial isolates were prevalent, with 55% frequency distribution and *Staphylococcus aureus* was frequent in different samples of patients.

INTRODUCTION

Bacterial infections are one of the leading causes of illness and death across the world. In this context, the spread of resistant microbes plays a key role. Due to insufficient treatment choices and the sluggish discovery of new antibiotic classes, the rapid emergence of multidrug resistance in bacteria poses a serious threat to global public health [1]. Antibiotic resistance is the ability of certain bacteria strains to develop tolerance to antibiotics that they were previously sensitive to [2]. Antibiotic resistance is a major public health concern that is linked to a high incidence rate of infectious illnesses. Bacterial resistance makes therapy more difficult and costly [3]. Bacterial infections account for one-fifth of all fatalities worldwide. In recent medicine, antibiotics' efficiency against bacteria-caused ailments has been a huge achievement. However, germs began to develop resistance to antibiotics, making them less effective when they were most needed. Antibiotic use can be limited to prevent the spread of drug-resistant microorganisms and maintain antibiotic potency [4].

Antibiotic overuse in the healthcare industry is a key factor in bacterial resistance. Antibodies are being used in such large quantities that microorganisms may develop resistance [5,6]. Antibiotic overuse has resulted in the formation of bacteria that are resistant to antibiotics, known as antibiotic-resistant bacteria (ARB), which are most common in underdeveloped nations where antibiotics are widely utilized. Antibiotic resistance is a natural adaptation that evolved as a result of the high selective pressure caused by antibiotic exposure [7].

Antibiotic resistance causes roughly 7 million deaths each year, and this number is expected to grow in the future years. Antibiotic resistance is causing more infectious illnesses than malignancies globally [8]. Antibiotic resistance has improved as a result of overuse and abuse of antibiotics, as well as present poor hygiene and contaminant control mechanisms [9].

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Antibiotic efficacy is becoming a worry as new antibiotic-resistant bacterial species arise. Despite an ever-increasing demand for new antimicrobial treatments, progress and development of antibiotics appear to have come to a halt in recent years [10].

Bacterial infections include Gram-positive and Gram-negative infections. Gram-positive bacterial infections, such as Methicillin-resistant Staphylococcus aureus (MRSA), Vancomycin-resistant Enterococci (VRE), and Coagulase-negative staphylococci (CONS), have become a serious problem in hospitals due to high morbidity and mortality, as well as a lack of treatment alternatives [11]. Gram-positive bacteria, such as Staphylococcus aureus and Streptococcus pneumonia, have been linked to a variety of bacterial infections, including bone and joint infections, upper and lower respiratory tract infections, bloodstream infections, central nervous system infections, and skin and soft tissue infections [12].

Bacterial infections are a leading source of illness and mortality in Pakistan. In patients getting medical treatment, nosocomial infections were the most common cause of serious disease. Nosocomial infections, often known as healthcare-associated illnesses, can develop as a result of a prolonged hospital stay. Antimicrobial resistance (AMR) is on the rise across the country, stifling all efforts to prevent hospital-acquired illnesses [13]. The majority of bacterial infections can currently be treated with simple antibiotics; however, as germs become more resistant to these medications, therapy becomes more difficult [14].

Antibiotic use is excessive in Pakistan for no apparent reason, and microorganisms are developing resistance to antibiotics as a result of this abuse. Pakistan is a developing country with limited financial and healthcare resources. Antibiotic resistance has increased due to a lack of essential healthcare services, an unclean lifestyle, and patient usage of inferior antibiotics. Antibiotic abuse is linked to bacterial resistance, and multidrug-resistant bacteria (MDR bacteria) are becoming more common [15]. Faster bacterial resistance to various medications leads to serious health issues, such as organ failure, which is widespread. Treatment options are also limited as a result of the growing number of novel bacterial strains [1]. The aim of this study was to determine the frequency of clinical isolates in various samples.

METHODS

A cross-sectional investigation was undertaken at the Pathology Department of the Fatima Memorial Hospital in Lahore, Pakistan. The study lasted one year. A total of 1,200 samples (sputum, wound swabs, blood, urine, pus, CSF, and semen) were collected. Each sample was taken in a sterile container. The sample container was labeled with the source, date, and time of collection within one hour of collection and brought to the laboratory for analysis. Inclusion criteria included patients who have an infection of any kind, patients of both genders, and patients who have never been treated before. Exclusion criteria included patients who are being given antibiotics, children, pregnant women, and patients who do not have any indications or symptoms of infection.

Samples were cultivated on selective media plates from the sample container (Mannitol Salt agar, TCBS Agar, Eosin thiazine Agar, MSA agar, MacConkey Agar, enteric bacteria Agar). After that, the plates were incubated for 24 hours at 37 degrees Celsius. The colonies were then divided and closely examined. For a handful of the plates, cfu/ml was computed, and a few of them exhibited a significant increase. The colonies were then streaked over agar plates to create pure cultures that could be stored. The growth of isolates on Cystine-Lactose-Electrolyte-Deficient (CLED) agar, Blood agar, and MacConkey agar base was used to determine their colonial morphology. The characteristics of the colonies were next examined. Susceptibility and standard identification techniques were used to identify more organisms. On MacConkey agar, E. coli colonies were dry and small pink in color, whereas on Cystine- Lactose-Electrolyte-Deficient (CLED) agar, yellow dry and smooth colonies were seen. On blood agar, mucoid hemolytic colonies were identified, indicating the presence of E. coli. Smooth, massive hemolytic colonies on blood agar were seen in the instance of Pseudomonas aeruginosa. On MacConkey agar, yellow-green non-lactose fermenting colonies were detected.

Biochemical identification of the clinical isolates was done by using various biochemical methods. Tests included were oxidase test, indole test, citrate utilization test, triple sugar iron test, catalase test, and coagulase test. Dark purple color organisms were found in gram-stained smears beneath the oil immersion lens of a microscope in the case of gram-positive bacteria and pink color organisms in the case of gram-negative bacteria. The data was tabulated and analyzed using SPSS 22.0 (Statistical Package for Social Sciences). The qualitative characteristics were reported using percentages and frequencies. A frequency and percentage were assigned to samples and microbiological isolates.

RESULTS

During the study period, a total of 1200 samples were received and evaluated for culture in the microbiology department of Fatima memorial hospital, Lahore.

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Frequency and Percentage of Clinical Isolates from samples: The frequency of total biological samples received and positive culture obtained had been shown in Table 1. Total biological samples of different types used in this study were Urine (380), Sputum (230), Blood (160), Pus (150), Wound (90), CSF (80), Ear Swabs (60) and Seminal Fluid (50). A total 750 (62%) positive cultures obtained from 1200 biological samples were 71.1% (270) from Urine, 57.4% (132) from Sputum, 61.3% (98) from Blood, 60.7% (91) from Pus, 63.3% (57) from Wounds, 55% (44) From Cerebrospinal fluid (CSF), 56% (28) from Seminal fluid, 50% (30) from Ear Swabs.

Samples	Frequency	Positive Culture	Percentage (%)
URINE	380	270	71.1
SPUTUM	230	132	57.4
BLOOD	160	98	61.3
PUS	150	91	60.7
WOUNDS	90	57	63.3
CSF	80	44	55.0
EAR SWABS	60	30	50.0
SEMEN	50	28	56.0
TOTAL COUNT	1200	750	62.5

Table 1: Positive Cultures in Total of Biological Samples

Distribution of Biological Samples: The gender-wise distribution of biological samples had been shown in Figure 1. Out of 1000 positive samples, 553 (55.3%) were from male patients and 447 (44.7%) were from female patients.

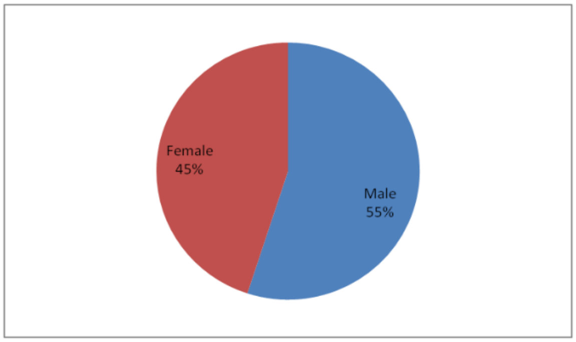

Figure 1: Genderwise distribution of biological samples

According to the gram category, the distribution of clinical isolates had been shown in Figure 2. Out of 750 clinical isolates, based on gram staining and microscopy 200 (27%) were Gram-Positive isolates and 550 (73%) were Gram-Negative isolates.

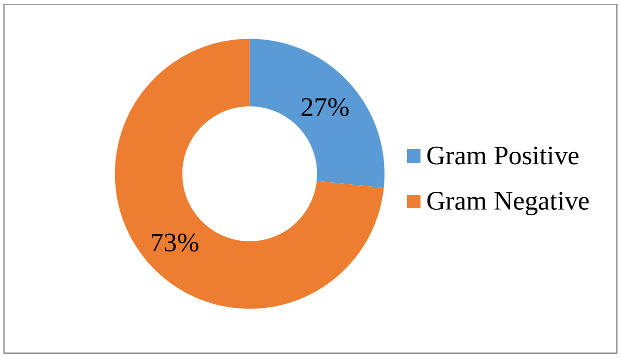

Figure 2: Gram Staining Characteristics of Clinical Isolates

Frequency of Clinical Isolates from Samples: The distribution of various clinical isolates from clinical samples had been shown in Table 2. Among Gram-negative isolates, the most common pathogen was Klebsiella species 236 (42.9%), Escherichia coli 124 (22.5%), Pseudomonas species 98 (17.8%), Proteus Vulgaris 55 (10%), Salmonella Typhi 25 (4.5%), and Acinetobacter species 12 (2.2%). Among Gram-Positive isolates, the most common pathogen was Staphylococcus Aureus 137 (68.5%) and Hemolytic Streptococci 63 (31.5%).

Micro-organisms	**BIOLOGICAL SAMPLES**								**TOTAL**
	Urine	**Blood**	**Pus**	**Sputum**	**Wounds**	**Semen**	**CSF**	**Ear Swab**	
Klebsiella species	126	18	16	32	8	10	12	14	236
Escherichia coli	44	22	12	6	6	16	10	8	124
Pseudomoas species	34	6	7	23	26	0	0	2	98
Proteus Vulgaris	22	4	6	21	0	2	0	0	55
Salmoella Typhi	3	14	8	0	0	0	0	0	25
Acinetobactor species	1	6	3	2	0	0	0	0	12
Staphylococcus aureus	34	22	27	27	14	0	9	4	137
Hemolytic Streptococci	6	6	12	21	3	0	13	2	63
TOTAL	270	98	91	132	57	28	44	30	750

Table 1: Represents Clinical Isolates in Biological Samples

Among all clinical isolates, Klebsiella species were the most common cause of urinary tract infection in patients, and Staphylococcus Aureus, a gram-positive pathogen was the most common cause of pyogenic infections.

DISCUSSION

Antibiotics are essential for more effective and timely treatment and management of bacterial illnesses. The phrase "every invention and discovery has its own drawback" is well-known, and infections are developing resistance to them. Bacteria are acquiring resistance to antibiotics as a result of abuse and overuse in the population, making them uncontrollable and difficult to treat.

Our findings revealed that 750 clinical isolates were collected from 1200 biological samples, with a 62.5 percent infection rate. This was higher than the infection rate reported in another study by Mehta A et al., which was 20% [16]. Urine samples had the highest percentage of clinical isolates (71.1%), followed by wound samples (63.3%), and blood samples (63.3%). (61.3 percent). 60.7 percent (91) of clinical isolates were found in Pus samples, 57.4 percent (132) in Sputum, 56 percent (28) in Seminal fluid, 55 percent (44) in Cerebrospinal fluid (CSF), and 50 percent (30) in Ear Swabs. These findings were in line with a previous study [16]. The number of biological samples collected from male patients 660 (55 percent) was

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larger than the number of samples obtained from female patients 540. (45 percent). A study conducted in Peshawar in 2014 found that males (58 percent) had a higher rate of clinical isolates than girls (42 percent) [13]. Among 750 clinical isolates, 200 (27%) were Gram-Positive and 550 (73%) were Gram-Negative, according to the gram category. In a 2007 study, similar findings were discovered [17]. In Riyadh, Saudi Arabia, Baddour et al., discovered that gram-negative isolates had a greater percentage (64.4%) than gram-positive isolates (35.6%) [18]. Our findings showed that among Gram-negative isolates, the most common pathogen was Klebsiella species 236 (42.9%) and among Gram-Positive isolates, the most common pathogen was Staphylococcus Aureus 137 (68.5%). Similarly, another study showed that among various bacterial species K. pneumonia was prevalent followed by E. coli [19].

Comparative antibacterial activity research will not only provide information on the current antimicrobial pattern but will also aid in advising on the best treatment for bacterial infections. The proliferation of bacterial diseases and the rise of antibiotic resistance have put a huge strain on hospitals [20].

CONCLUSIONS

It is concluded that positive cultures obtained from 1200 biological samples were mostly from Urine (71.1%). 55.3% of the positive sample were male and 73% of the clinical isolates were gram-negative. Among Gram-negative isolates, the most common pathogen was Klebsiella species (42.9%) and among Gram-Positive isolates, the most common pathogen was Staphylococcus Aureus 68.5%. Gram-negative bacterial isolates were prevalent, with 55% frequency distribution and Staphylococcus Aureus was frequent in different samples of patients.

Appropriate prophylactic measures should be implemented to prevent clinical isolates from developing resistance to particular antibiotics and the spread of antibacterial resistance. Appropriate diagnostic facilities can improve results by supporting focused therapy. Policies for keeping track of doctors’ prescriptions should be implemented.

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