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Original Article

Frequency and Gram Categorywise Distribution of Clinical Isolates from Patient samples in tertiary care Hospital of Lahore

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ABSTRACT

Antibiotics form an important tool in the treatment of various bacterial infections but their overuse and misuse are resulting in antibacterial resistance among bacteria. **Objective:** to outline the frequency and gram categorywise distribution of clinical isolates from patient samples **Methods:** In this study, 1000 samples were collected from the patients attending the pathology department of Fatima memorial hospital, Lahore, Pakistan. Identification of bacteria was done through conventional culture and biochemical tests **Results:** one thousand clinical isolated were identified **Conclusions:** Gram negative bacterial isolates were prevalent, with 55% frequency distribution and *Staphylococcus aureus* was frequent as it was identified in 410 different samples of patients.

INTRODUCTION:

Antibiotic resistance is the capacity of certain strains of bacteria to develop a tolerance to specific antibiotics to which they were once sensitive [1]. Increased antibiotic resistance is a great health issue which is directly associated with high incidence rate of infectious diseases. Bacterial resistance also make treatment complicated and expensive [2].

Extensive use of antibiotics in health care sectors is the major contributor of bacterial resistance. This extensive use of antibodies could develop resistance in bacteria [3,4].

Mortality rate due to antibiotic resistance is about 7million per year and may double in upcoming years. Globally the ratio of infectious diseases due to antibiotic resistance is greater than cancers [5].

Now a days antibiotic resistance has become an emerging issue due to the occurrence of novel bacterial strains. With the increasing demand of antibiotics, antibiotic resistance creates a huge hindrance in the development and discovery of new antibiotic drugs and treatments [6]. In Pakistan the use of antibiotic without any cause is high and due to overuse of the antibiotics bacteria are getting resistance against these antibiotics. Very few reports were revealed about the occurrence of antibiotic resistance in bacteria linked to different infections in Pakistan. Some examples of antibiotic resistance occurring in Pakistan at different places had been documented. *Acinetobacter* species had shown resistance to numerous kinds of antibiotics at high level, especially showed resistance to Carbapenem. The resistance to Ceftriaxone and Quinolone in Salmonella species was also increasing in Pakistan [7].

Worldwide bacterial infections diseases are major causes of mortality and morbidity rate. Resistant microorganisms are playing a crucial role in the prevalence of these diseases.

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Faster resistance of bacteria towards different drugs further leads toward major health complications such as organ failure is common. With variety of new bacterial variants treatment options are also reduced [8].

METHODS

Total one-thousand samples (blood, swabs, urine, sputum, pus) were collected. Each sample was collected in a sterile container. After sample collection, samples were cultured on selective media plates (Mannitol Salt agar, TCBS Agar, Eosin thiazine Agar, MSA agar, MacConkey Agar, enteric bacteria enteric bacteria Agar) from the sample container. After that plates were incubated for 24 hours at the temperature of 37ºC. Then separated colonies were carefully observed. cfu/ml was calculated for a few of the plates and a few showed large growth. Then the colonies were streaked on agar plates to induce pure cultures for storage. Isolates Colonial morphology was determined by their growth on Cystine-Lactose-Electrolyte-Deficient (CLED) agar, Blood agar and MacConkey agar base. Then colonies characteristics were also observed. Further oganisms were identified by susceptibility and standard identification techniques. On MacConkey agar dry and small pink color colonies were observed in the case of *E. coli* but yellow dry and smooth colonies were observed on Cystine-Lactose-Electrolyte-Deficient (CLED) agar. While mucoid haemolytic colonies were identified on blood agar that showed the presence of *E. coli*. In the case of *Pseudomonas aeruginosa* smooth, large hemolytic colonies on blood agar were observed. Yellow green non-lactose fermenting colonies on MacConkey agar were observed.

RESULTS

Out of 1000 samples, frequency and percentage of clinical isolates are shown in table 1. According to gram category clinical isolates distribution had been shown in Figure 1. Out of thousand clinical isolates, based on gram staining and microscopy 450 were Gram Positive isolates and 550 were Gram Negative isolates. Total 553 (55.3%) were male patients and 447 (44.7%) were female patents. Frequency of clinical isolates from samples is shown in table 2.

Blood	9.3%, (n=92)
Breast fluid	1.4%, (n=14)
CSF	1.7% (n=17)
Ear swabs	2.0% (n=20)
Fluids	4.1% (n=41)
Folet tips	2.2% (n=22)
HVS	3.7% (n=37)
Pleural tips	2.0% (n=20)
Pus	42.1% (n=421)
Semen	3.8% (n=38)
Sputum	4.5% (n=45)
Swab	3.7% (n=37)
Throat swab	1.2% (n=12)
Urine swab	13.9% (n=139)
Wound swab	4.4% (n=44)

Table 1: Frequency of clinical isolates

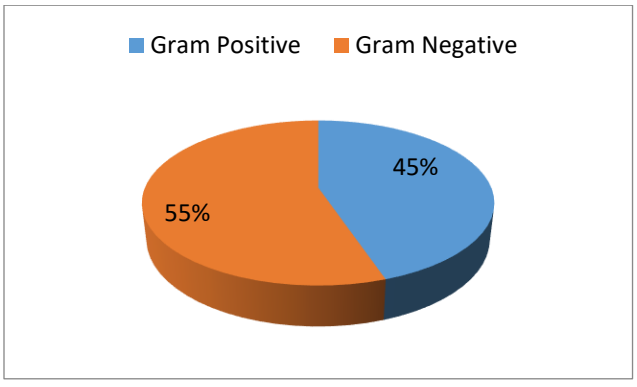

Figure 1: Represents Gram Staining Characteristics of Clinical Isolates

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Microorganism	Frequency	Percent
Actinobacter	6	0.6
Citrobacter	2	0.2
E.coli	131	13.1
E.coli, Enterobacter	9	0.9
E.coli, Klebsiella	1	0.1
E.coli, Pseudomonas	13	1.3
E.coli, Pseudomonas, Klebsiella	1	0.1
Enterobacter	64	6.4
Enterobacter, Proteus	27	2.7
Enterobacter, Pseudomonas	75	7.5
Hemolytic Streptococci	6	0.6
Klebsiella Species	31	3.1
Klebsiella,Hemolytic Streptococci	1	0.1
Klebsiella, Proteus Species	2	0.2
Klebsiella, Pseudomonas	2	0.2
Klebsiella, Pseudomonas, Staphylococcus Aureus	1	0.1
Proteus Species	9	0.9
Proteus Species, E.coli	3	0.3
Proteus Species, Klebsiella	1	0.1
Proteus species, Pseudomonas	7	0.7
Proteus Species, Staphylococcus aureus	3	0.3
Pseudomonas	144	14.4
Pseudomonas, Klebsiella	2	0.2
Pseudomonas, Proteus	1	0.1
Staphylococcus Aureus	410	41.0
Staphylococcus Aureus, E.coli	3	0.3
Staphylococcus Aureus, E.coli, Pseudomonas	1	0.1
Staphylococcus Aureus, Enterobacter	6	0.6
Staphylococcus Aureus, Klebsiella	2	0.2
Staphylococcus. Aureus , Proteus Species, Pseudomonas	1	0.1
Staphylococcus Aureus, Pseudomonas	35	3.5
Total	1000	100.0

Table 2: Frequency and Percentage of Clinical Isolates

DISCUSSION

Antibiotic resistance is not a latest phenomenon, since the introduction of antibiotic (penicillin), bacteria are noted to posses some resistance. The potential of bacteria to defend against the effects of an antibiotic is called antibiotic resistance. Antibiotic resistance occurs when bacteria or other microbes become resistant to the special effects of an antibiotic after being exposed to them. Antibiotic resistance results in bacteria due to change by some approach that eliminates or reduces the effectiveness of antibacterial agents intended for treatment of infections. The bacteria cause more damage to human body by survival and continuous multiplication [9].

The frequencies that are found in clinical isolates in Lahore are lesser than the past studies from Pakistan (60.4%) [10], 33.5% Russia, 32% in Kuwait, 69.1% in western Nepal 71.4% in Sudan [11], 42% in Iran and 13% in Sri Lanka [12]. Gram positive bacterial infections [13,14]. Gram positive bacteria, *Streptococcus pneumoniae* and *Staphylococcus Aureus* were linked with large ratio of bacterial infections at various sites such as bone, respiratory track, joints, CNS, skin and , bloodstream [15,16]. In a study on prevalence of clinical isolates e.g. *Staphylococcus Aureus*, causing nosocomial infections,

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were more in men than in women [17]. The maximum isolates were obtained from pus samples 42.1% followed by urine samples 13.9% and 9.3% in blood samples. The other clinical isolates were 4.5% for sputum samples, 4.4% for wound swabs samples, 4.1% for fluids. These results were in line with the previous studies [18]. A change in the frequency distribution of clinical isolates among Pakistani population, about 2% to 61% [19] and throughout the world [20].
Regarding effectiveness of antibiotics for gram positive isolates, Vancomycin, Klaricid, Fusidic acid, Vibramycin, Erythromycin were much effective. This sensitivity of gram-positive bacteria to Vancomycin was observed similarly in previous study [21]. Gram positive isolates showed resistance with various degrees to Amoxicillin (46.2%), Cefotaxime (12.7%), Ciprofloxacin (4.0%) and Ampicillin (3.1%). Resistance to Amoxicillin was also noted by Shrestha et al [22]. Few of gram positive isolates showed a low resistance to Ampicillin similar to Matute et al [23].

CONCLUSIONS

Non-empirical and inappropriate use of antibiotic has increased the emergence of antibiotic resistance within bacteria both Gram negative and Gram positive. In Pakistan, most of the antibiotics are prescribed inappropriately to patients in hospital.

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