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COMPARISON OF ANTIBIOTIC RESISTANCE AND SENSITIVITY WITH REFERENCE TO AGES OF ELDERS

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Abstract: Antibiotic resistance is one of the emerging and challenging areas in the communities worldwide. The present study was designed to investigate antibiotic sensitivity and resistance pattern against currently available antibiotics. Samples were collected from 28 patients and were subjected to culture sensitivity testing following CLSI standard protocols. Antimicrobial susceptibility testing was performed by Kirby Bauer’s disc diffusion method using standard antibiotic discs from different antibiotic groups including Macrolids, Sulphonamides, Quinolones and Fluoroquinolones, Carbapenem, Monobactums and Aminoglycosides. Isolated colonies were identified by different biochemical tests before culture sensitivity tests and were preserved in freeze-dried condition at 4°C in stab slant agar until later use. The common microorganisms isolated were Escherichia coli (75%), Morganella spp (10.71%), Staphylococcus aureus (7.14%), Klebsiella and Proteus (3.57%) respectively. Clinical isolates of Escherichia coli (64.28%) and Staphylococcus aureus were found highly resistant against Ciprofloxacin, whereas Morganella exhibited moderate resistance profile against it. The highly resistant antibiotics profile shows irrational prescription of broad spectrum antibiotics which ultimately results in emergence of resistance.

Key words: Antibiotic resistance, Escherichia coli, Morganella, Ciprofloxacin, Nalidixic acid.

1. Introduction
Microorganisms from clinical and non-clinical settings are becoming more and more resistant to conventional antibiotics. Clinical microbiologists now agree that multidrug resistant Gram-negative bacteria pose the greatest risk to public health. A major issue confronted by organized health care today is that of controlling the increase in antimicrobial resistance [1]. Although multiple factors play a role in this problem, the selective pressures induced by inappropriate and widespread use of antibiotics is considered important contributor. Several studies have reported higher rates of antimicrobial resistance among isolates from intensive care units (ICUs) than among isolates from general-patient-care areas [2]. These studies have provided important information about changes in the spectrum of microbial pathogens and trends in antimicrobial resistance patterns in nosocomial and community-acquired infections within time. The information generated by surveillance programs, associated with an increased awareness about evolving resistance patterns, has proved helpful for the development of empirical approaches for the treatment of serious infections [3].

Klebsiella species particularly Klebsiella pneumoniae are important opportunistic nosocomial pathogens causing a variety of infections including urinary tract infections, pneumonia, septicaemia, wound infections and infections in the intensive care units. It has been estimated that Klebsiella spp cause 5 - 7% of the total bacterial nosocomial infections [4]. Escherichia coli (E. coli) is the most common cause of both community-acquired and nosocomial transmitted UTIs. National Nosocomial Infections Surveillance [5] data indicate that 26% of all hospital-associated UTIs are caused by E. coli in the USA.

Antimicrobial resistance has been recognized as an emerging worldwide problem both in developed and developing countries [6]. Staphylococcus aureus (S. aureus) exhibits three problematic features that, taken together, are not found among most other clinically relevant bacteria. This species is capable of expressing a variety of virulence factors and thus is almost always considered medically
relevant when encountered in clinical specimens; the organism continues to demonstrate the ability to develop and expand resistance to include a broad array of antimicrobial classes, and *S. aureus* is a prominent pathogen in both the hospital and the community settings [7]. *Proteus mirabilis* as well as other members of the *Enterobacteriaceae* family are a leading cause of infectious diseases in both the community and acute care settings [8]. Among *Enterobacteriaceae*, a trend of increasing numbers of organisms resistant to several antimicrobial agents has been documented worldwide, attracting escalating concern [9].

Resistance has emerged even to newer, more potent antimicrobial agents. Antimicrobial resistance surveillance is necessary to determine the size of problem and to guide empirical selection of antimicrobial agents for treating infected patients as antibiotic resistance is highly increasing these days [10].

2. Materials and Methods

2.1 Chemicals and Drugs

Antibiotics used were Amoxicillin clavulanic acid (AMC 20 µg), Gentamicin (CN 10 µg), Pipemidic acid (PPM 50 µg), Ciprofloxacin (CIP 5 µg), Amikacin (Ami/AK 30 µg), Aztreonam (ATM 30 µg), Cefixime (CFM 5 µg), Meropenem (MEM 10 µg), Imipenem (IMP 10 µg), Trimethoprim sulfamethoxazole (SXT 1.25 µg), Cefepime (FEP 30 µg), Cephradine (CE 30 µg), Vancomycin (VA 30 µg), Clarithromycin (CLR 15 µg) Tigecycline (TGC 15 µg).These antibiotic discs were obtained from Oxoid Limited, UK.

2.3 Samples collection and identification

Samples were collected from the medical ward of Khyber Teaching Hospital Peshawar. Total 28 fresh midstream urine samples were taken in sterile bottles from the male patients that were referred to Clinical Microbiology Laboratory of Khyber Teaching Hospital Peshawar. Primary isolation was done on Cysteine Lactose Electrolyte Deficient (CLED) agar (Oxoid, UK, CM 0301) to allow the growth of all bacteria from the urine. Incubation was done by sterilized wire loop. Streaking was carried on Cysteine Lactose Electrolyte Deficient (CLED) agar media. Agar plates were incubated at 37°C for 24 - 48 hours for growth. Different types of biochemical tests like citrate (Oxoid, UK, CM 0155), triple sugar iron agar test (Oxoid, UK, CM 0277) and urease test (Oxoid, UK, CM 053) were performed to confirm the specific type of bacterial species.

2.4 Standardization of Bacterial Suspensions

Bacterial cultures were grown for 24 hours at 37°C and suspensions with cell density of 1X10^8 CFU/ml, were prepared using McFarland standard and was further diluted to a cell density of 1×10^6 CFU/ml using a UV visible spectrophotometer (Thermo electron corporation USA) at 625 nm and the standardization was maintained for the period of the study.

2.5 Antibiotics sensitivity Pattern

Resistance to antimicrobial agents was determined by Disc Diffusion method of Kirby Bauer method on Muller-Hinton agar (Oxoid, UK, CM 0337) as described by the Clinical Laboratory Standard Institute [11]. For the efficacy of selected antibiotics, zone of inhibition was measured. A clear zone of inhibition around the disc indicates sensitivity and their absence of resistance.

![Figure 1: Pie graph showing the percentages of isolated organisms.](image-url)
3. Results

3.1 Demography of bacterial isolates
A total 5 bacterial species (Escherchia coli, Morganella morganii, Klebsiella spp, Proteus spp and Staphylococcus aureus) were isolated from 28 samples (Figure 1)

3.2 Susceptibility pattern of E. Coli
The antibiotics like ciprofloxacin and pipemidic acid showed great resistance against E.Coli which were 64.28 and 57.14 %, respectively. Whereas, the resistance observed to Amoxicillin clavulanic acid and Aztreonam was 53.57 and 42.85 %, respectively. Furthermore, the sensitivity of Imipenem (53.57%) and Gentamicin (50%) was found to be high against E. Coli (Figure 2).

![Figure 2: Chart showing percentages of sensitive and resistant antibiotics used against E.coli.](image)

3.3 Susceptibility pattern of Klebsiella spp
Susceptibility pattern of Klebsiella against the tested antibiotics is summarized in Figure 3. Klebsiella spp were found resistant to Gentamicin and Ciprofloxacin which was 3.57% while the sensitivity against Klebsiella spp was offered by other antibiotics shows average results.

![Figure 3: Chart showing percentages of sensitive and resistant antibiotics used against Klebsiella.](image)
3.4 Susceptibility pattern of *Morganella morganii*

The isolated microbe *Morganella morganii* showed high resistance to antibiotics aztreonam and cefixime which was 10.71% while the sensitivity of meropenem and cefepime was found to be high against *Morganella morganii* which was 10.71% as shown in the figure 4.

![Figure 4](image)

**Figure 4:** Chart showing percentages of sensitive and resistant antibiotics used against *Morganella.*

3.5 Susceptibility pattern of *Proteus* species

The resistance and sensitivity of *Proteus* showed the average results against all the antibiotics tested as shown in Figure 5.

![Figure 5](image)

**Figure 5:** Chart showing percentages of sensitive and resistant antibiotics used against *Proteus.*

3.6 Susceptibility pattern of *Staphylococcus aureus*

The resistance offered to ciprofloxacin by *S. aureus* was found to be high which was 7.14% while the sensitivity and resistance of remaining antibiotics showed average result as shown in figure 6.
4. Discussion
Antibiotic resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the pre-eminent public health concerns of the 21st century. During last four decades antibiotics were used as most successful therapeutic agents to cure infectious microbial diseases. Antibiotics were successfully used against infection and wound healings and are proved more efficient in clearing disease pathogens. In the beginning antibiotics worked more effectively against majority of pathogens [12] and considered most successful chemotherapeutic agents in the history of medicine. Later on due to misuse of antibiotics microbes have shown wider resistance against most of them and its resistant strains spread in community hospitals residing in different environments and causing new types of infections [13]. Due to development of resistance, most of the broad spectrum antibiotics have lost their lethal action against microbes.

In the present study, *E. coli* was found to be leading cause of resistance against antibiotics. We have got the percentages of isolated microorganisms as *E.coli* 75, *Morganella* 10.71, *Klebsiella* and *Proteus* 3.57 respectively. *S.aureus* 7.14.

5. Conclusion
The susceptibility and resistance profile of all isolates in this study have shown that *Escherichia coli* was highly resistant against ciprofloxacin, pipemidic acid and amoxicillin clavulanic acid while Imipenem and gentamicin is highly sensitive to it. *Klebsiella spp* was resistant to gentamicin and ciprofloxacin and highly sensitive to various antibiotics as shown in figure 3. Similarly *Morganella morganii* was highly resistant to ciprofloxacin and aztreonam and showed moderate sensitivity to several antibiotics. *Proteus spp* showed moderate resistance and sensitivity to all antibiotics used against it. *S.aureus* showed high resistance to ciprofloxacin and have moderate sensitivity to several antibiotics.

This highly resistance antibiotic profile shows that in our irrational recommendation of broad spectrum antibiotics, which results in emergence of antibiotic resistance. Large scale use of antibiotics should be avoided in case of mild infections. Furthermore, an alternate way should be searched to minimize the use of antibiotics without prescription.

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