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Study of Trends of Antimicrobial Resistance in Clinical Bacterial Isolates from Three Private Tertiary Healthcare Facility in Pakistan

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Abstract: *Purpose of this study was to determine the prevalence of antimicrobial resistance in microbial pathogens from different clinical samples. The current study was conducted in Lahore, Faisalabad, Rawalpindi and Islamabad as these cities are ranked as highly populated cities of Pakistan. Isolation and identification of pathogens from almost 750 diverse urine, blood and pus samples by aid of selected media cultures, gram staining and various biochemical tests. Isolated pathogens were checked for antimicrobial susceptibility patterns against frequently prescribed antibiotics. In present study, Gram-negative isolates were found in maximum percentages (80%) while the remaining (20 %) were Gram-positives. The isolate which was most recurrently recognized from Gram-positives was S. aureus and most frequently identified isolate from Gram-negative was E. coli and P. aeruginosa. The prevalence of E. coli was 20 % in urine, 14% in blood, 12% in stool and 4 % in pus. Furthermore, the resistance rate of E. coli for Streptomycin, Nalidixic acid, Ciprofloxacin, Levofloxacin, Doxycycline, Moxifloxacin, Amikacin, Gentamycin, and Amoxicillin were between 12 % to a maximum of 100%. Gram positive S. aureus was found considerable in urine (9%), blood and pus cultures. The resistance shown by the isolates ranged from 12% to 100% against ceftriaxone, ampicillin, chloramphenicol, doxycycline, co-trimoxazole, penicillin, erythromycin, vancomycin and tetracycline. Moreover, the antibiotics like Augmentin (71%) and Moxifloxacin (60%) showed highest resistance so these are considered as poor choices for treating S. aureus infections. We concluded from this study that strict legislation and better management techniques in context to use of antimicrobials for therapeutic purposes in human and animals can minimize the chances of development of antimicrobial resistance.*

Key Words: Antimicrobial Resistance, Clinical Isolates, Pathological Specimens

Introduction

Improper prescription patterns and prolonged inappropriate use of broad-spectrum antimicrobial agents adding up to emerging resistance among bacterial pathogens generate a hazard to public health by escalating morbidity and mortality rates. Antimicrobial resistance (AMR) is the capability of microbes to persevere or reproduce even when the drug is present to hinder or kill them. Resilient microorganisms can survive attack by antimicrobial medicines henceforward designated treatment is useless and consequently infection continues. Stride of finding novel antibiotics is

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categorically time taking and practice of antimicrobial drugs is rising leading to resistance which became a sweeping problem in recent years. These complications have augmented costs to humanity, predominantly to the health care system due to protracted hospitalization [1].

At the present time, antimicrobial resistance (AMR) is considered as a main public health hazard. Drug resistant strains typically appear because of overuse or inappropriate use of antimicrobial drugs. Unsatisfactory guidelines for the control of infection, deprived hygienic settings and unsuitable food management are crucial perilous features contributing to additional escalation of antimicrobial drug resistance. Antimicrobial resistance where upsurges the monetary expenses to patients, but at the same time it also intensifies the brutality and mortality rates of certain infections that might have been tapered off by practical and balanced handling of new innovative antimicrobial agents [2]

Based on an available study, due to AMR 700,000 deaths are reported per annum, and it can be predicted that without apposite control and prevention measures AMR would turn into one of the major causes of death in developing countries. According to the global action plan on AMR sanctioned by WHO, to elevate consciousness on AMR through monitoring and research programs in numerous parts of the world is need of the hour which can serve quite a lot of benefits including: 1) data provision on bacterial resistance rate, 2) Following Safe medication practices and consequently reducing AMR rate, 3) Avoiding hospitalization rate and lowering treatment expenses, and 4) decrease in mortality rate [3-4]. Therefore, the current study assesses the epidemiology and AMR profile of the main pathogenic bacteria isolated.

While assembling statistics about the AMR in an area, unremitting scrutiny was ensured that engages the compilation of antimicrobial susceptibility testing (AST) outcomes of clinical specimens. The attained data made available an understanding into the frequency of antibiotic-resistant bacteria and aided in policymaking, primarily in the choice of antibiotics to be useful either for pragmatic handling of sick patients or prophylaxis in patients at greater risk of catching infection. Utilizing this new data, the national treatment guidelines must be efficient to cope with the danger. Data on infection epidemiology and antimicrobial resistance proposals can be proved a noticeable tool to assist physicians for the treatment of life-threatening infectious ailments through empirical observation. The focal goal of the present study was to identify various AMR patterns of bacterial isolates from diversified specimens at a lot of health setups. Verdicts of this study will contribute in creating strategies for the balanced practice of the prevailing antimicrobials and designing an ideal empirical treatment plan.

Methods

Study Setting

The present study was steered in Lahore, Faisalabad, Rawalpindi and Islamabad. According to a survey conducted by Statistics Division, Population Census Organization and Government of Pakistan in 2017 (Approved in 2020) [5]. Lahore, Faisalabad and Rawalpindi metropolitan cities are graded as the 2nd, 3rd and 4th extremely populated cities of the Pakistan, respectively. We have also included Islamabad along with Rawalpindi as both are considered as Twin cities and due to the high interdependence and intertwined areas of the two cities and mixed population. Among these cities, we have selected four renowned tertiary care private hospitals where records of in-patient s case files, prescription papers and AM sensitivity reports were appropriately maintained. Unfortunately, none of the government hospital has fulfilled above criteria.

Sample Size and Sampling Technique

For present study, the assessed sample size is derivative of an online Rao soft sample size calculator. The planned sample size was based on a response rate of 50%, a confidence interval of 99%, and a considering the margin of error of as 5%, among the total population of around 19030000 (i.e. Lahore (10.5 million), Rawalpindi and Islamabad City (3.25 million), Faisalabad (5.28 million) the principal obligatory sample size is 664. Therefore, this study encompassed a fitting sample of 750 individuals. Simple random sampling technique was used for data collection. [6]

Study Design and Study Population

This study was carried out after receiving approval from the Pharmacy research and Ethical Committee (PREC/55/20). The study population consists of critically ill patients. Blood, urine and pus samples were collected from CCUs (critical care unit) and medical ICUs (Intensive care units) from four renowned private hospitals of four major cities.

Study Period and Data Collection

The study was conducted from August 2020 to August 2021. From the case record section in-patient s prescription papers, case files, and AM sensitivity reports of ICUs and CCUs were collected. Information collected comprise of demographic information about the patient, admission unit, of hospital stay period, diagnosis and kind of infection (nosocomial or primary). Moreover, practical treatment, suggestive usage of the AM, collected specimen, contributing agent, and sensitivity or resistance pattern data was also composed. A case record form was generated to enter all the provided information.

Sample Collection and Processing

Clean catch midstream urine was collected in sterile 20 mL calibrated plastic container from each patient. When delivery was not possible for more than 2 hours boric acid was added as a preservative which allows the sample to be kept at room temperature. Tryptone soya broth (TSB) containers were used for dispensing of Blood samples which are than incubated for 72 hours (3 days) before subjecting to antibiotic susceptibility testing. Disposable syringes were used to collect anaerobic pus samples for testing and were used on the same day.

We used nutrient, blood and MacConkey agar for growth of bacterial isolates. The isolates were identified by morphology of colony by standard biochemical tests. [7] The specimens were subjected to Gram stains for further identification [8]. After isolation samples were processed for AM sensitivity or susceptibility and resistance testing was executed by disc diffusion method (modified Kirby Bauer method on Muller Hinton agar [9-10]. Evaluation by biochemical tests, culture media, disc diffusion and Gram stain methods were carried out day-to-day in triplicate manner to avoid any errors as per Clinical and Laboratory Standard Institute (CLSI) guidelines and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [11]. These experiments can lead to the foundation of antibiotics classes as resistant, sensitive and highly sensitive according to specifications of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [12].

Results

In current study, over-all 750 samples (stool, urine, blood, pus) of diseased patients were handled. These samples were cultured on specific media to obtain bacterial isolates. Antimicrobial susceptibility patterns were scrutinized by using colony morphology and Gram staining. Amongst all diverse clinical samples sent to the laboratory, a total of 500 (67%) were found evident of bacterial growth. Mainstream of bacterial strains were isolated from urine culture (40 %) followed by blood (30%), pus (18 %) and stool (12%).

In current study, maximum number of identified isolates were Gram-negative (80 %) while the remaining were Gram-positives (20 %). The most commonly known isolate from Gram-positives was *S. aureus* and most frequently identified isolate from Gram-negative was *E. coli* and *P. aeruginosa*. Figure 1 shows the prevalence of *E. coli, S. aureus, P. aeruginosa* in biological samples. It is obvious from figure 1, the prevalence of *E. coli* was 20 % in urine, 14% in blood, 12% in stool and 4 % in pus. Figure 2 indicates antimicrobial resistance and susceptibility patterns of *E. coli*. As shown in Figure 2, the resistance rate of *E. coli* for streptomycin, nalidixic acid, ciprofloxacin, levofloxacin, doxycycline, moxifloxacin, amikacin, gentamycin, and amoxicillin were between 12 % to a maximum of 100%. Whereas isolates of *E. coli* showed sensitivity to vancomycin at 85%, levofloxacin at 88 % and ceftriaxone at 90% [13]. The gastrointestinal tract gram negative *E coli* is said to be the deep-rooted causes of maximum reported urinary tract infections around the globe. With respect to the antimicrobial resistance profile it is found fully resistant (100%) to Augmentin and cotrimoxazole. The study indicated that ceftriaxone showed highest susceptibility against *E-coli*. Hence Order of prescribing these drugs will be according to efficacy of these drugs i.e. Ceftriaxone >Levofloxacin > vancomycin [14].

Figure 3 indicates antimicrobial resistance and susceptibility patterns of *P.aeroginosa* . It is obvious from figure 3, the prevalence of *P. aeruginosa was* 9 % in urine, 18% in blood and 3 % in pus. Isolated samples of *P. aeruginosa* from blood and urine culture shown resistance to ceftriaxone, gentamicin, norfloxacin, co-trimoxazole and tetracycline, respectively. Moreover, the results of resistance are well supported by another study. [15]. Our study indicates that *P. aeruginosa* is among greatest known causes for nosocomial infections and is also documented for its complex antimicrobial resistance in most health care settings. Antibiotic susceptibility profile showed that imipenem> ceftazidime> trimethoprim-sulfamethoxazole will be preferable sequence while treating *P. aeruginosa* infection as the order of susceptibility towards these antibiotics is 97%, 94% and 82% respectively [16]. Moreover, tobramycin and gentamycin showed maximum resistance and shouldn t be prescribed.

Figure 4 indicates antimicrobial resistance and susceptibility patterns of *S. aureus*. In our study, urine isolates from Gram positive *S. aureus* were (9%), blood and pus cultures. The isolates showed resistance from 12% to 100% against ceftriaxone, ampicillin, co-trimoxazole, chloramphenicol, doxycycline, penicillin, erythromycin, tetracycline and vancomycin. It is known that *S. aureus* is a familiar multi-drug resistant pathogen which can be one of the most communal bases of serious and even life-threatening bacterial infections in most health care settings. The general load of staphylococcal disease largely triggered by *S. aureus* that is methicillin resistant is snowballing in several countries and mainstream of its isolates were found multi drug resistant which might be due to hospital hygiene, sanitary condition and repeated use of antibiotics. It was found sensitive to linezolid (100%) and trimethoprim sulfamethoxazole (95%) which clearly indicated linezolid as drug of choice against *S. aureus* infections [17-18]. Moreover, the antibiotics like Augmentin (71%) and moxifloxacin (60%) showed highest resistance so these are considered as poor candidates for treating *S. aureus* infections.

In developing countries and economies such as Pakistan, AMR have increased the chances of treatment catastrophes and deficiency of effective therapy which can in return increase rates of mortality and morbidity. Lack of general AMR surveillance programs in developing and several developed countries have led to inappropriate use of antibiotics among patients and health care staff. [19] Thus, exploring AMR patterns is very critical and imperative, specifically in developing countries where no systematic guidelines for antibiotic usage are provided. On the other hand, it is necessary to analyze the antibiotic resistance patterns which can result in a valuable model for both clinicians and policy makers in executing safe and effective empirical therapy.

The statistics was composed from four renowned hospitals of four major cities. The distribution and occurrence of bacterial isolate varied in different clinical specimens. The high variability of isolated pathogens from diversified specimens showed the uselessness of currently available empirical antibiotic therapy and reinforced the repetition of susceptibility testing before any antibiotic therapy prescription. In our study, isolates recovered from clinical specimens were of urine, blood and pus origin. The most frequent strains recovered were Gram-negative bacteria, with the record of *P. aeruginosa* and *E. coli* being most common. [20]. Most predominant Gram-positive organisms recovered from ICU were *S. aureus*.

Strategies for antibiotic therapy had better be developed for each hospital and then well adjusted for every unit within the hospital as depending on the unit and susceptibility patterns of different pathogens may be involved. Furthermore, knowing AMR patterns can help physicians and policy makers towards finding choosing appropriate antibiotics, discovering effective empiric therapy and thus avoiding the development of drug resistance [21 - 22]. In this study we reported the patterns found in the critically ill patients of ICU/CCU. Unsuitable, uncontrolled empiric therapy or cross acquisition of resistance rather than the expansion of natural resistance could be serving as potentials for higher resistance rates. These reasons rationalize the obligation for initiation of immediate infection control strategies in our hospitals. Knowledge of the current local resistance patterns can pave a way to maintain guidelines for empirical therapy for ICU/CCU patients. We should seriously ponder implementation of the strategies to avert antimicrobial resistance in health care settings, which are listed as avoid infection, diagnose and treat infection effectually, use antimicrobials sensibly and stop transmission of infection wisely.

The constraints of the study consist of: investigation for some bacterial strains that can cause significant infections in hospitalized patients couldn t be included. Expedient sampling was used to hand-pick study contributors that can permit absence of cases. Nevertheless, these statistics can be a guide for pragmatic antibiotic therapy for the maximum major pathogens for diverse specimens. The verdicts illustrate the bacterial resistance patterns for the particular antibiotics.

Conclusion

Contemporary study exposed various microorganisms with noticeably higher resistance rates against numerous antibiotics. Being developing country Pakistan provides very inadequate opportunities for benign, operative and realistic antimicrobial therapies. Thus, there is a prerequisite to mature a home-grown plan to outspread the prevailing numbers on stuff like drug sensitivity patterns of bacteria. The misuse of antimicrobial drugs should be constrained in human, veterinary and agriculture medicine. Demanding mindfulness and training programs should be implemented to instruct both patients and physicians. Establishment of operative Antibiogram countrywide and worldwide is the finest answer for balanced and practical usage of antimicrobial drugs.

References

Touat, M., Brun-Buisson, C., Opatowski, M., Salomon, J., Guillemot, D., Tuppin, P., & Watier, L. (2021). Costs and Outcomes of 1-year post-discharge care trajectories of patients admitted with infection due to antibiotic-resistant bacteria. *Journal of Infection, 82*(3), 339-345.

World Health Organization. (2015). Antimicrobial resistance Fact sheet N19 Updated April 2015

Heredia, N., & García, S. (2018). Animals as sources of food-borne pathogens: A review. *Animal nutrition, 4*(3), 250-255.

Engda, T., Moges, F., Gelaw, A., Eshete, S., & Mekonnen, F. (2018). Prevalence and antimicrobial susceptibility patterns of extended spectrum beta-lactamase producing Entrobacteriaceae in the University of Gondar Referral Hospital environments, northwest Ethiopia. *BMC research notes, 11*(1), 1-7.

Al-Balas, M., Al-Balas, H. I., Jaber, H. M., Obeidat, K., Al-Balas, H., Aborajooh, E. A., & Al-Balas, B. (2020). Distance learning in clinical medical education amid COVID-19 pandemic in Jordan: current situation, challenges, and perspectives. *BMC medical education, 20*(1), 1-7.

Wani, P. A., Rafi, N., Wani, U., Bilikis A, H., & Khan, M. S. A. (2021). Simultaneous bioremediation of heavy metals and biodegradation of hydrocarbons by metal resistant Brevibacillus parabrevis OZF5 improves plant growth promotion. *Bioremediation Journal*, 1-12.

Boyanova, L. (2018). Direct Gram staining and its various benefits in the diagnosis of bacterial infections. *Postgraduate medicine, 130*(1), 105-110.

Derrick, C., Bookstaver, P. B., Lu, Z. K., Bland, C. M., King, S. T., Stover, K. R., & Justo, J. A. (2020). Multicenter, observational cohort study evaluating third-generation cephalosporin therapy for bloodstream infections secondary to Enterobacter, *Serratia, and Citrobacter species. Antibiotics, 9*(5), 254.

Buzˇn-Durßn, L., Capita, R., & Alonso-Calleja, C. (2018). Antibiotic susceptibility of methicillin-resistant staphylococci (MRS) of food origin: A comparison of agar disc diffusion method and a commercially available miniaturized test. *Food microbiology, 72*, 220-224.

Kassim, A., Omuse, G., Premji, Z., & Revathi, G. (2016). Comparison of Clinical Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing guidelines for the interpretation of antibiotic susceptibility at a University teaching hospital in Nairobi, Kenya: a cross-sectional study. *Annals of clinical microbiology and antimicrobials, 15*(1), 1-7.

Vanstokstraeten, R., Belasri, N., Demuyser, T., Crombé, F., Barbé, K., & Piérard, D. (2021). A comparison of E. coli susceptibility for amoxicillin/clavulanic acid according to EUCAST and CLSI guidelines. *European Journal of Clinical Microbiology & Infectious Diseases, 40*(11), 2371-2377.

Antonoplis, A., Zang, X., Wegner, T., Wender, P. A., & Cegelski, L. (2019). Vancomycin–arginine conjugate inhibits growth of carbapenem-resistant E. coli and targets cell-wall synthesis. *ACS chemical biology, 14*(9), 2065-2070.

Wang, S. S., Ratliff, P. D., & Judd, W. R. (2018). Retrospective review of ceftriaxone versus levofloxacin for treatment of E. coli urinary tract infections. *International Journal of Clinical Pharmacy, 40*(1), 143-149.

Abebe, M., Tadesse, S., Meseret, G., & Derbie, A. (2019). Type of bacterial isolates and antimicrobial resistance profile from different clinical samples at a Referral Hospital, Northwest Ethiopia: five years data analysis. *BMC research notes, 12*(1), 1-6.

Nguyen, L., Garcia, J., Gruenberg, K., & MacDougall, C. (2018). Multidrug-resistant Pseudomonas infections: hard to treat, but hope on the horizon?. *Current infectious disease reports, 20*(8), 1-10.

Bowen, A. C., Carapetis, J. R., Currie, B. J., Fowler Jr, V., Chambers, H. F., & Tong, S. Y. (2017). *Sulfamethoxazole-trimethoprim (cotrimoxazole) for skin and soft tissue infections including impetigo, cellulitis, and abscess*. In Open forum infectious diseases 4(4), p. ofx232). US: Oxford University Press.

Verma, A. K., Bauer, C., Yajjala, V. K., Bansal, S., & Sun, K. (2019). Linezolid attenuates lethal lung damage during postinfluenza methicillin-resistant Staphylococcus aureus pneumonia. Infection and immunity, *87*(10), e00538-19.

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. *Pathogens and global health, 109*(7), 309-318.

Axente, C., Licker, M., Moldovan, R., Hogea, E., Muntean, D., Horhat, F., & Baditoiu, L. (2017). Antimicrobial consumption, costs and resistance patterns: a two year prospective study in a Romanian intensive care unit. *BMC infectious diseases, 17*(1), 1-9.

Faizullah, M., Umar, M. I., Anwar, M., & Sarfraz, M. K. (2017). A cross-sectional study on knowledge, attitude and practices of medical doctors towards antibiotic prescribing patterns and resistance in Khyber Pakhtun Khawah, Pakistan.

Farah, S. M., Alshehri, M. A., Alfawaz, T. S., Alasmeri, F. A., Alageel, A. A., & Alshahrani, D. A. (2019). Trends in antimicrobial susceptibility patterns in King Fahad Medical City, Riyadh, Saudi Arabia. *Saudi medical journal, 40*(3), 252.

Appendix

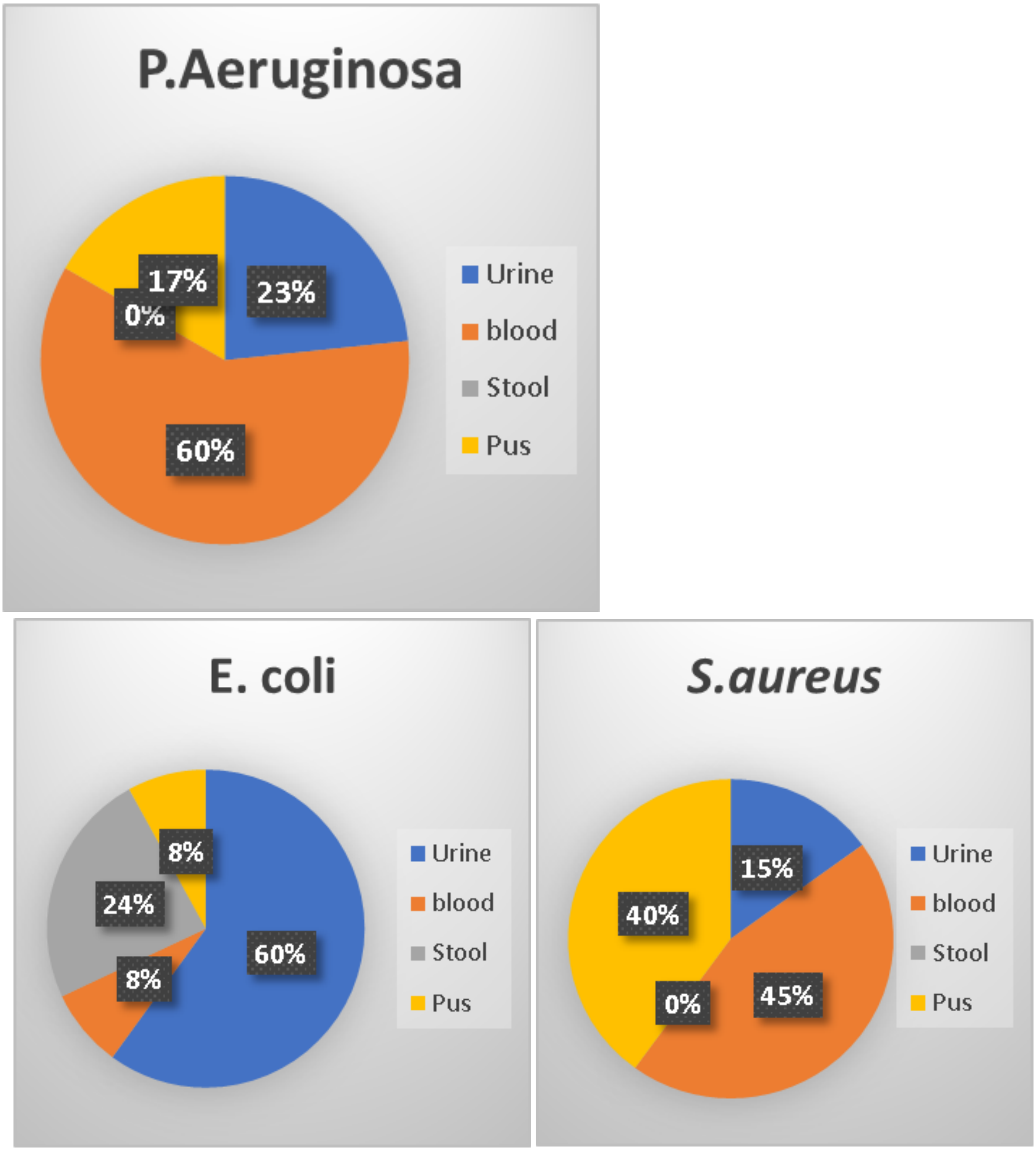

Figure 1: The Prevalence of *E. coli, S. aureus, P. Aeruginosa* in Urine, Blood and Pus Samples

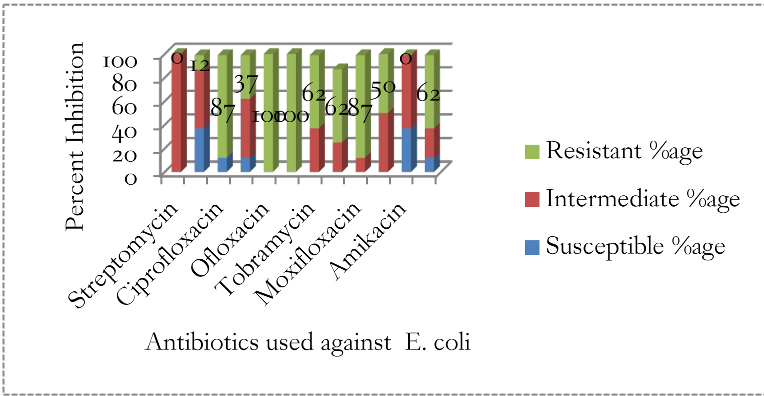

Figure 2: Antimicrobial Resistance and Susceptibility Patterns of *E. Coli*

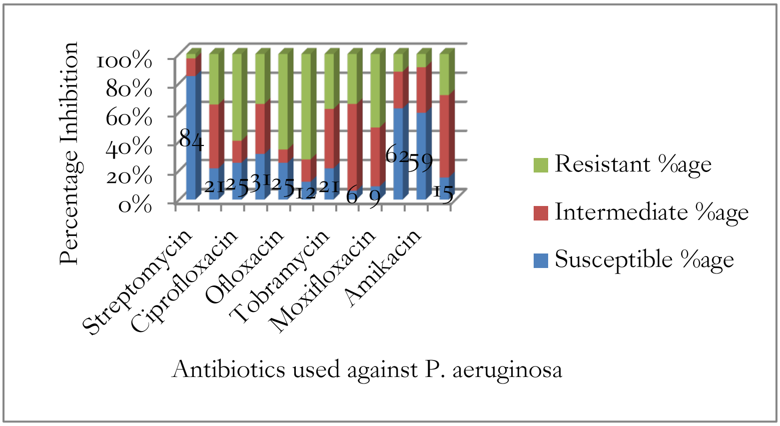

Figure 3: Antimicrobial Resistance and Susceptibility Patterns of *P.aeruginosa*

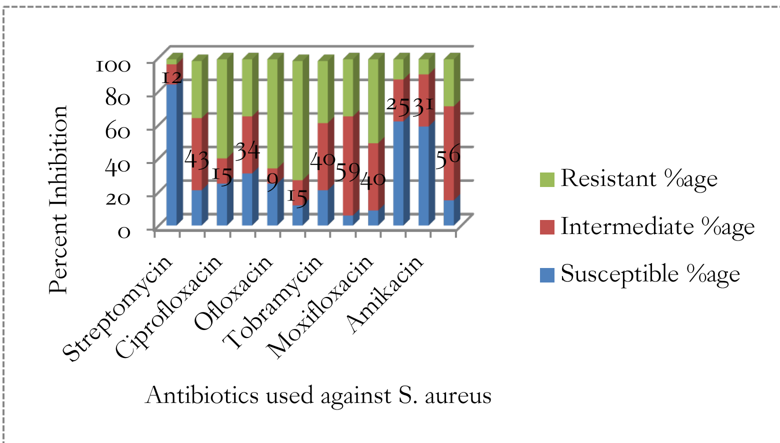

Figure 4: Antimicrobial Resistance and Susceptibility Patterns of *S.aureus*