Anti-microbial suseptability of Escherichia coli and Klebsiella spp: A review of 126 clinical isolates.

Fatima Iqbal Khan[1], Sheikh Abdul Khaliq[2], Bilqees Fatima[3]

ABSTRACT

Objective: To analyze the resistance patterns of antibiotics against infectious agents causing blood, urine and pus infections.
Study Design: Prospective experimental study.
Place and Duration: Pharmaceutical Microbiology Lab, Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University, Karachi from 8th January to 21st June 2018.
Methodology: As 126 Clinical isolates of E.coli and Klebsiella spp. were collected from various pathological laboratories of Karachi. Antimicrobial susceptibility testing was performed by Kirby-Bauer method for disks of four antibiotics; Imipenem, Cefotaxime, Nalidixic acid and Gentamicin.
Results: Among 126 clinical isolates, (66%) are Escherichia coli, (34%) are Klebsiella species. (63%) isolates have been obtained from the urine culture, (33%) from blood and (4%) from pus causing urinary tract infection, bacteremia and soft tissue infection respectively. Imipenem is found to have significantly (p=0.0001) highest susceptibility against E.coli (87%) and Klebsiella specie.(91%). However, E.coli (40%) and Klebsiella specie. (93%) are highly resistant from Cefotaxime, while almost 50% organism are resistant from Gentamicin (53%) and Nalidixic acid (33%).
Conclusion: Imipenem has been found to be the most effective of all tested antibiotics while Cefotaxime has developed resistance from these microorganisms.
Keywords: Antimicrobial susceptibility, Escherichia coli, Klebsiella, Disk diffusion method, Resistance, Antibiotics

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INTRODUCTION

Microbial resistance has been described as the insensitivity of the microorganisms towards the standard dosage of antimicrobial substances. It has been considered as an instinctive characteristic that is either irreversible or very slow to reverse[1]. Such pathogenic aggressiveness on global scale leads to a health crises resulting in a pharmaco-economic distress and increased mortality[2].

Due to negligence, resistance of organisms against antibiotic is escalating in developing region such as South East-Asia. The ignorance towards different studies pertaining to antimicrobial resistance and the extensive use of antibiotics has contributed substantially to the persistence of infections and a major cause of morbidity and mortality in developing countries including Pakistan[1]. Another study reported that routine antibiotics are irresponsive in Pakistan due to resistance[3]. The treatment of infections has now limited options due to emergence of antimicrobial resistance[4]. The emergence of Multi Drug Resistant (MDR) strains particularly in hospitalized children is noted against Klebsiella pneumonia[5]. It also has been reported that the gram negative organisms causing community infections are now resistant to third generation cephalosporin[6]. The decreased sensitivity of Nalidixic acid against Escherichia coli and similarly reduced sensitivity of Gentamicin and Nalidixic acid reported against Klebsiella spp. In another study, resistances of uro-pathogens to third generation cephalosporins and aminoglycosides have been acknowledged[7]. Resistance to carbapenems has also been

1. M.Phil. Scholar, Hamdard University, Sharae Madinat Al Hikma, Karachi, Pakistan
2. Professor Of Pharmaceutics & Pharmacy Practice, Hamdard University, Sharae Madinat Al Hikma, Karachi, Pakistan
3. Assistant Professor of Pharmaceutics, Senior Lecturer of Pharmaceutics, Barrett Hodgson University

Correspondence:
Bilqees Fatima
Senior Lecturer of Pharmaceutics,
Barrett Hodgson University
Email: bilqeesfatema08@gmail.com

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recognized in particularly difficult-to-treat infections associated with high mortality[8].

Infections associated with Escherichia coli and Klebsiella spp. are increasing drastically[9]. Almost 70-80% Escherichia coli has been reported to resistant from Cephalosporins in Pakistan[3]. Another study reported 91% Escherichia coli resistance to Nalidixic acid[4]. Based upon such alarming situation of antibiotics resistance, a study was designed to determine the sensitivity patterns of bacteria causing blood, urine infections and forming pus against Nalidixic acid, Cefotaxime, Gentamycin and Imipenem. We conducted this study with an objective to analyze the resistance patterns of antibiotics against infectious agents causing blood, urine and pus infections.

METHODOLOGY

This Prospective experimental study was conducted in Pharmaceutical Microbiology Laboratory, Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University, Karachi, Pakistan from 8th January 2018 to 29th June 2018. Clinical isolates (n=126) of E.coli and Klebsiella specie were collected from various pathological laboratories of Karachi. The clinical isolates were obtained from various diagnostic laboratories of Karachi. The Bacterial strains have been identified by examining morphological characteristics of colonies[10]. Sample size of study is determined by precision analysis technique[11]. Antimicrobial resistance was evaluated via Clinical and Laboratory Standard Institute (CLSI, formally NCCLS) guidelines. Disk diffusion (Kirby-Bauer) method was used for the evaluation of antimicrobial resistance[12].

The Inclusion criteria for the study was to collect clinical isolates on the basis of geographical locations in Karachi and of those patients who have either Urinary tract, wound or blood infections. Exclusion criteria for the study was anything that could impede with the success of the study or escalate the risk for an unfavorable outcome.

Inoculums were prepared via direct colony suspension method. The microorganisms were simply suspended in tryptone soy broth (CM0129 Oxoid UK). The density of inoculums was adjusted to a turbidity equivalent to a 0.5 McFarland standard by comparing the test and standard against a white background with black lines[10]. The cotton swab spread the inoculums evenly over the entire Mueller-Hilton Agar (MHA) (CM0337 Oxoid UK) surface in every direction[10]. Mueller-Hilton Agar MHA was suggested by Bauer, Kirby, Sherris and Tuck for the performance of antibiotic susceptibility testing[13]. Nalidixic acid 30 mcg (Oxoid UK), Cefotaxime 30 mcg (Oxoid UK), Gentamycin 10 mcg (Oxoid UK) and Imipenem 10 mcg (Oxoid UK) discs were used in the study. The discs were placed using sterile forceps on the surface of the agar and the distance between each disc were 24 mm and between the plate and antibiotic of 12 mm[10]. The plates have been incubated with agar side up at 34-36 ^{o}C for 16-20 hours.[10] Vernier Caliper has been used to measure the diameter of the zones of inhibition as per Clinical and Laboratory Standard Institute (CLSI)[12] guidelines.

Data Analysis: The data have been analyzed based upon zone of inhibition of all clinical isolates against different antibiotics. IBM SPSS Statistics 21 version was applied on collected data.

RESULTS

126 clinical isolates have been collected from various pathological laboratories of Karachi. Among them 83 (66%) were Escherichia coli and 43 (34%) were Klebsiella species. As shown in Fig-1, 80 (63%) isolates have been obtained from the urine culture, 41 (33%) from blood and 5 (4%) from pus causing urinary tract infection, bacteremia and soft tissue infection respectively.

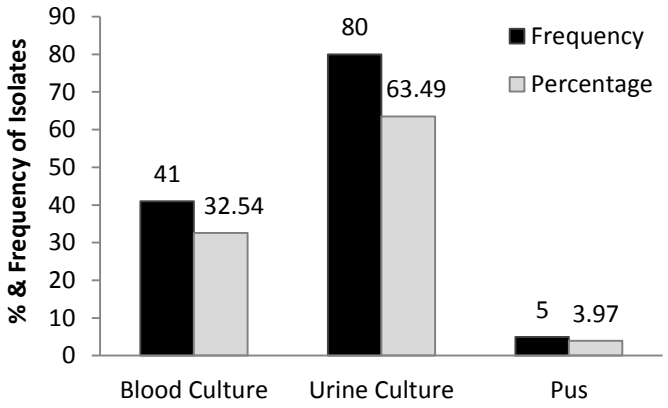

Figure-1: Prevalence of Isolates in various specimens (N=126)

Imipenem has been found to be the most effective of all tested antibiotics while Cefotaxime has been indicated as resistant. (Fig-2)

The Least Significant Differences (LSD) in zone of inhibition was compared among antibiotics to find most and least susceptible by IBM SPSS Statistics 21 version. (Table-I)

Multiple comparison by ANOVA reveals that zone of inhibition of Nalidixic acid is significantly better compare to Cefotaxime (p=0.038) and non-significant versus Gentamicin (p=0.968). Zone of inhibition of Gentamicin is significantly better compare to Cefotaxime (p=0.035). Zone of inhibition of Imipenem is found to be significantly better compare to Nalidixic acid (p=0.0001), Cefotaxime (p=0.0001) and Gentamicin (p=0.0001).

DISCUSSION

Antibiotic resistance is a growing concern all over the globe as it is creating hurdles in treating even the commonest infections generally found in a community[2]. The rate at which the resistance occurs to any antibiotic by any bacterial isolate vary from country to country and also depends upon the population and geographical area, where the antibiotic resistance is being found[14]. Escherichia coli are the most prevalent pathogen and are responsible for a wide variety of diseases including enteritis, urinary tract infection, and septicemia among other clinical infections[15].

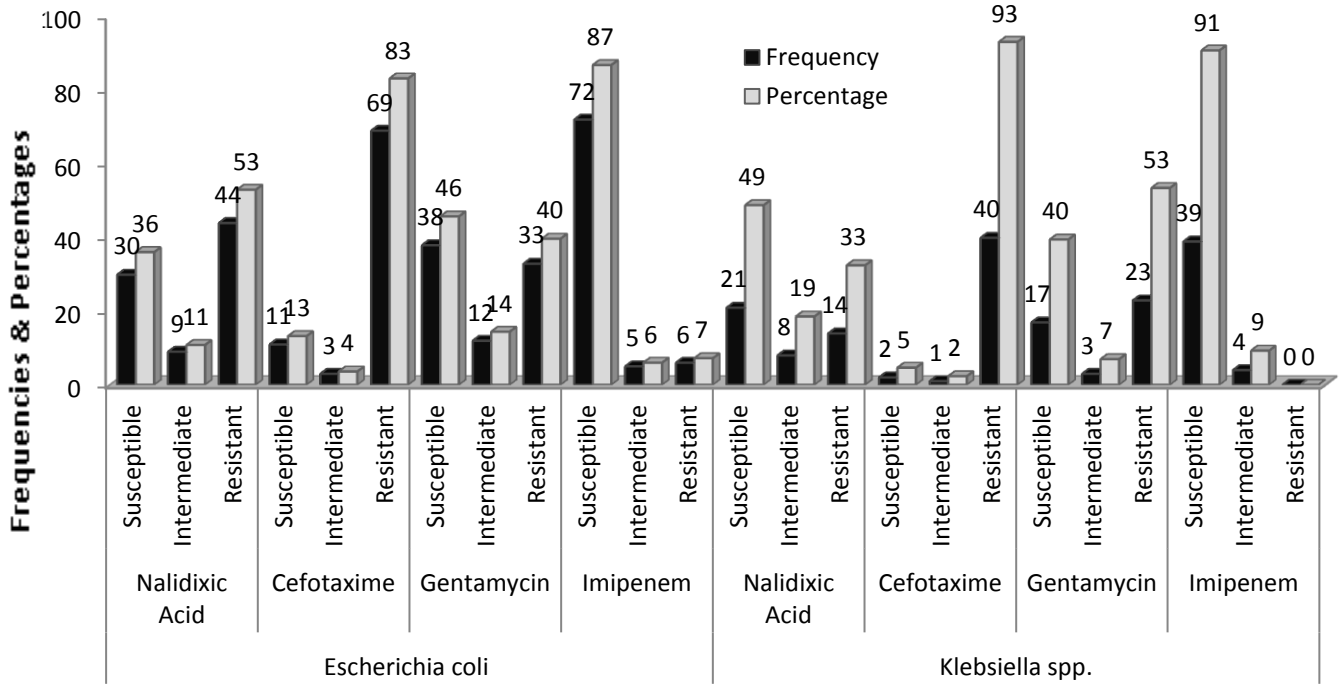

Figure-2: Frequency of Susceptibility Pattern of Antibiotics against Escherichia coli and Klebsiella spp. (N=126)

In the fore mentioned study various strains of Escherichia coli are obtained from pus, urine and blood have shown highest resistance against Cefotaxime and lowest against Imipenem. That is why highly efficient antibiotic against Escherichia coli is Imipenem with 87% sensitivity. The another study supported the findings of current study where E.coli showed resistance to cefotaxime and ceftazidime but exhibit susceptibility to imipenem and cefepime[16]. The activity pattern of Escherichia coli strains amongst all of the antibiotics are suggested as per the current CLSI guidelines[12]. As Escherichia coli has shown a considerably high resistance to Cefotaxime, this raises questions on the indiscriminate use of third generation cephalosporins because resistance of Escherichia coli against Cefotaxime cannot be neglected. The increased rate of resistance to third generation cephalosporins among gram-negative bacteria is causing community infections that could have a detrimental impact on clinical outcomes. Such outcome might leave us with only limited options for treating patients with gram-negative bacteremia, in general carbapenem is considered as the treatment of choice[6]. This has been consistent with the current study in which Imipenem has been proved to be highly sensitive against Escherichia coli. Furthermore, Gentamicin is also reported to be effective against most of the Escherichia coli strains[12].

Klebsiella pneumoniae is another common gram-negative pathogen. Klebsiella pneumonia has also developed resistance worldwide against extended-spectrum cephalosporin by producing extended-spectrum β-lactamases[17]. Acquired from the fore mentioned study, multiple strains of Klebsiella Pneumonia have shown highest resistance towards Cefotaxime followed by no resistance towards Imipenem. In other words, highest susceptibility has been observed with Imipenem against Klebsiella species. Similar to findings of current study, resistance towards Cefotaxime has also been reported in various studies. The rapid spread of multidrug-resistant Klebsiella pneumoniae has caused major concerns among health care professionals. During the past few years, cases of infections caused by K. pneumoniae strains resistant to clinically important classes of antibiotics, including third-generation cephalosporins[18]. Due to this reason an increased resistance to third generation cephalosporin in local population leaves us no choice except Imepenem. It is also observed in the current study that Nalidixic acid has moderate susceptibility against Klebsiella, however, statistically that is significant. This leaves us with another choice to treat Klebsiella species infections with Nalidixic acid. However, a report mentioned that treatment options for carbapenem-resistant Klebsiella pneumonia infections are limited[19]. That is why Imipenem is a choice of antibiotic which is also supported by findings of current study.

Analysis of zone of inhibitions reveals that Imipenem is always a first choice of antibiotic because it is significantly better in bactericidal effect compare to Nalidixic acid, Cefotaxime and Gentamicin. Similarly, Nalidixic acid is significantly better than Cefotaxime and Gentamicin is significantly better than Cefotaxime. However, non-significant difference has been found among zone of inhibitions of Nalidixic acid and Gentamicin, so both should not be use as first line of therapy. In order to limit the increasing antimicrobial resistance of Klebsiella pneumoniae further monitoring and efforts must be made as it may become a major problem for the public health and the hospital-acquired infections control[20]. Antimicrobial resistance has threatened the therapeutic treatment of Escherichia coli infections[15].

CONCLUSION

The most susceptible antibiotics was Imipenem against Escherichia coli and Klebsiella species while Cefotaxime has developed resistance from these microorganisms.

CONTRIBUTION OF AUTHORS

Khan FI: Conceived idea, Literature search, Data collection.
Khaliq SA: Designed research methodology, Data interpretation, Statistical analysis, Manuscript writing.
Fatima B: Literature review, Manuscript writing.

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