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Examining the Pattern of Susceptibility and Antibiotic Resistance in *Klebsiella pneumoniae* Strains Isolated from Urine Samples of Children with Urinary Tract Infections from the Children's Hospital of Tabriz in 2015

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Abstract

Background and Purpose: *Klebsiella pneumonia* is the most important causes of urinary tract infections in patients. Resistant strains of *K. pneumoniae*, especially to several antibiotics of different drug classes, have become very prevalent. In most cases, this resistance is such that causes illness exacerbation. The aim of this study is to evaluate the pattern of susceptibility and resistance of *K. pneumoniae* to some antibiotics isolated from urinary tract infections.

Materials and Method: In this cross-sectional study, we collected 198 strains of *K. pneumoniae* from patients with urinary tract infections (UTI). Initially, we selected these strains using standard laboratory and microbiology methods as well as culture in specific media. Muller Hinton agar was cultured on the medium by sterile cotton swab in three directions to evaluate the antibiotic susceptibility patterns of the strains, and Kirby-Bauer disk diffusion method was used for antibiogram.

Results: The highest degree of resistance of *K. pneumoniae* isolates were, respectively, to gentamicin (32.83%) and tetracycline (27.78%) antibiotics, and the highest susceptibility was to amikacin (98.98%) and imipenem (98.96%) antibiotics.

Conclusion: Considering the high prevalence of resistance to antibiotics, early and timely detection of resistant strains seems necessary to select appropriate treatment options and to prevent the spread of resistance.

Keywords: *K. pneumonia*; Urinary tract infections; Drug susceptibility; Resistance; Microorganisms

Introduction

This bacterium, such as other species of *Klebsiella*, is immobile and produces a prominent polysaccharide capsule. These organisms are urease, lysine decarboxylase-positive. In Triple Sugar Iron Agar (TSI), this organism is acid without producing hydrogen sulfide gas and produces gas. The optimal growth temperature of this organism is 37°C and can grow at temperatures ranging from 4 to 43°C [1]. One of the unique characteristics of *K. pneumoniae* is nitrogen fixation. It is the only genus in the family Enterobacteriaceae able to fix nitrogen in the atmosphere and to convert it into ammonia and amino acids [2].

Gram-negative bacteria resistant to antibiotics are connected to increased mortality, prolonged hospital stay, and increased costs due to production of Extendad Spectrum b-lactamases (ESBL) [3]. In humans, *Klebsiella* species colonize on the skin, throat, or digestive system. This bacterium colonizes in sterile wounds and urine and may be found as normal flora of some parts of intestines and bile ducts [4]. Among the many factors effective on *in vitro* antimicrobial activity are environment pH, components of the environment, stability of the drug, amount of sample incubation, incubation time, and metabolic activity of microorganisms [5].

In the body of living creatures, there are not only drug and parasite but also a third factor called host. In most cases, latent microorganisms survive the contact with high concentrations of the drug and later may bring clinical infection back. Tissue response induced by microorganisms may attract drug and prevent its exposure to the bacteria. In the body, microorganisms are mostly within tissue cells. Drugs enter cells in different ratios. Some drugs (e.g., tetracyclines) within monocytes reach the same concentration of extracellular fluid. In the case of some other drugs (e.g., gentamicin), drugs may not enter into host cells. Some drugs have poor penetration in certain tissues.

Accordingly, after systemic administration, drug concentration for effective treatment may not be enough. In superficial wounds or mucous membranes, topical application of the drugs with poor absorption leads to effective local concentration of drugs well absorption of them topically. The concentration of drug in the urine in most cases is significantly more than its concentration in the blood. Time-dose relationship should be considered for maintaining the appropriate concentrations of

the drug for optimum time. If in prescribing an antimicrobial drug, the fate of an infection changes, immune response of host may also change.

Methodology

This study was a cross-sectional study in which children with UTI were studied at Children's Hospital of Tabriz in 2015. We collected isolates of *K. pneumoniae* from urine samples (by infants' urine bag) of hospitalized and outpatients referring to Children's Hospital of Tabriz over a period of 5 months from September 2015 to January 2016 by simple random sampling. All samples were identified using laboratory standard tests and microbiology. Grown colonies that had features like *K. pneumoniae* were identified by biochemical differentiation tests including growth in Kligler Iron Agar (KIA), Urea Agar, and SIM media and then the isolated bacteria, after diagnosis, were kept in Tryptic Soy Broth (TSB) containing 20% glycerol at -80°C until further studies.

Then antibiotic resistance patterns of isolates of *K. pneumoniae* were determined by disc diffusion method in agar according to CLSI for 13 discs antibiotics including ciprofloxacin, gentamicin, chloramphenicol, imipenem, ceftazidime, cefotaxime, amikacin, tetracycline prepared from Padtan Teb Co. The antibiogram results of each sample were compared after 24 hours of incubation at 37°C according to CLSI table, and recorded as Susceptible (S), Intermediate (I) and Resistant (R). At this stage, isolates that were resistant to at least three antibiotics from different families were selected as MDR strains. For quality control of antibiogram stages in each series of tests, along with clinical isolates of *K. pneumoniae*, test of determining susceptibility was conducted exactly the same way on the standard strain of *K. pneumoniae* ATCC 700603, and results are compared with standard values [6]. Finally, data from the study was analyzed using SPSS 13.

Results

The findings show that out of 325 samples tested, after conducting differential tests, 198 cases were positive regarding *K. pneumoniae* (60.92%), out of whom 119 were female (60.10%) and 79 cases were male (39.90%). Outpatient section had 120 (60.60%) subjects and inpatient ward had 78 samples (39.40%). The average age of the patient was 8.64 2.89, varying from at least 5 years to a maximum of 14 years. Based on antibiotic susceptibility test, *K. pneumoniae* strains have the most resistance to gentamicin, tetracycline, chloramphenicol, ciprofloxacin antibiotics, and the highest susceptibility to amikacin, cefotaxime and most susceptible to antibiotics, imipenem, ceftazidime, cefotaxime and ciprofloxacin (Table 1 and Figure 1).

Concerning antibiotic resistance pattern, the results showed that out of 198 samples of *K. pneumoniae*, 96 (48.48%) cases had multi-drug resistance (MDR). Sixty cases of the isolated bacteria from females (65.62%) and 33 cases of isolated bacteria from male individuals (34.38%) had MDR. Out of 96 isolates with MDR, 14 (14.58%) were completely (100%) resistant to all antibiotics investigated.

Table 1: Antimicrobial resistance pattern of strains isolated from antibiotics used (%).

R	I	S	Antibiotics
18.18	4.05	77.77	Ciprofloxacin
32.83	6.06	61.11	Gentamicin
25.76	11.11	63.13	Chloramphenicol
1.52	0	98.48	Imipenem
2.52	0.52	96.96	Ceftazidime
15.15	5.05	79.8	Cefotaxime
0	1.01	98.99	Amikacin
27.78	29.29	42.93	Tetracycline

Figure 1: Antibiogram for *K. pneumonia.*

Discussion and Conclusion

UTI is one of the most important human infections that concerning frequency is in the second rank after respiratory infection [7]. After *Escherichia coli* and *K. pneumoniae*, Proteus and Enterobacter species have been reported to be the most important factors in UTI [8]. However, Shahcherai et al. reported more prevalence of *K. pneumoniae* isolated from urine samples compared to other urine pathogens [9]. In the present study, urine pathogens were isolated from 325 urine samples of outpatients and inpatients. Of these patients, 198 cases were positive *K. pneumoniae* (60.92%), of whom 119 were female (60.10%) and 79 cases were male (39.90%). Khalil et al. reported positive urine culture results of the women 82.8%, and UTI in men as 17.2% [10]. Therefore, the results of this study, such as many previous studies, show that women are more exposed to UTI such as *K. pneumoniae* more than men are. In this regard, different reasons have been proposed such as shortness of urethra and being close to anus in women, which is one of the main causes of UTI. On the contrary, men have different anatomical system of urinary tract with prostate secretions

containing inhibitory substances like cationic proteins that overall could play an important role in the fight against the invasion of urine pathogenic bacteria [11]. Due to the indiscriminate use of antibiotics in recent years that has led to the emergence and spread of resistant bacteria, especially MDR resistant strains, treatment of many infections caused by important pathogens such as *K. pneumoniae* has been associated with numerous problems that increases mortality risk. According to the results, the most resistance of *K. pneumoniae* isolates was to gentamicin (45.45%), tetracycline (27.7%), and chloramphenicol (25.75%). According to the study by Jazayeri from Semnan, rate of resistance to ciprofloxacin has been 92.6% and gentamicin 93 [12]. In an article by Taslima et al. published in Bangladesh, the resistance of these bacteria to ceftazidime is 36%, gentamicin 27%, tetracycline 27, and ciprofloxacin 45% [13].

In a study, Mozaffari et al. (2007) determined that 61.2% of *K. pneumoniae* isolated has drug resistance, of which 20.4% had 100% drug resistance to all cephalosporins (cefixime, ceftriaxone, ceftazidime, ceftizoxime, etc.) [12]. In a study, Shahcheragi et al. reported the resistance of *K. pneumoniae* to carbenicillin (98%), piperacillin (55%), cefotaxime (32%) and ceftazidime (31%) and reported no strains resistant to imipenem [14]. In a study, Jafari-sales et al. reported the resistance of *K. pneumoniae* Cefotaxime (24%), Ciprofloxacin (11%), Ceftazidime (10%), Amikacin (7%), Gentamicin (6%) [15]. Therefore, based on the results of this study, the rate of resistance of the isolates investigated, in comparison with the studies mentioned, regarding all antibiotics have some differences, which can be considered due to sample size or sampling method. In addition, high percentage of resistance to ceftazidime and tetracycline antibiotics, compared to other countries, shows a very sobering situation in terms of the spread of antibiotic resistance among clinical isolates of our country and our region. In this study, of 198 isolates of *K. pneumoniae*, 96 (48.48%) cases had MDR that is less than the results reported by Subha et al. from India in 2001, who had reported the rate of MDR of isolates of *K. pneumoniae* as 100% [16]. Increasing prevalence of these strains has many reasons and may be due incorrect administration of antibiotics in the treatment of various infections or transfer of resistance genes by various transport factors such as plasmids R, bacteriophage, transposons and integrons [17].

Conclusion

These findings show the thought provoking status of antibiotic-resistance of *K. pneumoniae* strains in the study area that in the absence of adequate attention in not a very distant future will bring irreversible consequences for health care.

Conflicts of Interest

There are no conflicts of interest.

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