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RESEARCH ARTICLE

Antibiotic Susceptibility of *Klebsiella pneumoniae* Strains Isolated from Clinical Samples

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Abstract: Antibiotic resistance in bacteria has become a worrying phenomenon in today's world. *K. pneumoniae* is a member of the *Enterobacteriaceae* family, which causes nosocomial infections as an opportunistic pathogen but inherently harboured as a part of the natural human microbiota. Carbapenem resistance of *K. pneumoniae* was a rare occurrence up to ten years ago, but in recent years many types of carbapenemase producing *K. pneumoniae* have become common. This retrospective study aims analysing susceptibility to various antibiotics, commonly used in treatment against *K. pneumoniae* strains isolated by using conventional methods from various infection sites. Antibiotic susceptibility tests were performed by using an automated system, the VITEK 2 Compact ®. In this study, 502 *K. pneumoniae* strains isolated from patients that treated at various services of a university hospital with 515-bed capacity were examined. When compared to the data available with studies of recent years in Turkey, especially in intensive care and inpatient services, the resistance of *K. pneumoniae* strains to antibiotics against most to carbapenems is rapidly increasing in the degree of high concern. Therefore, in all hospitals, antibiotic management policies should be implemented with a multidisciplinary approach.

Keywords: *Enterobacteriaceae*, *Klebsiella pneumoniae*, Drug Resistance, Bacterial, Retrospective Studies.

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INTRODUCTION

Increasing resistance of bacteria is reported with each passing day. This global threat now requires the implementation of antibiotic management policies in all hospitals. In antibiotic management, there are packages of measures aimed at improving the quality of anti-infective therapy with regulations on dosage, method of administration, and duration of treatment. While the best possible treatment is configured for each patient individually, resistance development is minimised and costs are reduced considering public health (1). Many international guidelines have defined the structural requirements and basic strategies of antibiotic management (2). However, for example in the USA, there is a legal requirement for antibiotic management only since 2017. In Germany, the Infection Protection Law was amended in 2011 and necessary measures were taken to rationalise antibiotic consumption by hospitals (1). Also in Turkey, there is a legal penalty for antibiotic sale without a proper prescription only since 2017 (3).

The genus name of *Klebsiella* comes from the German scientist Edwin Klebs (1834-1913) who discovered them. It is a genus of bacteria that is

often isolated from health facilities such as hospitals, nursing homes, and long-term treatment centers. The capsules of *Klebsiella* species are responsible for the mucoid appearance of bacterial colonies and the increased virulence of the microorganism *in vivo*. The most commonly isolated forms of these bacteria are *Klebsiella oxytoca* and *Klebsiella pneumoniae*, which cause nosocomial or community-acquired lobar pneumonia. Symptoms vary according to which part of the body is affected by the bacteria. Besides, these symptoms are the same regardless of which bacteria are the causes. For example, a *Klebsiella*-induced pneumonia patient and a patient suffering from pneumonia for another reason have the same symptoms as high fever, chills, chest pain, and difficulty of breathing (4).

Although *K. pneumoniae* is a bacterium that is resistant to many antibiotics, it is reported that *K. pneumoniae* has a natural resistance to ampicillin (4). Mostly cephalosporin and aminoglycoside antibiotics were chosen for the treatment of the infections caused by it, but *K. pneumoniae* isolates have developed resistance to cephalosporin by the production of extended-spectrum beta-lactamase (ESBL) enzymes and limited their use. Therefore, carbapenems are now preferred (5). With the introduction of carbapenems, resistance to these antibiotics has been reported too. Determining the appropriate treatment protocol for *K. pneumoniae* isolates and analysing the resistance status to prevent the development of resistance has become essential. High mortality rates have been seen in infections caused by *K. pneumoniae* strains resistant to carbapenems, especially septicemia. Most of these infections occur asymptomatic and the risk of comorbidity increases by previous surgeries, invasive devices and apparatuses and colonies commonly found in the body (6).

Although it is a rare phenotype in many geographical regions, carbapenem resistance of *K. pneumoniae* was first reported in 1983. Then carbapenemase-producing *K. pneumoniae* (primarily in Greece) emerged and shown that they were producing Metallo-beta-lactamase (7). *K. pneumoniae carbapenemase* (KpC), a new family of enzymes, were first identified in the USA and caused epidemics, mainly in New York. Carbapenemase-producing *Enterobacteriaceae* members are susceptible only to polymyxins and tigecycline, so they pose a serious threat in hospitals and should be carefully monitored for carbapenem resistance (8).

In this study, 502 *K. pneumoniae* isolates were examined which have been taken from the various clinical specimens. The isolates were transferred by medical personnel to the medical microbiology laboratory of a university hospital. Data were listed in the Microsoft Excel spreadsheets considering their susceptibility to antibiotics.

The aim of this retrospective study is analysing susceptibility to various antibiotics, commonly used in treatment, against *K. pneumoniae* strains isolated from various infection sites.

MATERIALS AND METHODS

Between 15.08.2017 and 10.11.2018, 502 microorganisms isolated from various regions of a university hospital were analysed retrospectively, considering patients' records. The study was approved by the ethics committee of İstanbul Medipol University.

Collection Of Isolates

K. pneumoniae culture samples were provided to a university hospital's clinical microbiology laboratory from over 33 separate outpatient clinics and inpatient wards; MacConkey and blood agar cultivation was carried out by microbiologists and samples were incubated at 37 °C for 17-24 hours. For the objectives of this research, only the cultures that tested positive for *K. pneumoniae* were included in the study. VITEK 2 Compact® (Biomerieux, France), for the identification of species of isolated microorganisms, an automated bacterial identification system, and common conventional methods have been used.

Susceptibility testing

All susceptibility testing was performed in a clinical microbiology laboratory. Antibiotic susceptibility of the isolated strains to 16 antibiotics (Amikacin, Ampicillin, Ampicillin-Sulbactam, Imipenem, Meropenem, Levofloxacin, Ciprofloxacin, Fosfomycin, Nitrofurantoin, Amoxicillin-Clavulanic Acid, Piperacillin-Tazobactam, Trimethoprim-Sulfamethoxazole, Cefotaxime, Cefepime) were tested. The method was coordinated with VITEK 2 Compact® (France, Biomerieux) automated antibiotic susceptibility testing system. Antimicrobial susceptibility of the isolates was examined by considering the recommendations of the European Antimicrobial Susceptibility Testing Committee (EUCAST) (9).

Data organisation & Statistical analysis

The data was imported into Microsoft Excel (Microsoft, Inc., U.S.A.) spreadsheet file and all-important patient identifiers were properly and securely discarded. The patients' data were classified according to type of clinic that they have been treated, nationality, sex, and age, microorganisms were classified according to the regions on patients they were colonised and antibiotic conditions were examined but not all

antimicrobials were tested against each isolate. In the study group, patients between 10 and 60 years of age were included in the sample in order to avoid inconsistency in the analysis results of age-related risk factors (10,11). Descriptive statistical analysis of isolates was performed using the program Prism (software version 8.3.0 (538); GraphPad Software, LLC). T-tests (two-tailed), Chi-square (and Fisher's exact) tests were used to assess any significant differences among the groups. A probability value of (P) <0.05 was considered statistically significant.

Comparison of materials and methods used in various studies

While analysing, regional differences in antibiotic susceptibilities should be considered as a result of antibiotics that used commonly in that region. Risk factors that play a role in the development of infections such as microbiota status, age, sex, postoperative status, implant and chronic diseases of the patients should be considered. When necessary, these patients should be excluded from the study sample in order not to cause meaninglessness in the study and thus to reduce reliability (10,11). Also, in studies involving large patient groups, separate studies should be conducted on the effects of risk factors on infection and bacterial resistance. Patients admitted to an institute as a tourist, especially in retrospective analysis, should be excluded from the research sampling when necessary, especially patients who cannot be treated in their own country because they are likely to have infections caused by bacteria with multiple drug resistance.

Many systems have been tried for continuous optimisation of treatment protocols, especially in the fight against nosocomial infections. However, in these studies, ones that placed the retrospective analysis method at the center, we encounter inconsistent results due to the risk factors mentioned. For example, systems such as the global anti-microbial resistance surveillance system (GLASS) have the disadvantage that they present too much data to a small staff to analyse, while not allowing them to validate results because of the distance from the environment where the data is obtained. The abundance of data distorts the study results and makes the analysis difficult. Single-center working groups should be formed and regional results should be combined (1).

Various programs (IBM SPSS, Stata, PSPP) are used for performing statistical analyses in clinical studies. Although statistical analyses can be performed manually by using the proper formulae for observed data (12). The program Prism was used in this study, and proper formulae were chosen according to literature sources that interest this study (13–15).

Clinical and statistical significance in medicine differs from each other. Statistical significance is making predictions about the population, which are based on the sample size of the patients, with clinical data gathered. So every statistically significant finding may not be clinically relevant, likewise for every clinically finding is subjected to the same situation. Considering this, various methods should be used to eliminate mistakes in clinical results and data should be organised in the best way possible for statistical analyses (16). For example, results obtained with automation systems such as VITEK 2 Compact and MALDI-TOF MS (e.g., carbapenem inactivation method (CIM) for Carbapenem resistance) should be provided manually at the center where the work is performed.

According to the data obtained in the centers where the studies are conducted, treatment protocols should be coordinated and implemented especially for the empirical treatment. The results of the studies should be compared according to the effectiveness of the treatments. Thus, another method can be obtained to validate the results of the studies (1).

RESULTS

The university hospital, which is the center of the study, accepts patients from many countries as shown in Figure 1. Thus, antibiotic resistance in different countries, Turkey is composed of comparisons with the data.

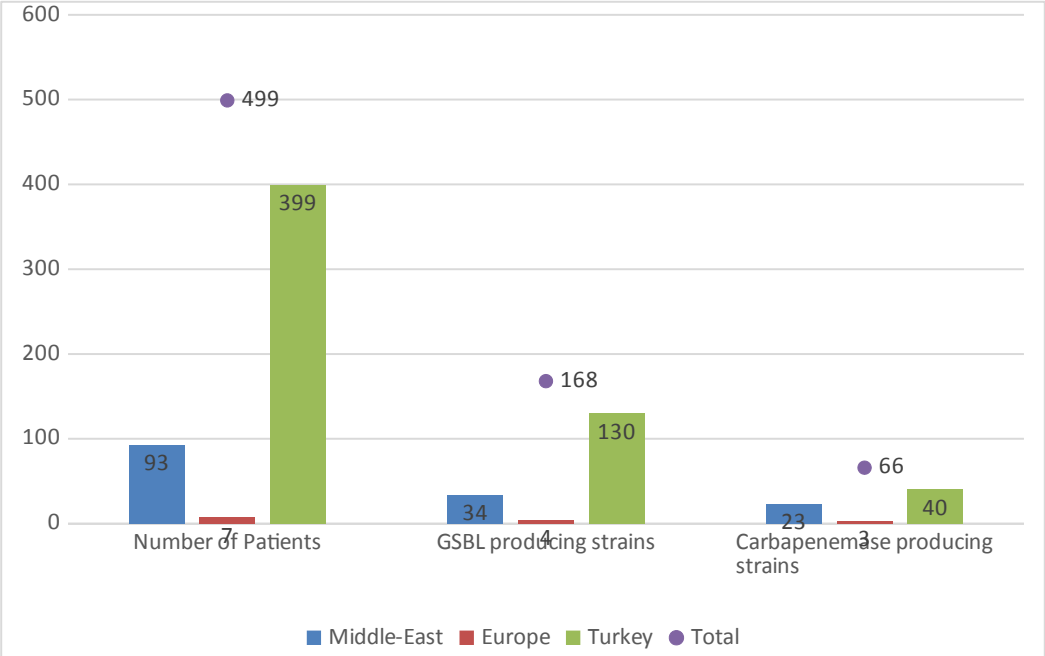

Figure 1: Patients sorted by their country.

As shown in Table 1, there is only one strain that does not express any beta-lactamase and probably this result was obtained due to an error; because *K. pneumoniae* has a natural resistance to ampicillin (4). In *K. pneumoniae*, resistance to beta-lactam group antibiotics is of concern. In addition, Cefepim, a member of 4th generation cephalosporins, has superiority over 3rd generation Cefotaxime as expected. All ESBL positive strains are considered resistant to cefotaxime and other third-generation cephalosporins (17). Clinical use of carbapenems for Turkey is still effective in the treatment of *K. pneumoniae* infection. However, in order to prevent the development of resistance to this group of antibiotics in the future, particular attention should be paid to its use in the treatment of empirical therapy.

Table 1: Antibiotic susceptibility and total sample numbers of strains that were isolated[#]

Antibiotic	Resistant Strains	Susceptible strains	Total
Amikacin	34	383	417
Ampicillin[*]	222	1	223
Ampicillin-Sulbactam	121	101	222
Imipenem	64	437	501
Meropenem	88	414	502
Levofloxacin	146	356	502
Ciprofloxacin	168	334	502
Fosfomycin	12	85	97

Nitrofurantoin	52	123	175
Amoxicillin-Clavulanic Acid	169	159	328
Piperacillin-Tazobactam	120	208	328
Trimethoprim-Sulfamethoxazole	160	168	328
Cefotaxime	224	193	417
Cefepime	133	284	417

[#]P<0,0001 (Chi-square), * Ampicillin is not included because of *K. pneumoniae's* natural resistance.

The higher smoking addiction in men than in women and the consequence of chronic pulmonary diseases such as COPD; It can be said that strains isolated from pulmonary infections in men cause more resistance as seen in Figure 2 (18).

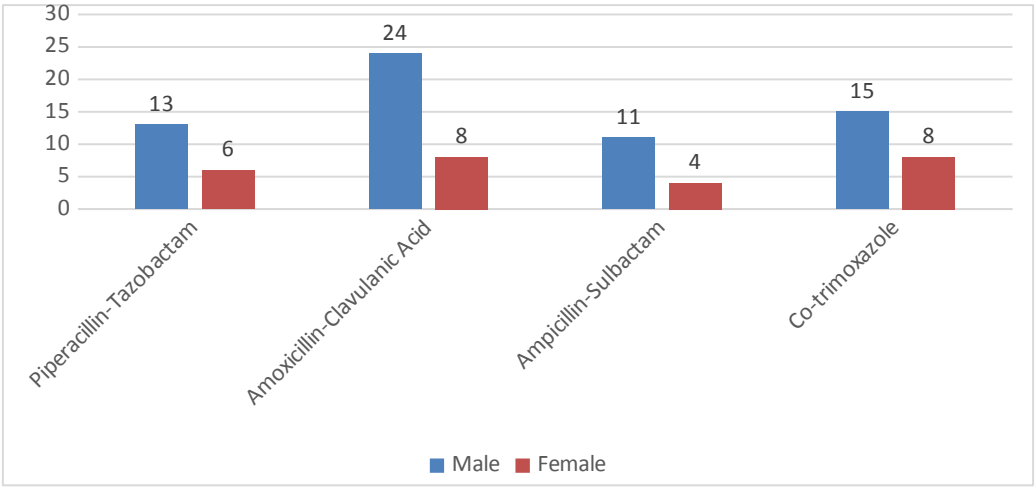

Figure 2: Gender distribution of antibiotic resistance of strains isolated from tracheal aspirate and bronchoalveolar lavage cultures, P=0.0261 (T-test).

Women are more prone to urinary tract infections due to their biological structure (19). Therefore, women are exposed to bacterial profiles more resistant than males because they use more antibiotics against infection. Likewise, the distribution of efficacy of strains isolated from urinary tract infections against broad-spectrum fluoroquinolone antibiotics and phosphomycin was seen with high resistance in females (Figure 3).

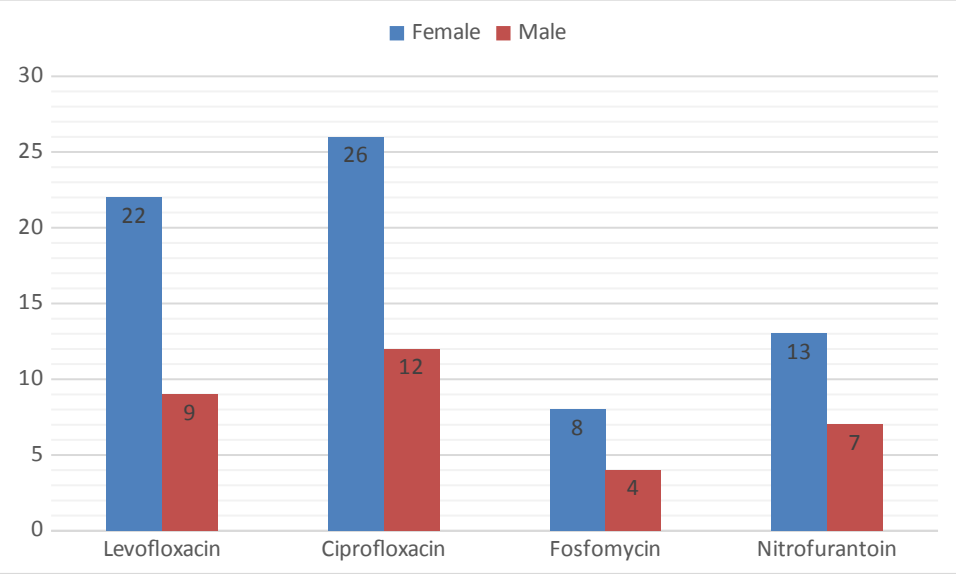

Figure 3: Resistance Distribution According to Gender in Urinary Tract Infections, P= 0.0341 (T-test).

DISCUSSION AND CONCLUSION

Today, due to the unnecessary and widespread use of antibiotics, gram-negative rods with multidrug resistance are reported all over the world. In recent years, the treatment of strains that become hypervirulent by producing ESBL (oxacylanase, high-level AmpC Beta-Lactamase, Carbapenemase) has become a major problem due to the multiple resistance strains encountered in intensive care patients (20).

In a multi-center study conducted in our country, "Meropenem Yearly Susceptibility Test Information Collection"2007 data, 40.5% of *K. pneumoniae* strains were found to be ESBL positive (21). Different results have been observed in different regional studies conducted in our country. ESBL rates in *K. pneumoniae* strains were found to be 42% in another study and 74% in another study (22). According to another study conducted between 2007 and 2008, 41 *K. pneumoniae strains* produced 24% ESBL (23). In this study, it can be stated that 33% of ESBL producing strains were detected.

Although it varies according to the region it is isolated, carbapenem resistance has started to be seen more frequently in our country. In a study that was conducted with 2903 strains isolated, Samasti *et al.* reported that carbapenem resistance of *K. pneumoniae* was found to be 3.13% (24). In another study conducted in 2014, they reported imipenem resistance to blood cultures as 47% and meropenem resistance as 45% (22). In this study, 13% of resistance was determined against carbapenems.

While analysing, regional differences in antibiotic conditions and the difference between the number of samples studied can be considered as the reason for the differences between susceptibility rates.

For further studies, susceptibility results should be provided by performing disc diffusion tests in the comparative analysis sections (e.g. analysing sex-related factors on susceptibility), the zone radius should be used instead of positive/negative results. Besides, sample numbers should be equal. This will improve statistical significance.

Amoxicillin-Clavulanic Acid, in Turkey, is a common antibiotic used as an empirical treatment choice for community-acquired infections. Data gathered in this study shows that this choice of treatment is still partially effective against *K. pneumoniae* with a rate of 48.5%.

Almost all of the studies in different geographies around the world show that the development of resistance to antibiotics is developing at an alarming rate. Therefore, to keep up with the bacteria in the fight against antibiotic resistance, sufficient personnel and resources need to be mobilised, but on the contrary, we see that some firms have terminated the budgets allocated for antibiotic research and even countries such as the USA are trying to implement national antibiotic management policies more recently (25).

ESBL production rates of *E.coli*, *K. pneumoniae* and other gram-negative enteric bacteria, which are expensive and difficult to treat, should be monitored by each center. In the treatment of infections preferred broad-spectrum beta-lactam

antibiotics should be used with caution. Inpatients should be isolated and surveillance studies should be performed in hospital departments at risk.

Identification of organisms causing nosocomial infections, conducting in vitro antibiotic susceptibility tests and rational use of antibiotics may increase the chance of treatment success, prevent the spread of resistant nosocomial infections and reduce treatment costs. Therefore, antibiotic management policies should be implemented in all hospitals with a multidisciplinary approach.

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SUPPLEMENTARY INFORMATION

1) Ethical council's decision
2) Decision form

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Konu : Etik Kurulu Kararı

21/12/2018

Sayın Ahmet Ozan ÖZGEN

Üniversitemiz Girişimsel Olmayan Klinik Araştırmalar Etik Kuruluna yapmış olduğunuz "Klinik örneklerden izole edilen Klebsiella pneumoniae suşlarının antibiyotik duyarlılıkları" isimli başvurunuz incelenmiş olup etik kurulu kararı ekte sunulmuştur.

Bilgilerinize rica ederim.

Prof. Dr. Hanefi ÖZBEK
Girişimsel Olmayan Klinik Araştırmalar
Etik Kurulu Başkanı

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BAŞVURU BİLGİLERİ	ARAŞTIRMANIN AÇIK ADI	Klinik örneklerden izole edilen Klebsiella pneumoniae suşlarının antibiyotik duyarlılıkları			
	KOORDİNATÖR/SORUMLU ARAŞTIRMACI UNVANI/ADI/SOYADI	Ahmet Ozan ÖZGEN			
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	Belge Adı	Tarihi	Versiyon Numarası	Dili
Değerlendirilen Belgeler	ARAŞTIRMA PROTOKOLÜ/PLANI			Türkçe ☐ İngilizce ☐ Diğer ☐
	BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU			Türkçe ☒ İngilizce ☐ Diğer ☐
Karar Bilgileri	**Karar No: 764**	**Tarih: 19/12/2018**		
	Yukarıda bilgileri verilen Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu başvuru dosyası ile ilgili belgeler araştırmanın gerekçe, amaç, yaklaşım ve yöntemleri dikkate alınarak incelenmiş ve araştırmanın etik ve bilimsel yönden uygun olduğuna **"oybirliği"** ile karar verilmiştir.			

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Dr. Öğr. Üyesi Sibel DOĞAN	Psiko-onkoloji	İstanbul Medipol Üniversitesi	E☐	K☒	E☐	H☒	E☒	H☐	
Dr. Öğr. Üyesi Mehmet Hikmet ÜÇIŞIK	Biyoteknoloji	İstanbul Medipol Üniversitesi	E☒	K☐	E☐	H☒	E☒	H☐	
Dr. Öğr. Üyesi Keziban OLCAY	Endodonti	İstanbul Medipol Üniversitesi	E☐	K☒	E☐	H☒	E☒	H☐	

* :Toplantıda Bulunma