Determination of Resistance in *Pseudomonas aeruginosa* Strains Against Beta-Lactam, Aminoglycoside and Ciprofloxacin Group Antibiotics

Pseudomonas aeruginosa Suşlarında Beta-Laktam, Aminoglikozid ve Siprofloksasin Grubu Antibiyotiklere Karşı Direncin Belirlenmesi

Research Article

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ABSTRACT

Nowadays, due to widespread use of broad range spectrum antibiotics in treatments, Pseudomonas *aeruginosa* developed high levels of resistance to these antibiotics. Therefore it is challenging to treat the infections caused by *P. aeruginosa*. In this study, multi-drug resistant strains and antibiotype groups were identified by investigating resistance in *P. aeruginosa* strains isolated from three different hospitals in Ankara, against beta-lactam, aminoglycoside and ciprofloxacin. Thus, it was aimed that the most effective antibiotics for treatment of *P. aeruginosa* sourced infections were determined. Sixty-nine *P. aeruginosa* strains were investigated for resistance against aztreonam, meropenem, imipenem, amikacin, tobramycin, piperacillin, ceftazidime, cefepime and ciprofloxacin. It was found that the highest resistance rate was against aztreonam (66.6%) and the most effective antibiotic was found to be amikacin (5.7%). Additionally, the rate of multi-drug resistance in *P. aeruginosa* strains was determined as 36%. Although, 18 antibiotype groups were identified in *P. aeruginosa* strains, the highest antibiotype rate was observed in antibiotype 8 which was intermediate to aztronem and sensitive to other 8 antibiotics. In this respect, the combined treatment of aminoglycoside and ciprofloxacin is thought to be effective against resistant *P.aeruginosa* strains.

Key Words
Pseudomonas *aeruginosa*, Antibiotic resistance, Antibiotype, Multi-drug resistance

ÖZET

Günümüzde geniş spekturumlu antibiyotiklerin yaygın kullanımına bağlı olarak Pseudomonas *aeruginosa*'da yüksek oranda antibiyotik direnci gelişmektedir. Böylece etken olduğu enfeksiyonların tedavisinde zorluklar yaşanmaktadır. Çalışmamızda Ankara' daki üç farklı hastaneden izole edilen *P. aeruginosa* suşlarının beta-laktam, aminoglikozid ve siprofloksasin grubu antibiyotiklere karşı olan dirençlilikleri incelenerek çoklu ilaç dirençli suşlar ve antibiyotip grupları belirlendi. Böylece etkeni *P. aeruginosa* olan enfeksiyonların tedavisinde kullanılabilecek en etkili antibiyotiklerin belirlenmesi amaçlandı. 69 *P. aeruginosa* suşu ile aztreonam, meropenem, imipenem, amikasin, tobramisin, piperasilin, seftazidim, sefepim ve siprofloksasine karşı direnç durumları incelendi. *P. aeruginosa* suşlarında en yüksek direnç oranın aztreonama (%66.6) karşı olduğu görülürken, en etkili antibiyotiğin amikasin (%5.7) olduğu belirlendi. Ayrıca, *P. aeruginosa* suşlarında %36 oranında çoklu ilaç direnci saptandı. Bu bağlamda, aminoglikozid ve siprofloksasinin kombine antibiyotik tedavisinin dirençli *P. aeruginosa* suşlarında etkili olacağı düşünülmektedir.

Anahtar Kelimeler
Pseudomonas aeruginosa, Antibiyotik direnci, Antibiyotip, Çoklu ilaç direnci

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INTRODUCTION

Pseudomonas *aeruginosa* is an important opportunistic pathogen that causes high mortality and morbidity rates by frequent and variable infections, especially in humans. Alginate, lipopolysaccharide, flagella, pilus and non-pilus adhesins are important particularly at surface colonization of *P. aeruginosa*. Besides, exoenzymes such as protease, elastase, phospholipase, exotoxin A, exoenzyme S, haemolysins also play a significant role in *P. aeruginosa* infections [1]. The reason of 10-20% of nosocomial infections are assumed to be caused by *P. aeruginosa*. In particular, immune suppressed patients are highly susceptible to *P. aeruginosa* infections. As well as, being naturally resistant to many antibiotics, also some problems may arise due to *P.aeruginosa* developing resistance during therapy [2, 3]. Low permeability of outer membrane, pumping antibiotics out of the cell by active pumping (efflux systems) and inducible chromosomal or derepressed AmpC type beta lactamase enzymes can be listed among the genetic natural resistance mechanisms of *P. aeruginosa* [4]. In addition, chromosome or plasmid encoded beta-lactamase production and chromosomal mutation in peniciline binding proteins are acquired resistance mechanisms in *P. aeruginosa* [5].

Recently, multi-drug resistance (MDR) developed by *P. aeruginosa* strains became a serious problem in worldwide [6]. The strains resistant to at least 3 of aminoglycosides, antipseudomonal penicilins, cephalosporins, carbapenems and floroquinolones groups, are regarded as multidrug-resistant strains in literature studies [7].

In our study, resistance of 69 *P. aeruginosa* strains against beta lactam, aminoglycoside and ciprofloxacin group antibiotics were determined. By this way, it was aimed to detect the most effective antibiotic group that can be an alternative for the treatment in *P. aeruginos* infections.

MATERIALS AND METHODS

Identification of *Pseudomonas* strains

This study was carried out by 69 Pseudomonas strains which were collected from three different hospitals in Ankara. Macroscobic and microscobic examinations of Pseudomonas strains isolated from different clinical wards and clinical specimens were performed. Identification of strains was carried out by phenotypic methods like lactose fermentation on EMB agar, beta-hemolysis on blood agar, oxidase, urea, three sugar iron, IMVC, nitrate reduction and growth at 42ºC tests. Specie level identification of strains was confirmed with cetrimide agar. *P. aeruginosa* strains were stored at -20ºC after inoculation into 10% glycerol included Brain-Hearth Infusion broth.

Determination of antibiotic resistance of *P. aeruginosa* strains

The resistance of *P. aeruginosa* strains against 9 different antibiotics, namely, aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), ceftazidime (30 µg), cefepime (30 µg), amikacin (30 µg), piperacillin (10 µg), ciprofloxacin (5µg) and tobramycin (10 µg) were investigated by Kirby-Bauer disc diffusion method. Overnight culture of strains were adjusted to 0.5 McFarland standard turbidity and inoculated on sterile Mueller-Hinton agar. Antibiotic discs were placed on petri surface and incubated at 37ºC for 24 hours. Inhibition zone diameters were measured and interpreted according to the CLSI criteria [8].

Determination of MDR *P. aeruginosa* strains and antibiotypes

Multi-drug resistant *P. aeruginosa* strains were determined according to antibiotic resistance patterns. Antibitoic resistance patterns of *P. aeruginosa* were based in determining the antibiotype groups. Strains which show the same antibiotic pattern were included into the same antibiotype group which are indicated with the same numbers, strains which show different antibiotic pattern were included into the antibiotype group which are indicated with different numbers.

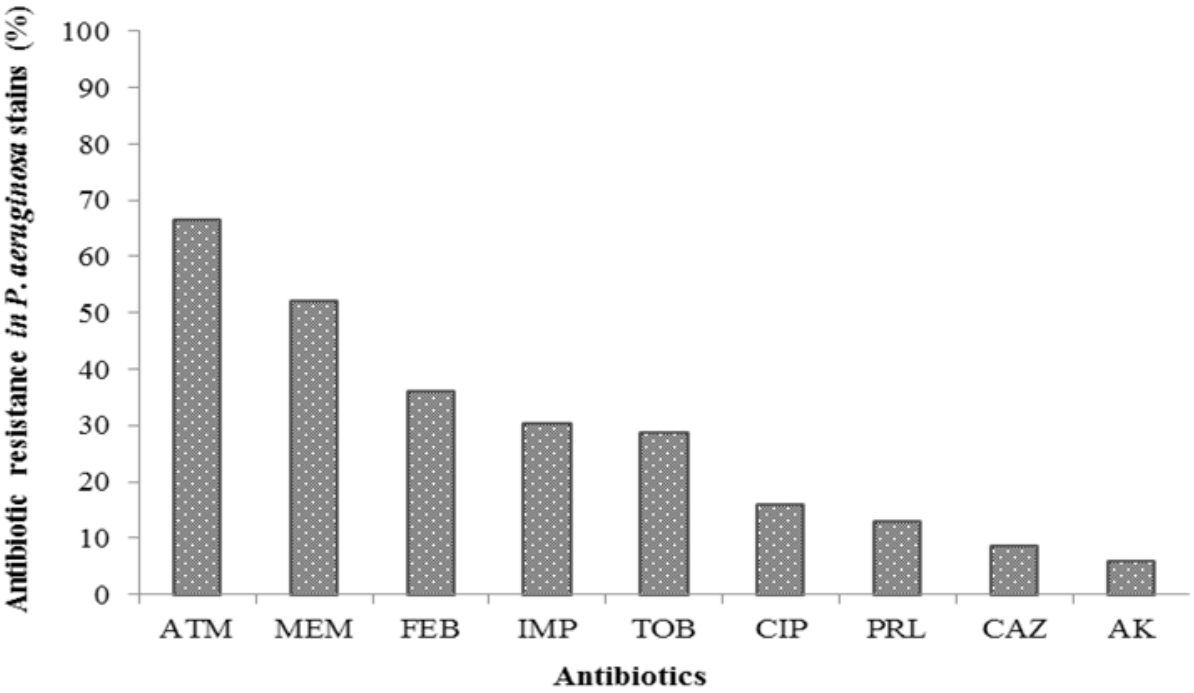

Figure 1. The distribution rates of antibiotic resistance in *P. aeruginosa* strains.
ATM: Aztreonam, MEM: Meropenem, FEB: Cefepime, IMP: Imipenem, TOB: Tobramycin, CIP: Ciprofloxacin, PRL: Piperacillin, CAZ: Ceftazidime, AK: Amikacin.

Following determination of the resistance of *P. aeruginosa* strains to antibiotics; the distribution rates of strains were determined according to the isolated clinical wards, clinical specimens and patient's gender.

RESULTS

In our study, 69 Pseudomonas strains isolated from different clinical wards and specimens from three different hospitals in Ankara, were identified with phenotypic methods. Gram negative cocobacil morphology was determined. Lactose fermentation, methyle red, voges-proskauer, TSI tests results were negative; citrate, nitrate, oxidase, growth at 42ºC and growth on cetrimide agar tests results were positive while urea test results showed differences between strains.

Determination of antibiotic resistance rates of *P. aeruginosa* strains

In our study, the highest resistance rate was observed against aztreonam (66.6%) and the most effective antibiotic was observed as amikacin (5.7%) (Figure 1). The rate of multi-drug resistance in *P. aeruginosa* strains was determined as 36%.

Determination of antibiotype patterns of *P. aeruginosa* strains

Totally 18 antibiotype patterns were determined on 69 *P. aeruginosa* strains after determining the antibiotic resistance rates of *P. aeruginosa* strains (Table 1). Although, in our study high rates of antibiotic resistance were ensured, the highest antibiotype pattern was found to be at antibiotype 8 which was intermediate to aztronem and sensitive to all other 8 antibiotics (Figure 2).

When the distribution rates of *P. aeruginosa* strains isolated from clinical wards, were investigated, it was found that the strains were more frequently isolated from pediatry, plastic and reconstructive surgery and intensive care unit (Figure 3).

When the distribution rates of *P. aeruginosa* strains isolated from clinical specimens were investigated, it was found that the strains were more frequently isolated from wounds and urine

Table 1. Antibiotype patterns identified by antibiotic resistance of *P. aeruginosa*.

Antibiotics	ATM	IMP	MEM	CAZ	FEB	AK	PRL	CIP	TOB
ANT 1	R	S	R	S	R	S	S	S	S
ANT 2	R	S	R	R	R	R	R	R	R
ANT 3	S	S	S	S	S	S	S	S	S
ANT 4	R	R	R	S	R	S	S	S	R
ANT 5	R	S	R	S	S	S	S	S	S
ANT 6	R	S	S	S	R	S	S	S	S
ANT 7	R	S	S	S	S	S	S	S	S
ANT 8	I	S	S	S	S	S	S	S	S
ANT 9	R	R	R	S	R	S	R	R	R
ANT 10	R	S	R	R	R	S	R	S	R
ANT 11	R	R	S	S	S	S	S	S	S
ANT 12	R	R	R	S	S	S	S	S	S
ANT 13	R	R	R	S	R	R	R	R	R
ANT 14	R	R	R	S	R	S	S	R	R
ANT 15	R	R	R	R	R	S	R	R	S
ANT 16	R	R	R	S	R	R	S	R	R
ANT 17	R	R	R	R	R	S	R	R	R
ANT 18	R	R	R	S	R	S	S	S	S

ATM: Aztreonam, IMP: Imipenem, MEM: Meropenem, CAZ: Ceftazidime, FEB: Cefepime, AK: Amikacin, PRL: Piperacillin, CIP: Ciprofloxacin, TOB: Tobramycin; S: Sensitive; I: Intermediate; R: Resistance; ANT: Antibiotype

(Figure 4). It was determined that *P. aeruginosa* strains were isolated from much more frequent from males compared with females.

DISCUSSION

The increase in multiple drug resistant *P. aeruginosa* strains in recent years causes serious problems in the treatment of *P. aeruginosa* infections. Especially long-term hospitilization, long term broad spectrum antibiotic treatment and chemotherapy in cancer patients increase the rate of *P. aeruginosa* infections. In this respect, totally 69 *P. aeruginosa* strains which were isolated from three different hospitals in Ankara, were investigated for their resistance against beta-lactam, aminoglycoside and ciprofloxacine group antibiotics.

In our study, it was determined that the highest resistance rates were observed against aztreonam and meropenem (Figure 1). Aztreonam; is the only antibiotic which is not effected by metallo-beta lactamases and very important in resistance to antibiotics in particular carbapenemes [9]. Although aztreonam is used in treatment of *P. aeruginosa* sourced infections, high (66.6%) aztreonam resistance was determined in our study. High rate of aztreonam resistance were observed in similar studies [10, 11]. Although carbapenems take rather important role in treatment of infections that are caused by multi-drug resistant *P.aeruginosa* strains, its effectiveness is under risk due to developed carbapenem resistance. In our study, 52% meropenem (one of the carbapenem group antibiotic) resistance was detected. Resistance to carbapenem usually has many

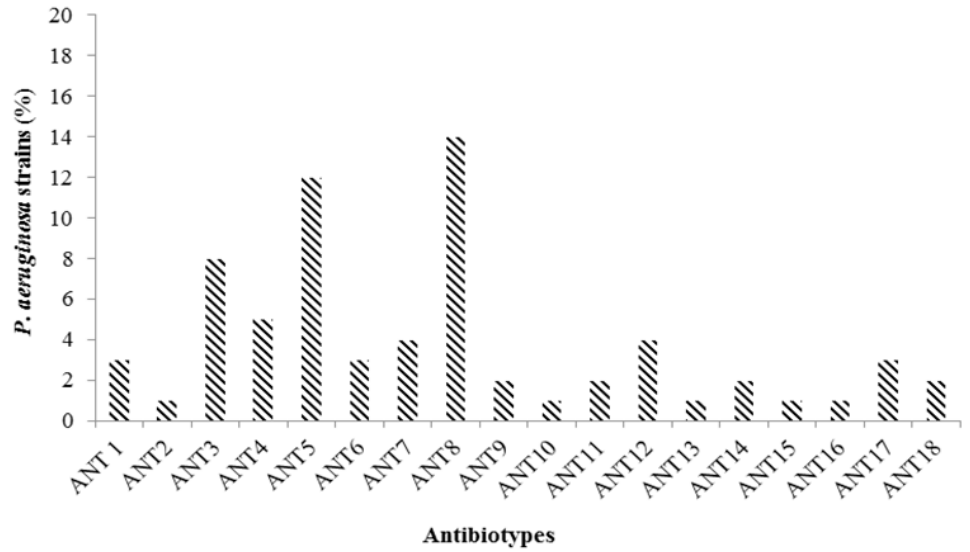

Figure 2. The distribution rates of antibiotic resistance in *P. aeruginosa* strains;
ATM: Aztreonam, MEM: Meropenem, FEB: Cefepime, IMP: Imipenem, TOB: Tobramycin, CIP: Ciprofloxacin, PRL: Piperacillin, CAZ: Ceftazidime, AK: Amikacin.

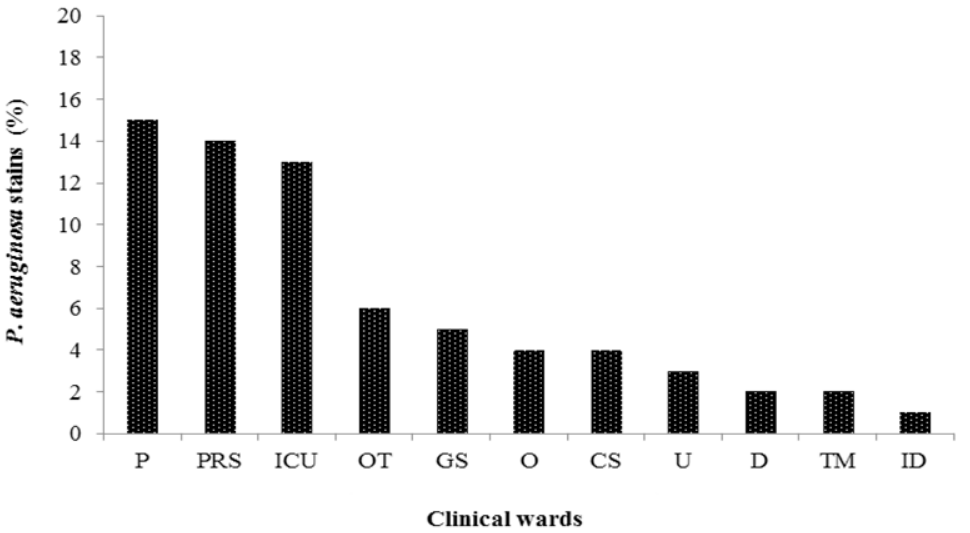

Figure 3. The distribution rates of antibiotype patterns in *P. aeruginosa* strains.
P: Pediatry, PRS: Plastic and reconstructive surgery, ICU: Intensive care unit, OT: Orthopedics and traumatology, GS: General surgery, O: Otorhinolaryngology, CS: Cardiovascular surgery, U: Urology, D: Dermatology, TM: Thoratic medicine, ID: Infection disease

factors besides metallo-beta lactamases play an important role in this resistance. In addition to this, increased chromosomal AmpC type beta lactamas production, decreased expression of outer membrane porin proteins (OprD) and many other related factors are known to contribute to carbapenem resistance [12, 13]. In particular the decrease in the sensitivity of *P. aeruginosa* to meropenem is related with decreased amount of OprD porin [14]. Similar results with our study were reported, which are performed with multi-drug resistance *P. aeruginosa* strains [15]. In our study 30% resistance was determined to imipeneme and this resistance rate was similar in other studies [16, 17].

In this study, amikacin and ceftazidime were determined as the most effective antibiotics for

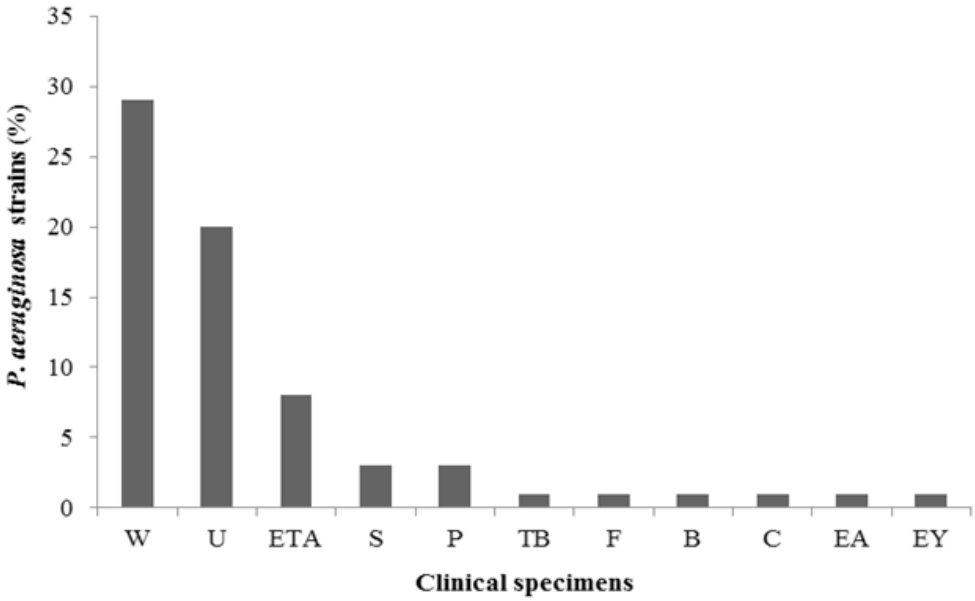

Figure 4. The distribution rate of *P. aeruginosa* strains according to clinical specimens .
W: Wound, U: Urine, ETA: Endotracheal aspirate, S: Sputum, P: Puy, TB: Tissue biopsy, F: Feces, B: Blood, C: Catheter, EA: Ear, EY: Eye.

treatment of *P. aeruginosa* infections (Figure 1). It was shown that amikacin is the most effective antibiotic against *P. aeruginosa* strains since only 5.7% of strains have resistance to amicacin. Amikacin; an aminoglycoside antibiotic, is known as much more effective in *P. aeruginosa* and other Gram negative bacteria infections than the same group antibiotics since it is effected slightly from aminoglycoside modifying enzymes which are intracellular enzymes different than other antibiotic inactivating enzymes. Thus, it becomes a an important antibiotic that is used in treatment of *P. aeruginosa* infections [18]. Effectiveness of amikacin against *P. aeruginosa* was also shown in other studies [19-21]. Combined therapy was recommended especially in treatment of *P. aeruginosa* infections and to prevent resistance development during treatment. In this sense, an antipseudomonal beta-lactam and aminoglycoside or quinolone combinations are usually used [22, 23]. Ceftazidime resistance was detected as 9% in *P. aeruginosa* strains in our study. Ceftazidime; is in third generation cephalosporin group and shows very good in vitro activity against *P. aeruginosa*, known as effective and safe in treatment of infections sourced by these microorganisms. Similar to our study, in some other studies ceftazidime resistance was found to be very low [24,25]. Contrary to our study, a study performed with *P. aeruginosa* strains; ceftazidim resistance was found rather high as 53.4% [26]. These different resistance rates were thought to be due to the antibiotic utilizaiton policies of the hospitals where the strains were isolated. It is known that antibiotic resistance rates vary by geographic regions, hospital to hospital, different regions of hospital and even among different years in the same hospital [27].

In our study, high rate of antibiotic resistance was detected in *P. aeruginosa* strains and 36% multiple drug resistance was determined. It was detected that multi-drug resistance strains were isolated from 28% pediatry and general intensive care unit; 44% urine and 28% wounds. It was observed in similar study that 44% of *P. aeruginosa* strains have multi-drug resistance while 39% of MDR strains were isolated from wound and 18% of MDR strains from urine [28]. It was determined in similar studies that 36% of *P. aeruginosa* strains have multi-drug resistance [29,30]. On the contrary, in another study high level (88%) of multi-drug resistance

was determined in *P. aeruginosa* strains [23].

Antibiotyping; is important for determining the spread of nosocomial infection sources and developing rational pathogen control precautions. For this purpose, 18 different antibiotype groups were identified after determining the antibiotic resistance profiles (Table 1). The highest antibiotype ratio was observed in antibiotype 8 which was intermediate to aztreonam and sensitive to other 8 antibiotics (Figure 2). Those identified similar antibiotypes point out the spread of similar profiled *P. aeruginosa* strains in different hospitals in Ankara. In similar study, 26 antibiotype groups were identified according to resistance rate of 81 *P. aeruginosa* strains against 7 different antibiotics (amikacin, aztronam, carbanicilline, ceftazidime, ciprofloxacine, imipenem, ticarcillin/clavulanic acid), antibiotype which shows the highest ratio is sensitive against all antibiotics that are used in the study and results were found to be compatible with our study [24]. 8 different antibiotype patterns were detected in another study which was carried out with 58 *P. aeruginosa* strains and 11 different antibiotics (amikacin, netilmicin, gentamicin, ceftazidime, cip rofloxacine,cefaperozone+sulbactam, piperacillin, piperacillin+tazobactam, aztreonam, imipenem, meropenem) [30].

When the distribution rates of *P. aeruginosa* strains were investigated through the clinical wards, clinical specimens and patient genders, it was observed that strains were frequently isolated from pediatry, plastic and reconstructive surgery and intensive care unit wards (Figure 3; Figure 4). *P. aeruginosa* causes serious bacteremia in children [31]. It was observed in an another study which was carried out with 108 pediatry patients that, *P. aeruginosa* strains cause septicemia, pneumonia and otorhinolaryngology infections and cause more infections at male children rather than female children [32]. In a similar study that was performed with *P. aeruginosa*, most of the strains were isolated from burn units and plastic and reconstructive surgery. Similar studies reported that multi-drug resistance *P. aeruginosa* strains were generally isolated from intensive care units [6, 33]. Threfore, improving conditions of intensive care units and strict infection control precautions, immediate discharge of recovered patients from intensive care unit, avoiding unnecessary and long period invasive surgery and especially applying right antibiotic treatment at respiratory tract, urinary system and wound infection developed patients, will be effective for decreasing incidence of *P. aeruginosa* strains isolated from those samples.

In our study, it was determined that the most of the *P. aeruginosa* strains were isolated from wounds, urine and endotracheal aspirate specimens. From the other studies, it was determined that the most of the *P. aeruginosa* strains were isolated from wounds and urine [6, 34]. In a study carried out in Turkey, *P. aeruginosa* strains, seen in patients hospitalized in intensive care unit, are usually isolated from mostly tracheal aspirates, urine and wound cultures [35]. These cases are sourced by applying surgeries (mechanic ventilation, catheter, tracheostomy, thraceal intubation, surgery) causing the colonization of *P. aeruginosa* strains [36]. In our study, it was determined that the majority of *P. aeruginosa* strains were isolated from male patients. It was observed in similar studies that *P. aeruginosa* strains were isolated mostly from male patients rather than female patients [6,37].

As a result, treatment of infections caused by *P.aeruginosa* strains is becoming more difficult since its natural resistance against most of the antibiotics, as well as developing new resistance mechanisms. Development of resistance against most of the antibiotics used during treatment and development of cross resistance cause multi-drug resistance. Therefore, the determination of antibacterial resistance rates in each hospital will be effective in ensuring infection control. Accordingly, the combined treatment of aminoglycoside (amikacin) and ciprofloxacin (ceftazidime) is thought to be efficient to prevent the high resistance rates observed in *P. aeruginosa* strains.

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