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# Antibiotic Susceptibility and Carbapenem Resistance of Gram Negative Bacilli Recovered from Various Specimens of Hospitalized Patients in a Training Hospital for Chest Diseases (*)

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***ÖZET***

***Bir Göğüs Hastalıkları Eğitim Hastanesinde Yatan Hastaların Çeşitli Örneklerinden Soyutlanan Gram Negatif Çomakların Antibiyotiklere Duyarlılıkları ve Karbapeneme Direnç Özellikleri***

*Bu çalışmada, İzmir Göğüs Hastalıkları Hastanesinde Ekim-Kasım 2000 aylarında izole edilen 44 Pseudomonas spp., 9 Klebsiella, 8 Escherichia coli, 6 Enterobacter, 4 Acinetobacter , 3 Gram olumsuz nonfermentatif çomak ve bir Proteus mirabilis suşu olmak üzere toplam 75 suş incelenmiştir. Pseudomonas ve Acinetobacter suşlarının dışında kalan suşların amoksisilin-klavulanik asite (AMC) direnç oranları ortalama % 30 olarak bulunmuştur. Oksiimino-beta laktamlara dirençli suşların oranı ortalama % 41-52 olarak saptanmıştır. Pseudomonas , Klebsiella ve Enterobacter suşlarında genişlemiş spektrumlu beta-laktamaz oluşumu, sırasıyla, 12 / 44 ( % 27.2), 1 / 9 (% 11.1) ve 3 / 6 ( % 50) oranlarında belirtilmiştir. Direkt indüksiyon testi ile Pseudomonas ve Enterobacter suşlarında indüklenebilir beta-laktamaz üretimi sırasıyla 15 / 44 ( % 34), 1 / 6 (% 16.6) oranlarında gösterilmiştir. Disk difüzyon testi ile karbapeneme direnç, imipenem için suşların 18' inde ( % 24), meropenem için ise 16'sında (% 21.3) saptanmıştır. İmipeneme disk difüzyon ile orta derecede duyarlı bulunan bir Acinetobacter ve bir Pseudomonas suşu, meropeneme karşı duyarlı bulunmuştur. Genel olarak, MİK değerlerine göre, Gram olumsuz nonfermentatif çomaklarda imipeneme direnç, meropeneme göre anlamlı derecede yüksek bulunmuştur (p<0.05). Bunun yanısıra, meropenem için disk difüzyon ile bulunan direnç oranları, MİK değerlerine göre bulunan oranlardan anlamlı derecede yüksek bulunmuştur (p<0.05).*

***Anahtar kelimeler:*** *Gram olumsuz çomak, antibiyotiklere duyarlılık, genişlemiş spektrumlu beta-laktamaz, indüklenebilir beta-laktamaz, karbapenemler, direnç.*

***SUMMARY***

*In this study, a total of 75 gram negative rods, comprising 44 Pseudomonas spp., 9 Klebsiella spp., 8 Escherichia coli, 6 Enterobacter spp., 4 Acinetobacter spp., 3 Gram negative nonfermentative bacilli and one Proteus mirabilis, which were isolated at the Training Hospital for Chest Diseases in Izmir during September-October were studied. Amoxicillin-clavulanic acid resistance for the bacteria out of Pseudomonas and Acinetobacter strains were found as approximately 30 % and for oxyimino beta-lactams as 41-52 %. Ratios for extended spectrum beta-lactamase production which was determined in Pseudomonas, Klebsiella and Enterobacter strains were 12 / 44 (27.2 %), 1 / 9 (11.1 %) and 3 / 6 (50 %), respectively. Inducible beta-lactamase production in Pseudomonas and Enterobacter strains by direct induction test was determined as the ratios ; 15 / 44 (34 %), 1 / 6 (16.6 %) respectively. Resistance for carbapenems by disk diffusion was found in 18 (24 %) strains for imipenem and in 16 (21.3 %) strains for meropenem respectively. One Acinetobacter and one Pseudomonas which were found as intermediately resistant against imipenem were determined as susceptible to meropenem. In general, according to the MIC values, imipenem resistance in gram negative nonfermentative rods was significantly higher than meropenem resistance (p< 0.05). Moreover, ratio of resistant strains for meropenem by disk diffusion was significantly higher than the ratio according to the MIC values (p < 0.05).*

***Key words :*** *Gram negative rod, antibiotic susceptibility, extended spectrum beta-lactamase, inducible beta-lactamase, carbapenems, resistance.*

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## INTRODUCTION

Antibiotic resistance to Gram negative bacilli in hospital infections is an important problem all over the world and the frequency of resistant strains differs among the hospitals from one region to another. Therefore, it is necessary to follow the surveillance , take the precautions and make the accurate therapeutic selections. The most important gram negative bacilli in hospital infections are *Pseudomonas aeruginosa, Escherichia coli, Klebsiella spp., Enterobacter spp., Acinetobacter spp*. and *Serratia spp*. Bacterial resistance are mainly due to production of a variety of beta-lactamases (1, 2) . Isolates which produce extended spectrum beta-lactamases (ESBL) and/or chromosomally encoded inducible beta-lactamases are widespread and the percentage of their presence differs in different species from hospital to hospital. Additionally, carbapenem resistance which has been increasing in Gram negative bacilli, especially in nonfermentative bacilli such as *Pseudomonas aeruginosa, Acinetobacter spp*. and *Stenotrophomonas maltophilia* causes a great problem in hospitalized patients (1). Resistance to carbapenems are mainly due to metallo beta-lactamase activity, deficiency of Opr D protein (outer membrane protein D2) and porin mutations (3-10). In the present study, it was intended to analyze the susceptibility of hospital-acquired Gram negative bacilli to various antibiotics and evaluate their resistance against carbapenems.

## MATERIALS AND METHODS

In this study, a total of 75 Gram negative bacilli isolated from various samples of patients hospitalized in internal and surgical wards of chest diseases and intensive care units at the Training and Research Hospital For Chest Diseases and Chest Surgery in Izmir-Turkey during October and November 2000, were analyzed. Distribution of isolation rates of various samples according to the strain species is shown in Table 1. Forty-four isolates were *Pseudomonas spp*., nine *Klebsiella spp*., eight *E.coli*, six *Enterobacter spp*., four *Acinetobacter spp*., three Gram negative nonfermentative bacilli and one Proteus mirabilis (11,12). Susceptibility patterns of these strains were investigated for meropenem (MEM 10 $\mu$g, Oxoid), imipenem (IPM 10 $\mu$g, Oxoid), ceftriaxone (CRO 30 $\mu$g, Oxoid), ceftazidime (CAZ 30 $\mu$g, Oxoid), aztreonam (ATM 30 $\mu$g, Oxoid), amoxicillin-clavulanic acid (AMC 20 $\mu$g / 10 $\mu$g, Oxoid), isepamicin (ISP 30 $\mu$g, Oxoid), gentamicin (CN 10 $\mu$g, Oxoid), levofloxacine (LEV 5 $\mu$g, Oxoid), ciprofloxacine (CIP 5 $\mu$g, Oxoid), piperacillin-tazobactam (TZP 75/10 $\mu$g, Oxoid) and cefoperazon-sulbactam (SCF 75 / 30 $\mu$g, Oxoid) by disk diffusion method on Mueller-Hinton agar according to the recommendations of " National Commitee for Clinical Laboratory Standards " (NCCLS) (13). Resistance to all the oxyimino-beta-lactams by disk diffusion method was evaluated as presumptive for ESBL (16-18). Double disk synergy test was used to confirm ESBL and direct induction test was used to define inducible beta-lactamase (14 -16).

Twelve of 18 strains were found as resistant (≤ 13 mm zone) and six as intermediately resistant (14 -15 mm zone) to IPM whereas 10 were resistant, six intermediate and two susceptible to MEM by the disk diffusion method. These strains which appeared to be intermediate and resistant to carbapenems by disk diffusion method were analyzed for MIC levels by agar dilution and microdilution methods for IPM and MEM, respectively. MIC levels of ≥ 16 $\mu$g / mL were accepted as resistance to carbapenems; ≤4 $\mu$g / mL and equivalent to 8 $\mu$g / mL (4 $\mu$g / mL < MIC < 16 $\mu$g / mL) as susceptible and intermediately resistant, respectively .

### Statistical Analysis

Comparison of the groups were performed by chi-square test with Yate's correction for statistical analysis. P value less than 0.05 was accepted as statistically significant.

## RESULTS

The resistance rates of Gram negative bacilli were found as 14.6 % (n=11) for MEM, 17.3 % (n=13) for IPM, 52 % (n=39) for CRO, 32 % (n=24) for CAZ, 38.6 % (n=29) for ATM, % 33.3 (n=25) for AMC, 26.6 % (n=20) for ISP, 40 % (n=30) for CN, 25.3 % (n=19) for LEV, 20 % (n=15) for CIP, 20 % (n=15)

for TZP and 8 % (n=6) for SCF. When strains which had intermediate level of antibiotic resistance were accepted as resistant, proportions of resistance rates were 16 %, 17 %, 42 %, 31 %, 38 %, 30 %, 26 %, 37 %, 20 %, 20 %, 28 %, 23 % for MEM, IPM, CRO, CAZ, ATM, AMC, ISP, CN, LEV, CIP, TZP and SCF, respectively. Distribution of antibiotic resistance rates of Gram negative bacilli is shown in Tables 2A and 2B.

Presence of ESBL was indicated in 12 of 44 (27.3 %) *Pseudomonas spp*., one of nine (11 %) *Klebsiella spp*. and three of six (50 %) *Enterobacter spp*. by

**Table 1. Distribution of strains isolated from different samples**

| Sample | Pseudomonas spp. Male n (%) | Pseudomonas spp. Female n (%) | Klebsiella spp. Male n (%) | Klebsiella spp. Female n (%) | Escherichia coli Male n (%) | Escherichia coli Female n (%) | Enterobacter spp. Male n (%) | Enterobacter spp. Female n (%) | Acinetobacter spp. Male n (%) | Acinetobacter spp. Female n (%) | Gram Negative Nonfermentative bacilli Male n (%) | Gram Negative Nonfermentative bacilli Female n (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Biopsy | | | | | | | 1 (1.3) | | | | | |
| Wound | 15 (20) | 11 (14.6) | 1 (1.3) | | | | | | | | | |
| Sputum | | | 3 (4.0) | 1 (1.3) | 3 (4) | | 3 (4.0) | 1 (1.3) | 1 (1.3) | 1 (1.3) | 2 (2.6) | |
| Urine | | | | 3 (4.0) | | 3 (4.0) | | | | | | |
| Drenage | 7 (9.3) | | | | 1 (1.3) | | | | | | 1 (1.3) | |
| Bronchial aspiration | 8 (10.6) | 3 (4.0) | 1 (1.3) | | | | 1 (1.3) | | 2 (2.6) | | | |
| Vaginal | | | | | | 1 (1.3) | | | | | | |
| | 30 (40) | 14 (18.6) | 5 (6.6) | 4 (5.3) | 4 (5.3) | 4 (5.3) | 5 (6.6) | 1 (1.3) | 3 (4.0) | 1 (1.3) | 3 (4.0) | - |
| Total(n=75) | 44 (58.6) | | 9 (12.0) | | 8 (10.6) | | 6 (8.0) | | 4 (5.3) | | 3 (4.0) | |

**Table 2A. Resistance of gram negative bacilli to various antibiotics**

| **Antibiotic** | | MEM | IPM | CRO | CAZ | ATM | AMC* | ISP | CN | LEV | CIP | TZP | SCF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Resistance rates** | **(%)** | 14.6 | 17.3 | 52 | 32 | 38.6 | 33.3 | 26.6 | 40 | 25.3 | 20 | 20 | 8 |
| | **(n)** | 11 | 13 | 39 | 24 | 29 | 25 | 20 | 30 | 19 | 15 | 15 | n=6 |

* AMC resistance was not taken into account for *Pseudomonas* and *Acinetobacter* strains. For AMC these strains were accepted as resistant.

**Table 2B. Resistances* of gram negative bacilli to various antibiotics**

| **Strain** | **Isolates n (%)** | **Number and rates of Isolates Resistant to Antibiotics** n (%)** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **MEM** | **IPM** | **CRO** | **CAZ** | **ATM** | **AMC*** ** | **ISP** | **CN** | **LEV** | **CIP** | **TZP** | **SCF** |
| **Pseudomonas spp.** | 44(58.6) | 13(29.5) | 13(29.5) | 30(68) | 22(50) | 26(59) | 44(100) | 19(43) | 24(54.5) | 13(29.5) | 13 (29.5) | 16(36.3) | 15(34) |
| **Klebsiella spp.** | 9 (12) | - | - | 1(11) | - | 1(11) | 1(11) | - | 1(11) | - | - | 3(33.3) | 1(11) |
| **E.coli** | 8 (10.6) | - | - | 2(25) | - | 2(25) | 1(12.53) | 2(25) | 4(50) | 2(25) | 2(25) | 1(12.5) | 1(12.5) |
| **Enterobacter spp.** | 6 (8) | 1(16.6) | 1(16.6) | 5(83.3) | 5(83.3) | 5(83.3) | 5(83.3) | 2(33.3) | 3(50) | 2(33.3) | 3(50) | 4(66.6) | 3(50) |
| **Acinetobacter spp.** | 4 (5.3) | 2(50) | 3(75) | 3(75) | 3(75) | 3(75) | 4(100) | 3(75) | 3(75) | 3(75) | 2(50) | 3(75) | 3(75) |
| **Gram negative nonfermenter** | 3 (4) | 1(33.3) | 1(33.3) | 1(33.3) | 1(33.3) | 1(33.3) | 1(33.3) | - | 1(33.3) | - | - | 1(33.3) | - |
| **P.mirabilis** | 1 (1.3) | - | - | - | - | - | - | - | 1(1.3) | - | - | - | - |
| **Total** | 75(100) | 16(21.3) | 17(22.6) | 42(56) | 31(41.3) | 38(50.6) | 56(74.7) | 26(34.6) | 37(49.3) | 20(26.6) | 20(26.6) | 28(37.3) | 23(30.6) |

* Intermediately resistant strains were accepted as resistant.
** AMC resistance againts microorganisms out *Psedomonas spp*. and *Acinetobacter spp*. was found as 8/27 (29.6 %)

double disk synergy test and evaluation of resistance in all oxyimino-beta lactams tested. Inducible beta-lactamase production was determined in 15 of 44 (34 %) *Pseudomonas spp*. and one of six (16.6 %) Enterobacter spp. by direct induction test. There was no visible beta-lactamase production in eight *E. coli* and three Gram negative nonfermentative bacilli. Acinetobacter strains were not taken into account as their mechanisms of detection of beta-lactamases are not clear enough phenotypically (18). Distribution of beta-lactamases detected in Gram negative bacilli is shown in Table 3.

Eighteen carbapenem-resistant strains of which the susceptibility patterns were analyzed were found as completely (n=12) or intermediately (n=6) resistant to IPM, whereas, 16 of these strains were found as completely (n=10) or intermediately (n=6) resistant to MEM by disk diffusion method. One Acinetobacter spp. and one Pseudomonas spp. which were intermediately resistant to IPM were found as susceptible to MEM by disk diffusion method. MIC levels of these strains were MICIPM > 16 $\mu$g/mL and MICMEM 2-4 $\mu$g/mL for Acinetobacter spp. and MICIPM > 16 $\mu$g/mL and MICMEM > 8 $\mu$g/mL for Pseudomonas spp. All the resistant strains to carbapenems which were tested for their MIC levels showed bacterial growth up to 2 $\mu$g/ml of drug concentration. Nine of 13 *Pseudomonas* strains were resistant (MIC > 16 $\mu$g/mL) and four Pseudomonas strains were intermediately resistant (4 $\mu$g / mL < MIC < 16 $\mu$g / mL) to IPM. Two of four Acinetobacter strains were resistant (MIC > 16 $\mu$g / mL) and two *Acinetobacter* strains were intermediately resistant (4 $\mu$g / mL < MIC < 16 $\mu$g /

**Table 3. Distribution of beta-lactamases detected in gram negative bacilli.**

| Strain | | ESBL* positive n | % | Inducible beta-lactamase positive n | % |
|---|---|---|---|---|---|
| Pseudomonas spp. | (n=44) | 12 | (27.2) | 15 | (34) |
| Klebsiella spp. | (n=9) | 1 | (11.1) | 1 | (11.1) |
| E.coli | (n=8) | - | | - | |
| Enterobacter spp. | (n=6) | 3 | (50) | 1 | (16.6) |
| Acinetobacter spp. | (n=4) | - | | - | |
| GNF | (n=3) | - | | - | |

**Table 4. Distribution of the MIC levels for meropenem and imipenem.**

| Strain | MIC (mg/ml) | Number of Isolates Detected For MIC levels | |
|---|---|---|---|
| | | IPM (n*=18) | MEM (n**=18) |
| **Pseudomonas spp. (n=13)** | 2-4 | | 2 |
| | >4 | 1 | 4 |
| | >8 | 3 | 4 |
| | >16 | 1 | 1 |
| | >32 | 1 | |
| | >64 | 7 | 2 |
| **Acinetobacter spp. (n=4)** | 2-4 | | 3 |
| | >4 | | 1 |
| | >8 | 2 | |
| | >16 | 2 | |
| | >32 | | |
| | >64 | | |
| **GNF (n=1)** | 2-4 | | |
| | >8 | | 1 |
| | >16 | | |
| | >32 | | |
| | >64 | 1 | |

Abbreviations: GNF : gram negative nonfermentative bacilli

* Twelve of 18 were found as resistant and six of them were found as intermediate by disk diffusion method.

** Ten of 18 were found as resistant, six of them were found as intermediate and two of them were found as susceptible by disk diffusion method.

mL) to IPM. One Gram negative nonfermentative bacillus which needed further identification showed resistance to IPM with the MIC level over 64 $\mu$g/mL. These findings had correlation with the results by disk diffusion test for IPM. Three strains among 13 *Pseudomonas spp*. were resistant to MEM according to the MIC levels (MIC > 16 $\mu$g / mL) and eight of them were intermediately resistant (4 $\mu$g / mL < MIC < 16 $\mu$g / mL) and two of them which had 2 $\mu$g / mL < MIC < 4 $\mu$g / mL of MIC levels were accepted as susceptible to MEM. One of four Acinetobacter strains were intermediately resistant (4 $\mu$g / mL < MIC <16 $\mu$g / mL) and three of them which had 2 $\mu$g / ml < MIC < 4 $\mu$g / ml of MIC levels were accepted as susceptible to MEM. One Gram negative nonfermentative bacillus was intermediately resistant (4 $\mu$g / mL < MIC < 16 $\mu$g / mL) to MEM. These findings didn't have correlation with the disk diffusion test. Ten strains were completely resistant to MEM by disk diffusion method when three strains were found as completely resistant according to the MIC levels. Six strains were found as intermediately resistant to MEM by disk diffusion method whereas 10 strains were found as intermediately resistant to MEM and five strains were susceptible to MEM according to the MIC levels (2-4 $\mu$g / mL) . Two strains were susceptible to MEM and intermediately resistant to IPM by disk diffusion method and MIC levels of these two strains were MICIPM >16 $\mu$g / mL and MICMEM 2-4 $\mu$g / mL for an Acinetobacter strain and MICIPM >16 $\mu$g / mL and MICMEM > 8 $\mu$g / mL for a Pseudomonas strain. Distribution of the MIC levels of the strains for MEM and IPM is shown in Table 4.

In general, it was found that IPM resistance among Gram negative nonfermentative bacilli was statistically higher than MEM according to the MIC levels ($p < 0.05$). Moreover, resistance rates for MEM which were found by disk diffusion method were statistically higher than the ones according to the MIC levels ($p < 0.05$).

## DISCUSSION

Antibiotic resistance against Gram negative bacilli has been studied so many times in previous reports (5,6,20-26). Investigation of beta-lactamase production has been carried out by different methods with modifications and different results have been taken (14,17,21,27). In the present study, total of beta-lactamase producing strains were found as 22.5 %. Four Acinetobacter strains were not taken into account as the phenotypical detection of beta-lactamase production in Acinetobacter strains has not been defined clearly (28).

In a study from Hungary (21) in the year of 2000 which has been made in approximately 3500 Enterobacteriaceae, resistance to AMC was 11-45 % whereas resistance to third generation cephalosporins in E. coli, *Klebsiella-Enterobacter*, *Proteus-Providencia-Morganella* was 3-8 %, 15-52 %, 16-27 % respectively. In the present study, AMC resistance against gram negative bacilli out of *Acinetobacter* and *Pseudomonas* strains was ~ 30 % and resistance to third generation cephalosporins was found as 41-56 %. In the same study from Hungary, isoelectric points, inhibitor profiles and substrate profiles have been analyzed and it has been thought that the resistance was mainly due to the hyperproduction of chromosomally encoded AmpC beta-lactamases.

In most of the strains which were found as ESBL positive by double disk synergy or resistance to all oxyimino beta-lactams, AMC resistance was observed as well suggesting that resistance against beta-lactamase inhibitors occurs mainly by the mechanisms: hyperproduction of beta-lactamases, production of beta-lactamases resistant to inhibitors and chromosomal cephalosporinases (2,28,29).

Various mechanisms and beta-lactamases might play a role in the occurence of multiple resistant strains. This may address the issue that inducible beta-lactamase production could be in higher rates than the amounts which were determined by direct induction test, especially in *Pseudomonas strains*. Practically, it is accepted that *Enterobacter cloacae*, *Pseudomonas aeruginosa* and *Acinetobacter spp*. always produce chromosomally inducible beta-lactamases at a certain level (30).

In a study from Brasil (25) in 2000, 608 gram negative isolates have been analyzed and identified as *Pseudomonas aeruginosa* (20 %), *E. coli* (17 %),

Acinetobacter spp. (16 %) most frequently. High level of resistance against all beta-lactams tested has been found in *Pseudomonas strains*. In the present study, resistance rates were higher in Gram negative nonfermentative bacilli and *Pseudomonas strains* (n=44) were identified most frequently (58.6 %) among them. Resistance and intermediate resistance for IPM were observed in nine and four of 13 Pseudomonas strains, respectively, whereas three and eight of 13 were found as completely and intermediately resistant to MEM according to the MIC levels. In addition with these, two Pseudomonas isolates were found as susceptible (2-4 $\mu$g/ml) for MEM according to the MIC levels. In Acinetobacter strains, two of them were resistant and two were intermediately resistant to IPM. Three of them were susceptible and one of them were intermediately resistant to MEM according to the MIC levels.

In studies from U.S.A (23,31) in 2000 and 2001, which included a surveillance program in 1998 in the patients with the diagnosis of pneumonia, the most frequent strains were *P. aeruginosa* (26.8 %), S. aureus (24 %), *Klebsiella spp*. (12.1 %) and *Acinetobacter spp*. (10.5 %). In the present study, the most frequent strains isolated were *Pseudomonas spp*. (58.6 %), *Klebsiella spp*. (12 %), *E.coli* (10.6 %), *Enterobacter spp*. (8 %), *Acinetobacter spp*. (5.3 %), other Gram negative nonfermenter bacilli (4 %) and Proteus mirabilis (1.3 %). Gram negative strains especially *Pseudomonas spp*. and *Acinetobacter spp*. have showed high resistance rates against the agents tested in these studies. In our hospital, Gram negative nonfermentative bacilli have been isolated most frequently as compared with other strains.

In recent years, *P.aeruginosa* strains producing plasmid-mediated ESBL have been reported increasingly (1,2,4,32). Most of them are non-SHV and non-TEM type of ESBLs such as; PER-1, IMP-1 and OXA type (33,34). Besides these, Acinetobacter strains have ARI-type enzymes which lead to clonal spread (33). Some SHV and TEM type of ESBLs in *Pseudomonas aeruginosa* from different countries such as Thailand and France have been reported in recent years (32,35).

In some studies reported from Turkey, ESBL positivity has been found 20-90 % in *Klebsiella spp*., 10 % in *P. aeruginosa*, 50 % in *Acinetobacter spp*. SHV type of ESBL in *Klebsiella spp*. and PER-1 in Pseudomonas spp. and *Acinetobacter spp*. are seen most frequently (27,28). In a study from Turkey (27) in 1999, ESBL positivity has been found as 30 % and inducible beta-lactamase production has been reported as 29 %. İnducible beta-lactamase production have been found in nine of 17 Pseudomonas strains and only seven of them have been designated by direct induction test. In 1998, in a multi-center study which has been carried out in Turkey, ESBLs have been found as 33-86 % in Klebsiella spp. and 0-27 % in *E.coli* (2). In the present study, ESBL and inducible beta-lactamase production were found as 27 % and 34 % in *Pseudomonas spp*. (n=44) and 50 % and 16.6 % in *Enterobacter spp*. (n=6) respectively. ESBL production was found as 11 % in *Klebsiella spp*. (n=9). ESBL was not determined in *E. coli* (n=8) strains. These results address the issue that beta-lactamase production have shown different frequencies in different studies.

*Pseudomonas aeruginosa* produces inducible AmpC enzyme (chromosomal B lactamase Group 1 Class C) like Enterobacter spp. as well. This is one of the reasons for the occurence of the resistant strains against beta-lactamase inhibitors. Hyperproduction of TEM enzyme, class B, C, and D enzymes resistant to inhibitors, loss of porins in addition to the classical beta-lactamases, mutants of TEM enzymes resistant to inhibitors are the reasons for the resistance against beta-lactamase inhibitors (4, 5, 7,10, 28, 29, 32, 36).

Increasing levels of carbapenem resistance have been being reported especially in *Pseudomonas aeruginosa* and *Acinetobacter spp*. in recent years (20, 37, 38). Mono or multi-resistant strains in *Pseudomonas spp*. have been occuring by means of porin-efflux system and chromosomal beta-lactamases. Resistance to carbapenems during therapy occurs frequently. Porin mutations and carbapenemases cause carbapenem resistance (5,6). In a study from Izmir-Turkey (3), carbapenem

resistance (and its mechanisms) have been reported as 92 %, 60 % and 100 % in *Acinetobacter baumannii*, *P.aeruginosa* and *S. maltophilia,* respectively. In another study from Ankara-Turkey (24), MEM resistance has been reported as 2 % and 0 % for MEM and IPM in Klebsiella spp. respectively. In the same study, resistance for MEM and IPM in P.aeruginosa have been reported as 4 % and 21 % respectively and there was no resistance among *E.coli, Proteus* and *Enterobacter* strains. In two reports from Turkey , carbapenem resistance in *P.aeruginosa* has been found as 33 % and 17-18 % and as 23.8 % in *Acinetobacter spp*. (20,22,27). In intensive care units, carbapenem resistance is much higher than usual and it has been reported as 66 % and 53 % for IPM and MEM respectively (38). In the present study, MEM and IPM resistance were found as 14.6 % and 17.3 % and if intermediately resistant strains were accepted as resistant, the resistance rates were 17 % and 18 % for MEM and IPM, respectively. Carbapenem resistance for both MEM and IPM was 27 % in *Pseudomonas spp*. including *P. aeruginosa*. There were carbapenem resistance in one of six *Enterobacter spp*., in two of four *Acinetobacter* against MEM and three of four *Acinetobacter* strains against IPM respectively and in one Gram negative nonfermenter bacillus against both MEM and IPM. When we searched for their MIC levels, higher concentrations of MIC levels were observed for IPM than the MIC levels for MEM in *Pseudomonas spp*. (n=13), *Acinetobacter spp*. (n=4) and in one gram negative nonfermenter bacillus. In the present study, there were no resistance to carbapenems in *Klebsiella spp., E.coli, Enterobacter spp*. and in one *P. mirabilis* which were taken into the study. Carbapenem resistance in gram negative nonfermentative bacilli have been reported from Turkey and other countries. In some of these studies, Pseudomonas strains have been found as more susceptible to MEM than IPM and there have been studies reporting IPM as more susceptible (22, 24, 25, 37). In the present study, there was no significant difference between MEM and IPM activities by disk diffusion method. But the MIC levels of MEM were in lower concentrations than the MIC level concentrations of IPM as well.

In conclusion, increasing production of beta-lactamases and resistance levels against carbapenems in gram negative bacilli especially in gram negative nonfermentative bacilli isolated from internal and surgical units and especially from the intensive care units indicates the widespread use of those antibiotics that leads to selection of resistant organisms and an endemic problem in some hospitals in different regions all over the world (25,28,38).

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