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Investigation of antibiotic susceptibility profile and minimal inhibitor concentration changes in *Pseudomonas aeruginosa* isolates that exposed to subinhibitory concentrations of antibiotic

Cetin Kilinc[1], Ridvan Guckan[2], Umut Safiye Say Coskun[3]*

Abstract

Objective: During antibiotic use some of the bacteria in our flora can be affected by the used antibiotic in subinhibitory concentrations in addition to pathogenic microorganisms. The aim of this study to investigate in-vitro effects of subinhibitory concentrations antibiotic on antibiotic susceptibility profile of *P.aeruginosa* which can be found in normal flora and be a pathogenic bacteria.

Material and Method: The antibiotic effective concentrations decrease with distance from the antibiotic disc and growth-inhibition zone ends with the effect of the antibiotic falls to subinhibitory concentrations; and growth starts.We accepted this growth starting region as the area in which bacteria exposed to subinhibitory concentrations of antibiotic are located and we developed a model. We separetely exposed the standard *P.aeruginosa* strain to eight different antibiotics (amikacin, gentamicin, imipenem, meropenem, ceftazidime, cefepime, ciprofloxacin, colistin) for seven days in subinhibitory concentrations. *P. aeruginosa* strain is susceptible to these antibiotics and we monitored susceptibility and minimal inhibitor concentration changes. Moreover, we also made these procedures in 20 different clinical *P.aeruginosa* isolates.

Results: We observed that a resistance was developed in the standard *P. aeruginosa* strain starting second day of meropenem exposure, third day of ceftazidime exposure, fifth day of amikacin exposure and sixth day of gentamicin exposure. There was no resistance development after colistin, cefepime, ciprofloxacin, meropenem exposure but significant MIC value increases were detected. This resistance was not only against exposed antibiotic or antibiotic group but also against antibiotics in different antibiotic groups.

Conclusion: It was shown that especially subinhibitory concentrations using carbapenem and aminoglycoside antibiotics triggered resistance development against themselves more than other antibiotic groups. Use of colistin was not shown to cause cross resistance.

Key words: Subinhibitory concentrations, *P. aeruginosa*, antibiotic susceptibility, MIC value changes

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is found in human body flora, may live in nutrient poor environments (distilled water… etc.) also colonize in hospitals, and cause infections with high mortality and morbidity ratios. Failure to treat infections caused by intrinsic resistance to many antibiotics as well as resistance to antibiotics that are susceptible even during treatment is encountered.Antibiotics that can be used in the treatment of *P. aeruginosa* infections with increasing resistance rates are limited(1-4).

Pathogenic bacteria can expose to nonlethal concentrations of antibiotic (subinhibitory concentrations) for days during the treatment of these infections although they are susceptible to that antibiotic because of using insufficient dose of that antibiotic or reaching of unsufficent concentrations of antibiotic to the area where bacteria locate. During the use of a systemic antibiotic not only infectious bacteria but also all other bacteria in normal body flora can be exposed to inhibitor or subinhibitory concentrations of antibiotics for days.

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1 Kastamonu State Hospital, Microbiology Laboratory Kastamonu, TR
2 Amasya University Training and Researh Hospital, Department of Medical Microbiology Amasya, TR
3 Tokat Gaziosmanpasa University School of Medicine, Department of Medical Microbiology Tokat, TR
*** Corresponding Author:** Umut Safiye Say Coskun **E-mail:** umut.saycoskun@gop.edu.tr **Phone:** +90 (505) 541 08 56

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In our study we aimed to investigate invitro effect of subinhibitory concentrations of antibiotic exposure on antibiotic susceptibility profile of *P. aeruginosa.*

Material and methods

In the Kirby Bauer disc diffusion method, the antibiotic effective concentrations decrease with distance from the antibiotic disc and growth-inhibition zone ends with the effect of the antibiotic falls to subinhibitory concentrations; and growth starts. We developed a model by accepting this region in which growth started as an area which includes bacteria that exposed to subinhibitory concentrations of antibiotics. In our study we used eight different antibiotic discs (Oxoid, U.K) amikacin (30 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), ceftazidime (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), colistin (10 µg) and *P. aeruginosa* ATCC 27853 isolate which are known to be susceptible to these antibiotics and other 20 clinical *P. aeruginosa* isolates. We identified minimal inhibitor concentration (MIC) values of 21 isolates against eight different antibiotics which they were susceptible and we exposed these isolates to subinhibitory concentrations of these antibiotics for seven days. *P. aeruginosa* ATCC 27853 isolate colonies which grow on Eosin Metilene Blue medium homogenized in saline adjusted to the turbidity of 0.5 McFarlandand streaked onto Mueller-Hinton agar (Oxoid, U.K) for Kirby Bauer disc diffussion method. We placed amikacin disc in the middle of medium and after 24 hour incubation at 37^0C and colonies were collected from the region which exposed to subinhibitory concentrations of antibiotic around the disc (Figure 1).

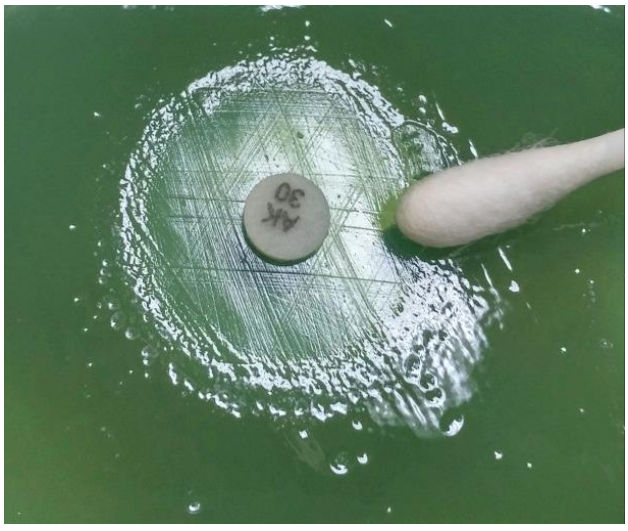

Figure 1: Colony intake from the region which exposed to sub-inhibitor doses of amikacin

Collected colonies were adjusted to 0.5 Mcfarland standard with saline and passaged to Mueller Hinton agar again and incubated for one day after placement of amikacin disc in the middle of passage. This process was repeated for 7 consecutive days.Thus we exposed this bacteria to subinhibitory concentrations of amikacin in vitro for seven consecutive days (Figure 2).

The susceptibility (Kirby Bauer disk diffusion method) and MIC (E test (Oxoid, U.K.)) values of *P. aeruginosa* ATCC 27853 isolate which is known against all antibiotics before amikacin exposure and amikacin susceptibility changes during exposure from day to day were monitored. End of the seventh day it is controlled changes of inhibition zone and MIC values of not only exposed amikacin but also all antibiotics (amikacin, gentamicin, imipenem, meropenem, ceftazidime, cecefepimeime, ciprofloxacin, colistin) which bacteria is susceptible.

Same procedure as above, which we performed with amikacin to *P. aeruginosa* ATCC 27853 isolate, was also applied to other seven antibiotics seperately.

Procedures that we use with *P. aeruginosa* isolate (ATCC 27853) was also applied with 20 different clinical isolates. Furthermore, in order to control whether repeated passages cause any changes for resistance profile of the bacteria; a standard isolate was passaged for seven consecutive days. In our study; antibiotic susceptibilities were controlled according to 2013 Clinical and Laboratory Standards Institute criteria(CLSI) (5). Mid-susceptible isolates were considered resistant.

Results

We detected changes in susceptibility of the *P. aeruginosa* ATCC 27853 isolate against antibiotic which was exposed to subinhibitory concentrations for seven days and MIC values before and after antibiotic exposure. For *P. aeruginosa* ATCC 27853 isolate which exposed to subinhibitory concentrations of antibiotic for seven days, resistance development were not determined after exposure to ciprofloxacin, cefepime, colistin, meropenem antibiotics. But an elevation of MIC values against these oantibiotics was observed. Earliest resistance development according to days was observed as imipenem (second day), ceftazidime (third day), amikacin (fifth day), gentamicin (sixth day) , respectively (Table 1).

P. aeruginosa ATCC 27853 isolate's susceptibility profile was observed not only for the antibiotic that the isolate was exposed but also the exposed antibiotic affects on other antibiotics and MIC values for seven days. (Table 2)

Furthermore, we made these procedures for 20 different clinical isolates in addition to the *P. aeruginosa* ATCC 27853 isolate, we identified susceptibility and MIC value changes of 20 clinical *P. aeruginosa* isolates which were exposed to subinhibitory concentrations of antibiotic for seven days (Table 3).

No changes were detected in antibiotic susceptibility profile of *P. aeruginosa* ATCC 27853 which was passaged for seven consecutive days.

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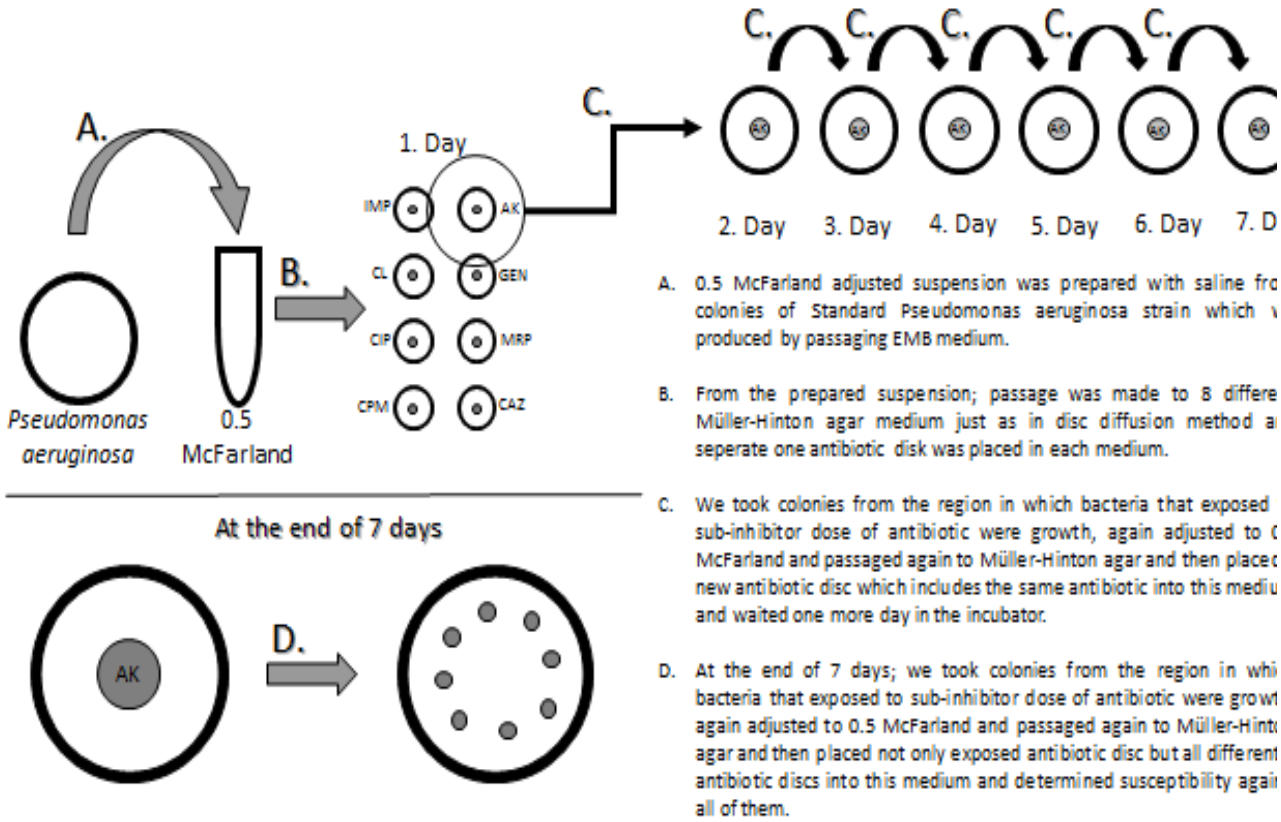

Figure 2: Antibiotic exposure of standard pseudomonas strain for 7 days

Table1: Susceptibility and MIC value changes of ATCC strains from day to day which exposed to sub-inhibitor doses of antibiotic.

Exposed antibiotic	1.day Zone diameter (Susceptibility) /MIC	2. day Zone diameter /Susceptibility	3.day Zone diameter /Susceptibility	4.day Zone diameter /Susceptibility	5. day Zone diameter /Susceptibility	6. day Zone diameter /Susceptibility	7. day Zone diameter (Susceptibility) /MIC
Cefepime	25(S)/<1	24(S)	22(S)	22(S)	20(S)	18(S)	16(S)/8
Ceftazidime	20(S)/2	19(S)	15(R)	13(R)	11(R)	11(R)	10(R)/32
Imipenem	26(S)/2	15(R)	14(R)	14(R)	15(R)	13(R)	12(R)/>16
Meropenem	26(S)/0,5	26(S)	22(S)	20(S)	18(S)	18(S)	17(S)/2
Gentamicin	28(S)/<2	29(S)	24(S)	21(S)	20(S)	14(R)	12(R)/8
Amikacin	26(S)/<2	25(S)	20(S)	18(S)	16(R)	15(R)	13(R)/32
Ciprofloxacin	34(S)/<0,2	33(S)	33(S)	29(S)	30(S)	28(S)	24(S)/1
Colistin	15(S)/<0,5	13(S)	14(S)	12(S)	13(S)	12(S)	11(S)/1

Table 2: Susceptibility and mic value changes status of exposed antibiotic (AK) and other antibiotics after exposure of ATCC strain to sub-inhibitor dose of susceptible antibiotics.

	Antibiotics whose susceptibility status was controlled at the end of seventh day							
	Cefepime	Ceftazidime	Imipenem	Meropenem	Gentamicin	Amikacin	Ciprofloxacin	Colistin
Cefepime	A	R	N	A	N	N	N	N
Ceftazidime	A	R	N	N	N	N	N	N
Imipenem	N	N	R	R	N	N	N	A
Meropenem	N	A	R	A	N	N	A	A
Gentamicin	N	A	N	N	R	R	N	N
Amikacin	N	N	N	N	R	R	N	N
Ciprofloxacin	A	N	R	A	N	N	A	N
Colistin	N	N	N	N	N	N	N	A

N: No changes for susceptibility and mic value, **R**: Resistant, **A**: MIC value increased although susceptibility continues

Table 3: Susceptibility and mic value changes of 20 clinical P.aeuroginosa isolates which were exposed to sub-inhibitor dose of antibiotic.

Exposed antibiotic for 7 days	Number of resistant isolates after antibiotic exposure and antibiotics to which resistance developed (%)	MIC values of isolates increased despite the lack of development of resistance after exposure to antibiotics (%)
Cefepime	In 11 strains cefepime (55%), in 10 strains ceftazidime (50%) , in 5 strains imipenem (25%), in 4 strains meropenem (20%), in 3 strains colistin (15%), in 2 strains ciprofloxacin (10%) resistance developed	Mic value increased in 9 strains against cefepime (45%), in 10 strains against ceftazidime (50%), in 4 strains against imipenem (20%), in 5 strains against meropenem (25%), in 5 strains against ciprofloxacin (25%), in 3 strains against colistin (15%).
Ceftazidime	In 13 strains ceftazidime (65%), in 10 strains cefepime (50%), in 3 strains imipenem (15%), in 2 strains meropenem (10%), in 1 strain colistin (5%) resistance developed	Mic value increased in 7 strains against ceftazidime (35%), in 10 strains against cefepime (50%), in 3 strains against imipenem (15%), in 3 strains against meropenem (15%), in 3 strains against ciprofloxacin (15%), in 2 strains against colistin(10%).
Imipenem	In 17 strains imipenem (85%), in 15 strains meropenem (75%), in 6 strains cefepime (30%), in 4 strains ceftazidime (20%), in 4 strains ciprofloxacin (20%), in 3 strains colistin (15%) resistance developed.	Mic value increased in 3 strains (15%) against imipenem, in 5 strains against meropenem (25%), in 2 strains against cefepime (10%), in 2 strains against ceftazidime (10%), in 3 strains against ciprofloxacin (15%) and in 2 strains against colistin(10%).
Meropenem	In 14 strains meropenem (70%), in 13 strains imipenem (65%), in 6 strains cefepime (30%), in 4 strains ceftazidime (20%), in 4 strains ciprofloxacin (20%), in 3 strains colistin(15%) resistance developed.	Mic value increased in 7 strains against imipenem (20%), in 6 strains against meropenem (30%) , in 2 strains against cefepime (10%) , in 2 strains against ceftazidime (10%) , in 4 strains against ciprofloxacin (20%) , in 4 strains against colistin (20%).
Gentamicin	In 20 strains gentamicin (100%), in 16 strains amikacin (80%), in 4 strains ciprofloxacin (20%), in 3 strains imipenem (15%), in 3 strains meropenem (15%), in 2 strains colistin (10%) resistance developed.	Mic value increased in 3 strains against colistin (15%) , in 5 strains against imipenem, in 2 strains against meropenem (10%) , in 4 strains against ciprofloxacin, and in 4 strains against amikacin (20%).
Amikacin	In 18 strains amikacin (90%), in 14 strains gentamicin (70%), in 4 strains imipenem (20%), in 3 strains meropenem (15%), in 3 strains colistin (15%) resistance developed.	Mic value increased in 3 strains against colistin (15%) , in 5 strains against imipenem (25%) , in 2 strains against meropenem (10%) , in 2 strains against amikacin (10%) , in 6 strains against gentamicin (30%).
Ciprofloxacin	In 7 strains ciprofloxacin (20%), in 3 strains imipenem (15%) , in 2 strains meropenem (10%), in 2 strains colistin (10%) , in 2 strains ceftazidime (10%) and in 2 strains cefepime(10%) resistance developed.	Mic value increased in 13 strains against ciprofloxacin (65%) , in 5 strains against imipenem (25%) , in 2 strains against meropenem (10%) , in 2 strains against colistin (10%) , in 2 strains against ceftazidime (10%) and in 1 strain against cefepime (5%) .
Colistin	In 2 strains colistin (10%) resistance developed.	Mic value increased in 3 strains against colistin (15%) .

Discussion

Antibiotics came into use in the past hundred years and have provided the most significant contribution to human life and make it possible to successfully cure many of deadly infectious diseases. Antibiotics are one of the most important inventions in human history and they have significantly lost their effects because of resistance particularly due to inappropriate and unnecessary use. Microorganisms gain oppositional force, namely resistance, sooner or later against antimicrobials which are used to destroy these microorganisms. Resistance to against antimicrobial agents, today is a very significant problem which will threaten humanity. In a kind of microorganism that has become resistant to an antimicrobial agent; resistance may develop against other antimicrobials which are similar with chemotherapeutic agent in terms of structure or effect (6). Pathogenic bacteria can survive despite exposure to subinhibitory concentrations of antibiotic during treatment for several days although they are susceptible to that antibiotic because of using insufficient amount of the antibiotic or reaching inadequate concentrationss of antibiotic to the area where bacteria locates. Besides bacteria that are members of the normal human flora might be exposed to subinhibitory concentrations of antibiotic during treatment. *P. aeruginosa* is also one of the bacteria which can be exposed to subinhibitory concentrations of antibiotic both as a pathogenic bacteria and member of normal human flora.

Susceptibility against exposed antibiotic and MIC value changes of *P. aeruginosa* ATCC 27853 isolate, which exposed to subinhibitory concentrations of antibiotic, was observed for seven days. It was seen that resistance developed starting from second day of meropeneme exposure, third day of ceftazidime exposure, seventh day of amikacin exposure and sixth day of gentamicin exposure. Although there was no resistance development after colistin, cefepime, ciprofloxacin, meropenem exposures; significant mic value increases were observed (Table1). Resistance development was not only against exposed antibiotic and antibiotic group, but also against antibiotics in different groups. In fact, antibiotics with increased MIC values were observed despite of no change in susceptibility status. In *P. aeruginosa* ATCC 27853 isolate, after ciprofloxacin exposure while imipeneme resistance developed, MIC values against cefepime and meropeneme increased. In the same isolate, amikasin resistance developed after gentamicin exposure, imipeneme resistance developed after meropenem exposure, ceftazidime resistance developed after cefepime exposure, respectively(Table2).

In the treatment of infections caused by *P. aeruginosa* isolates; different groups of antibiotics are used. As carbapenems which are one of the most broad-spectrum b-lactam antibiotics, are resistant against hydrolysis of various beta-lactamase such as extended spectrum beta-lactamases (ESBLs); they can be effectively used for the treatment of infections caused by resistant Gram negative bacteria as *P. aeruginosa* but in the last years increased carbapenem resistance was reported in *Pseudomonas* isolates (7, 8).

Carbapenem resistance of *P. aeruginosa* can be due to OprD pore loss, MexABOprM active efflux pumping system, permeability mutations, excessive production of chromosomal AmpC beta-lactamase and production of metallo-beta-lactamase enzymes (9). In case of OprD pore loss; meropenem can be susceptile while imipenem is resistant. In MexAB-OprM active efflux pumping system; resistance to develope all beta-lactamases except for imipenem. In togetherness of MexEF-OprN efflux pumping and oprD pore loss; imipenem and quinolone resistant, meropenem susceptible isolates are seen. For development of meropenem resistance during treatment; both pore protein loss and mutation of active efflux pumping system are needed (10, 11).

In our study, among 20 different *Pseudomonas* isolates which exposed to subinhibitory concentrations of imipenem for seven days resistance developed in 17 isolates for imipenem, 15 isolates for meropenem, 6 isolates for cefepime, 4 isolates for ceftazidime, 4 isolates for ciprofloxasin and 3 isolates for colistin, respectively.Besides, although susceptibility resumed in three isolates against imipenem, in five isolates against meropenem, in two isolates against cefepime, in two isolates against ceftazidime, in three isolates against ciprofloxacine and in two isolates against colistin; significant increases for MIC values of these isolates against these antibiotics were observed. Among 20 different *Pseudomonas* isolates which exposed to subinhibitory concentrations of meropenem for seven days resistance developed in 14 isolates for meropenem, 13 isolates for imipenem, 6 isolates for cefefim, 4 isolates for ceftazidime, 3 isolates for ciprofloxacin and 3 isolates for colistin, respectively. Besides, although susceptibility resumed in seven isolates against imipenem, in six isolates against meropenem, in two isolates against cefepime, in two isolates against ceftazidime, in four isolates against ciprofloxacine and in four isolates against colistin; significant increases for mic values of these isolates against these antibiotics were observed (Table 3).

It was suggested that subinhibitory concentrationss of carbapenem exposure in *Pseudomonas* isolates might trigger resistance mechanisms such as pore loss, beta-lactamase activation, permeability mutations, active efflux pumping system and as a result can cause resistance development against both the used antibiotic and different antibiotic groups such as cephalosporin and quinolone.

Another group of antibiotics with activity against *P. aeruginosa* is aminoglycoside. Aminoglycoside resistance can be due to change of affinity against ribosomes (cause resistance in only aminoglycosides), active efflux pump, mutations that can cause membrane permeability changes and aminoglycoside modifying enzyme mutations (6,12,13). Aminoglycoside resistance in *P. aeruginosa* is generally due to aminoglycoside-modifying enzymes and decrease in membrane permeability (14).

Among 20 different *P. aeruginosa* isolates which exposed to subinhibitory concentrations of amikacin for seven days resistance developed in 18 isolates for amikacin, 14 isolates for gentamicin, 4 isolates for imipenem, 3 isolates for meropenem and 3 isolates for colistin, respectively. Besides, although susceptibility resumed in 5 isolates against imipenem, in 2 isolates against meropenem, in 2 isolates against amikacin, in 6 isolates against gentamicin and in 3 isolates against colistin; significant increases for mic values of these isolates against these antibiotics were observed. Among 20 different *P. aeruginosa* isolates which exposed to subinhibitory concentrations of gentamicin for seven days resistance developed in 20 isolates for gentamicin,16 isolates for amikacin, 4 isolates for ciprofloxasin,3 isolates for imipenem, 2 isolates for meropenem and 2 isolates for colistin, respectively. Besides, although susceptibility resumed in six isolates against gentamicin five isolates against imipenem, in two isolates against meropenem, in four isolates against ciprofloxacin, in four isolates against amikacin and in three isolates against colistin; significant increases for MIC values of these isolates against these antibiotics were observed (Table 3).

It is suggested that exposure to subinhibitory concentrationss of aminoglycosides in *P. aeruginosa* isolates can cause aminoglycosides resistance via triggering ribosomal mutations and release of aminoglycosides modifying enzymes and in addition to that aminoglycosides, carbapenems and quinolone resistance via permability mutations and activation of active efflux pump. Main mechanism for resistance to quinolones is mutation of DNA gyrase enzyme and in addition to that the change in outer membrane permeability due to defects of outer membrane proteins such as OmpF, OmpC and active efflux pumping systems can also cause quinolone resistance. Changes in outer membrane porins and efflux pumping systems due to chromosomal mutations can cause resistance to other antimicrobial agents in addition to quinolone resistance (6,15).

Among 20 different *P. aeruginosa* isolates which exposed to subinhibitory concentrations of ciprofloxacine for seven days resistance developed in 7 isolates for ciprofloxacine, 3 isolates for imipenem, 2 isolates for meropenem, 2 isolates for cefepime, 2 isolates for ceftazidime, 2 isolates for colistin, respectively. Besides, although susceptibility resumed in 13 isolates against ciprofloxacin, in five isolates against imipenem, in two isolates against meropenem, in two isolates against ceftazidime, in one isolate against cecefepimeime and in two isolates against colistin; significant increases for MIC values of these isolates against these antibiotics were observed (Table 3).

Ciprofloxacin resistance developed in *P. aeruginosa* isolates due to DNA gyrase mutation caused by exposure to subinhibitory concentrations of quinolone and in addition to that this exposure can cause mutations of outer membrane porins and efflux pumping systems which results with resistance to carbapenems and cephalosporins in adition to quinolone resistance. It has been reported that resistance against cephalosporins in *P.aeruginosa* isolates is increasing. In *P. aeruginosa*, resistance to beta-lactam antibiotics may develop due to AmpC enzyme, ESBL, carbapenemases, efflux, permeability changes (16, 17).

Among 20 different *P. aeruginosa* isolates which exposed to subinhibitory concentrations of ceftazidime for seven days resistance developed in 13 isolates for ceftazidime, 10 isolates for sefepim, 3isolates for imipenem, 2 isolates for meropenem, 1 isolates for colistin,respectively. Besides, although susceptibility resumed in seven isolates against ceftazidime, in ten isolates against cecefepimeime, in three isolates against imipenem, in three isolates against meropenem, in three isolate against ciprofloxacine and in two isolates against colistin; significant increases for MIC values of these isolates against these antibiotics were observed.

Among 20 different *P. aeruginosa* isolates which exposed to subinhibitory concentrations of cefepime for seven days resistance developed in 11 isolates for cefepime, 10 isolates for ceftazidime, 5 isolates for imipenem, 4 isolates for meropenem, 3 isolates for colistin and 2 isolates for ciprofloxasin,respectively. Besides, although susceptibility resumed in nine isolates against cecefepimeime, in ten isolates against ceftazidime, in four isolates against imipenem, in five isolates againstmeropenem, in five isolate against ciprofloxacine and in three isolates against colistin; significant increases for mic values of these isolates against these antibiotics were observed (Table 3).

Subinhibitory concentrations of cephalosporin exposure in *P. aeruginosa* isolates; can trigger resistance mechanisms such as AmpC enzyme, ESBL, carbapenemases, efflux pumping, permability changes and can cause resistance against beta-lactam antibiotics such as cephalosporins and carbapenems due to these resistance mechanisms. In addition to that changes in permability and efflux pump systems can also cause resistance against quinolones. Especially resistance development via various mechainsms against colistin can be seen which are used against multi drug resistant gram negatives. Resistance development is related with decrease of binding points for colistin on cell and decrease of outer membrane polarity. In resistance development PmrA-PmrB and PhoQ-PhoP regularotry systems play role. Besides cross-resistance can be seen between polymyxins (18, 19).

Among 20 different *P. aeruginosa* isolates which exposed to subinhibitory concentrations of colistin for seven days, colistin resistance deveoped in two isolates. Furthermore, although susceptibility resumed in three isolates against colistin; significant increases for MIC values of these isolates against these antibiotics were observed (Table 3).

Exposure to subinhibitory concentrations of colistin in *P. aeruginosa* isolates caused a decrease in binding points of colistin to bacteria and outer membrane polarity. This effect led to colistin resistance with low ratio. Colistin resistance, which develops after exposure to subinhibitory concentrations of other antibiotic groups mentioned above, is related with changes of outer membrane polarity and colistin binding points. Moreover, it was observed that

colistin exposure did not cause any changes of resistance rates of bacteria against other antibiotic groups.

In different studies; it was shown that subinhibitory concentrations of antibiotic can trigger slime formation in *P. aeruginosa* isolates (20-22). This finding suggests that increased resistance after antibiotic exposure can be due to slime formation.

As it was seen in this study; bacteria which can not be killed after exposure to antibiotics can become a much more dangerous infection potential. In studies resistance development of *P.aeruginosa* in a short period was shown in vitro against subinhibitory concentrations of carbapenem or quinolones (23, 24).

In our study cross-resistance development against not only the exposed antibiotic but also various other antibiotics in different groups was shown in most of the isolates which exposed to subinhibitory concentrations of antibiotics and isolates with mult-drug resistance occured. It was shown that especially subinhibitory concentrations use of carbapenem and aminoglycoside antibiotics triggered resistance development against themselves more than other antibiotic groups. This ratio was lower for ciprofloxacin and colistin. Cross-resistance did not develop in isolates which exposed to subinhibitory concentrations of colistin. It was shown that use of different antibiotic groups in subinhibitory concentrations can cause colistin resistance or increase in MIC ratios. *P. aeruginosa* isolates were susceptible against all antibiotics used in our study at the beginning but after exposure of these bacteria to non-lethal concentrationss of these antibiotics; isolates have emerged which are resistant to various antibiotics and the antibiotics used. So, use of appropriate antibiotics with inappropriate amounts can also cause serious problems.

In conclusion, the effect of antibiotics on the bacteria is not limited to just killing them. Subinhibitory concentrations use of antibiotics might change a isolate which is infectious agent into a isolate with multi-drug resistance during treatment and disrupt treatment or some of the bacteria in our flora can turn into a more resistant bacteria after subinhibitory concentrations antibiotic exposure, even become dominant in flora after natural selection and could become a severe infection potantial for the future.

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