Risk factors for infection with colistin-resistant gram-negative microorganisms: a multicenter study

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BACKGROUND: Knowing risk factors for colistin resistance is important since colistin is the only remaining choice for the treatment of infections caused by multi-drug resistant microorganisms.

OBJECTIVE: Evaluate risk factors associated with infection by colistin-resistant microorganisms.

DESIGN: Retrospective study.

SETTINGS: Tertiary healthcare centers.

PATIENTS AND METHODS: An e-mail including the title and purpose of the study was sent to 1500 infectious disease specialists via a scientific and social web portal named "Infeksiyon Dunyasi (Infection World)". Demographic and clinical data was requested from respondents.

MAIN OUTCOME MEASURE(S): Colistin-resistance.

RESULTS: Eighteen infectious disease specialists from twelve tertiary care centers responded to the invitation. Data was collected on 165 patients, 56 cases (39.9%) and 109 (66.0%) age- and sex-matched controls. The colistin-resistant microorganisms isolated from cases were 29 Acinetobacter baumannii (51.8%), 18 Pseudomonas aeruginosa (32.1%) and 9 Klebsiella spp. Colistin, carbapenem, and quinolone use in the last three months were risk factors for colistin resistance in the univariate analysis. Previous quinolone use in the last three months (P=.003; RR:3.2; 95% CI:1.5-6.7) and previous colistin use in the last three months (P=.001; RR: 3.6; 95% CI:1.63-7.99) were significant risk factors in the multivariate analysis.

CONCLUSION: Clinicians should limit the use of quinolones and remain aware of the possibility of resistance developing during colistin use.

LIMITATIONS: The lack of a heteroresistance analysis on the isolates. No data on use of a loading dose or the use of colistin in combination.
The increased prevalence of infections related to multi-resistant microorganisms has led to a search for alternative antibiotics in recent years. Colistin, which was first used in clinical practice in the 1960s, was abandoned due to nephrotoxicity and neurotoxicity. However, in the last fifteen years, colistin has gained an important role as salvage therapy for patients infected with microorganisms susceptible only to colistin. As with all antibiotics, the increased use of colistin has resulted in the development of colistin resistance.

Adaptive or mutational mechanisms may play a role in resistance to colistin. In colistin resistance, different gene mutations cause resistance by changing the outer membrane of gram-negative bacteria. Although data on resistance mechanisms is limited, the regulator systems of PmrA-PmrB and PhaP-PhOq are known to play a key role in resistance development.

Statistical analysis
Data collected from twelve centers were combined and analyzed using SPSS 15.0 (SPSS, Chicago, IL, USA) program. In the analysis of categorical variables the chi-square test was used. In the analysis of continuous variables either the t test or the Mann Whitney U test were used. A P value less than .05 was considered statistically significant. Statistically significant variables in the univariate analysis were included in a stepwise backwards logistic regression analysis.

RESULTS
Twelve tertiary care centers, consisting of six university and six training and research hospitals, replied via email to the request for data. Eighteen infectious disease specialists responded. The analysis used data from 56 cases and 109 controls, after exclusion of 3 control patients that could not be matched to cases. Colistin-resistant microorganisms isolated were 29 A baumannii (51.8%), 18 P aeruginosa (32.1%) and 9 Klebsiella spp. No sta-
A statistically significant difference was detected in the demographic characteristics and diagnosis on admission between the case and control groups (Table 1). In a univariate analysis for risk factors associated with colistin resistance, antibiotic use in the last three months ($P=.003$), colistin use in the last three months ($P=.016$), carbapenem use in the last three months ($P=.027$), quinolone use in the last three months ($P=.001$) and the length of hospital stay before the isolation of the multi-resistant microorganism ($P=.04$) were statistically significant (Table 2).

A multivariate analysis that included these sig-
significant parameters showed that quinolone use in the last 3 months increased the risk of infection 3.2 times ($P = .003$, 95% CI:1.50-6.74) and colistin use in the last three months increased risk 3.6 times ($P<.001$, 95% CI:1.63-7.99). Further subgroup analysis by microorganisms could not be performed because of low numbers.

**DISCUSSION**

An increase in carbapenem resistance in multi-resistant microorganisms such as *A baumannii*, *K pneumoniae* and *P aeruginosa* has led to the increased use of colistin.² As an inevitable outcome of increased colistin use, colistin-resistant strains have developed through selection of heteroresistant strains.¹,² Colistin has been widely used in Turkey since 2010. Colistin resistance is threatening as it can be related to a poor prognosis.³² Not clarifying an optimal dosage and therefore using colistin in different dosages may induce inappropriate usage, the emergence of resistance and its continuous spread.¹³

Published reports on risk factors for colistin resistance are limited.¹,³,³² Matthaiou et al reported in 2008 on 41 cases including 35 infected and 6 colonized with colistin-resistant *K pneumoniae* (33), Acinetobacter spp (6), and *P aeruginosa* (2). In a univariate analysis, colistin use, duration of previous colistin use, patient age, history of previously performed surgical procedures, duration of stay in the ICU, monobactam use and duration of antifungal agent use were reported as risk factors. In the multivariate analysis of the same study, only colistin use was determined to be statistically significant and an independent risk factor.³ In colistin resistance, the selection of strains plays a role. In another study, in colistin-susceptible Acinetobacter types isolated from the patient group using colistin, heteroresistant strains were shown to be significantly higher than in Acinetobacter types isolated from the patient group not using colistin.³⁴ Colistin heteroresistance was defined as an isolate with colistin an MiC of 2 mg/L, in which detectable subpopulations were able to grow in the presence of >2 mg/L colistin.³³ In *K pneumoniae* types resistant to carbapenem, the existence of heteroresistant strains has been shown. In the multivariate analysis of our study, colistin use in the last three months was found to be a risk factor for colistin resistance. This points to the possibility that heteroresistant strains were selected by selective depression. However, a limitation of our study was in not performing a heteroresistance analysis on the strains. A heteroresistance analysis might have identified selection of heteroresistant strains as an important mechanism of colistin resistance in our study. In the multivariate analysis, another parameter found

### Table 2. Univariate analysis of risk factors as invasive devices and antibiotic usage associated with infection of colistin-resistant gram-negative microorganisms and prognosis.

| Invasive devices and treatment | Colistin S n=109 (%) | Colistin R n=56 (%) | P |
|--------------------------------|----------------------|---------------------|---|
| Mechanical ventilation         | 71 (65.1)            | 39 (69.6)           | .56 |
| Central venous catheter        | 79 (72.5)            | 40 (71.4)           | .89 |
| Urinary catheter               | 91 (83.5)            | 45 (80.4)           | .38 |
| Steroid use                    | 18 (16.5)            | 14 (25.0)           | .21 |
| Hemodialysis                   | 13 (12.0)            | 12 (21.4)           | .09 |
| H2 receptor blocker or proton pump inhibitor use | 89 (81.7) | 48 (85.7) | .33 |
| Total parenteral nutrition     | 55 (50.5)            | 30 (53.6)           | .70 |
| Nasogastric entubation         | 67 (61.5)            | 36 (64.3)           | .43 |
| Previous antibiotic use (last 3 months) | 73 (67.0) | 49 (87.5) | .003 |
| Colistin                       | 16(14.7)             | 17 (30.4)           | .16 |
| Carbapenem                     | 38 (34.9)            | 29 (51.8)           | .027 |
| Glycopeptide                   | 25 (22.9)            | 11 (19.6)           | .39 |
| Cephalosporin                  | 39 (35.8)            | 25 (44.6)           | .17 |
| Aminoglycoside                 | 7 (6.4)              | 4 (7.1)             | 1.00 |
| Quinolone                      | 19 (17.4)            | 24 (42.9)           | .001 |
| Sulbactam                      | 24 (22.0)            | 17 (30.4)           | .16 |
| Tigecycline                    | 10 (9.2)             | 13 (23.2)           | .15 |
| Piperacillin-tazobactam        | 32 (29.4)            | 22 (39.3)           | .13 |
| Metronidazole                  | 18 (16.5)            | 8 (14.3)            | .45 |
| Linezolide/daptomycine         | 21 (19.3)            | 18 (32.1)           | .051 |
| Anti-fungal agent              | 19 (17.4)            | 12 (21.4)           | .33 |
| Clinical cure                  | 63(57.8)             | 27 (48.2)           | .24 |
| Microbiological cure           | 61 (56.5)            | 29 (51.8)           | .07 |

-MDR: multidrug resistant; Contributors: Husnu Pullukçu, Meltem Tasbakan, Ozlem Tunccan, Yasemin Tezer, Mehmet Ozden, Otge Turhan, Rahmet Guner, Yasemin Cag, Fatma Bozkurt, Fatma Yılmaz Karadag, Elif Doyuk Kartal, Gokhan Gozel, Cemal Bulut, F. Şebnem Erdinç, Cibali Ackgoz, Mehmet A. Tasyaran.
to be significant was quinolone use in the last three months.

In Matthaiou’s study, no significant difference in mortality was reported between the group infected and/or colonized with colistin-resistant and colistin-susceptible microorganisms. In our study, mortality rates were higher in infected patients, but the difference was not statistically significant (53.6% to 45%) (Table 1).

Colistin resistance in *A. baumannii* is thought to be related to insufficient dosage. In our study, there was no significant difference in two different dosages used in patients infected with colistin-resistant gram-negative microorganisms (3×75 mg to 2×150/3×100 mg). A loading dose of colistin might be useful in critical patients, and most experts think that colistin should be used in combination antibiotic therapy to prevent colistin resistance. However, our study was limited in that we were unable to collect relevant data relating to the loading dose or the use of colistin in combination. We could only find one case-control study in medical literature that investigated risk factors for colistin resistance in *P. aeruginosa*. According to the results of this study, risk factors in terms of pandrug-resistant *P. aeruginosa* were reported as combined use of carbapenem for over 20 days, colistin combination use for more than 13 days, and more than 78 open suctioning procedures. The global spread of resistant *K. pneumoniae* and the limited number of treatment options have given rise to substantial concerns. Although results are contradictory, some studies suggest there is a relationship between colistin use and carbapenem-resistant *K. pneumoniae*. Colistin resistance can be seen in *K. pneumoniae* and it has been reported that the resistance develops during treatment. In a case-control study published by Zarkotou et al, risk factors for colistin-resistant carbapenemase producing *K. pneumoniae* included referral to the study center from another hospital and long term use of beta-lactam/beta-lactamase inhibitor combination (18.7 [6.5] days to 10.5 [5.2] days, *P*=.002). Thirteen patients, of whom 8 were infected and 5 colonized with colistin-resistant strain, were included in the study as the case group. Although there was a greater ratio of colistin-resistant in the patient group infected or colonized with colistin-resistant *K. pneumoniae* (30.8% to 20.5%), no significant difference was detected. The duration of colistin use was not associated with colistin resistance. However, in the same study the authors speculated that the effect of colistin use could be underestimated due to clonal transfer identified by molecular analysis. In the univariate analysis of a study from Greece, colistin treatment, the average stay in the ICU and the length of colistin treatment were significant variables. In the multivariate analysis, colistin treatment was a statistically significant risk factor. Ninety percent of the patients who were colonized with colistin-resistant *K. pneumoniae* had been treated with colistin. This rate was reported as 56% for the control group. Previous colistin use in the last three months was a risk factor in our study, but we could not perform subgroup analysis for each microorganism because of low numbers. In an epidemic of colistin-resistant *K. pneumoniae*, the cases were older than the controls and imipenem MIC levels were higher. Mortality and length of hospital stay were higher in the case group, but the difference was not statistically significant. In another recent study, colistin-resistance was independently associated with a poor prognosis in infections due to carbapenem-resistant *K. pneumoniae*. When evaluating all microorganisms concomitantly for colistin resistance, quinolone use and receiving colistin were found to be significant risk factors.

We conclude that limiting the use of quinolone and being aware that resistance can develop during colistin use are important.

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