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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Determination of the Presence of *Klebsiella pneumoniae* and Phenotypic Antibiotic Resistance Profiles in Budgerigars and Parrots, Istanbul, Turkey

A. Ilgın KEKEÇ* **Barış HALAÇ** **Beren BAŞARAN KAHRAMAN** **Seyyal AK**
Istanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, Department of Microbiology, Büyükçekmece, Istanbul, Turkey

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*: https://orcid.org/0000-0002-0821-8376
: https://orcid.org/0000-0002-3067-9937
: https://orcid.org/0000-0002-1736-093X
: https://orcid.org/0000-0002-6687-8401

***Corresponding author's:**
A. Ilgın KEKEÇ
Istanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, Department of Microbiology, Büyükçekmece, Istanbul, Turkey
✉: ilginkekec@iuc.edu.tr

Abstract: In this study, it was aimed to investigate the presence of *Klebsiella pneumoniae* and phenotypically carbapenemase, extended-spectrum β-lactamase (ESBL), acquired-AmpC beta-lactamase, and multiple antibiotic resistance of the isolates in the faeces of budgerigars and parrots. A total of 96 faecal samples belonging to 54 budgerigars and 42 parrots were used in the study. Cultivation was performed on various media for the identification of *K. pneumoniae* from the collected stool samples. Biochemical properties of the presumptive isolates were determined by conventional methods. Besides, antibiotic susceptibility tests and ESBL, carbapenem, acquired AmpC screening and confirmation tests were applied to the identified isolates to phenotypically determine beta-lactam resistance. Beta-lactam, aminoglycoside, fluoroquinolone, tetracycline, chloramphenicol, and sulphonamide groups were used to determine the multidrug resistance. Isolates with resistance to 3 or more of the antibiotic and sulphonamide groups were accepted as multidrug-resistant isolates. *K. pneumoniae* was isolated from 2 (3.7%) of 54 budgerigars, and 1 (2.3%) of 42 parrot faecal samples from 3 (3.1%) of 96 faecal samples in total. No phenotypic resistance was detected in any of the isolates as a result of screening and confirming tests for ESBL, carbapenem, and acquired-AmpC resistance to determine phenotypic antibiotic resistance of the isolates. Multidrug resistance was detected in only one isolate. The presence of multi-resistant *K. pneumoniae* in cage birds that have close relationships with humans has been revealed.

Keywords: Budgerigar, faeces, *Klebsiella pnemoniae*, multidrug resistance, parrot.

Muhabbet Kuşu ve Papağanlarda *Klebsiella pneumoniae* Varlığının ve Fenotipik Direnç Profillerinin Saptanması, İstanbul, Türkiye

***Sorumlu yazar:**
A. Ilgın KEKEÇ
İstanbul Üniversitesi-Cerrahpaşa Veteriner Fakültesi Mikrobiyoloji Anabilim Dalı, Büyükçekmece, İstanbul, Türkiye
✉: ilginkekec@iuc.edu.tr

Öz: Bu çalışmada, muhabbet kuşu ve papağan dışkılarında *Klebsiella pneumoniae* varlığının ve fenotipik karbepenemaz, genişletilmiş spektrumlu β-laktamaz, kazanılmış- AmpC, beta-laktam ve çoklu antibiyotik direncinin belirlenmesi amaçlanmıştır. Çalışmada, 54'ü muhabbet kuşuna, 42'si papağana ait toplam 96 dışkı örneği kullanıldı. Toplanan dışkı örneklerinden *K. pneuomiae*, çeşitli besiyerleri kullanılarak izole edildi. Şüpheli izolatların biyokimyasal özellikleri konvansiyonel yöntemlerle belirlendi. Ayrıca beta-laktam direncini fenotipik olarak belirlemek için belirlenen izolatlara antibiyotik duyarlılık testleri ve ESBL, karbapenem, edinilmiş AmpC tarama ve doğrulama testleri uygulandı. Çoklu ilaç direncini belirlemek için beta-laktam, aminoglikozid, florokinolon, tetrasiklin, kloramfenikol ve sülfonamid grupları kullanıldı. 3 veya daha fazla antibiyotik ve sülfonamid grubuna dirençli izolatlar çoklu ilaç direncine sahip izolatlar olarak kabul edildi. *K. pneumoniae*, 54 muhabbet kuşu dışkı örneğinin 2'sinden (%3,7), 42 papağan dışkı örneğinin 1'inden (%2,3) olmak üzere toplamda 96 dışkı örneğinin 3'ünden (%3,1) izole edildi. İzolatların fenotipik antibiyotik direncini belirlemek için ESBL, karbapenem ve edinilmiş AmpC direncinin taranması ve doğrulanması sonucunda izolatların hiçbirinde fenotipik direnç saptanmadı. Yalnızca bir izolatta çoklu ilaç direnci tespit edildi. İnsanlarla yakın ilişkileri olan kafes kuşlarında çoklu ilaç direncine sahip *K. pneumoniae*'nin varlığı ortaya konuldu.

Anahtar kelimeler: Çoklu ilaç direnci, dışkı, *Klebsiella pneumoniae*, muhabbet kuşu, papağan.

INTRODUCTION

Companion birds like budgerigars and parrots are high in the ranking of popular pets worldwide. They have an important place for supporting children's love for animals, are preferred by families due to their close relationship with people and ease of feeding. Therewithal, the practice of keeping birds as pets has increased globally as these animals are used as companions, enjoyment, or psychological support (Ahmed et al., 2021). Resistance to antimicrobials frequently encountered among pathogen and commensal bacteria of animal origin is a growing concern in both veterinary and human medicine (Teuber, 2001). Bacterial pathogens carried by companion birds are considered a risk for birds, pet owners and also potential risks remain for human beings who come into close contact with companion birds in the home environment. The spread and contamination of commensal bacteria is thought to pose a risk to the release of caged birds, and also factors such as defecation in the home environment, frequent kisses by their owners, and oral feeding increase this risk. *Klebsiella pneumoniae* is considered one of the most important Gram-negative opportunistic pathogens and worrisome multidrug-resistant bacteria in nosocomial infections. The presence of *K. pneumoniae* in psittacines can create a potential risk to other birds and human beings (Davies et al., 2016). The increase in bacterial antimicrobial resistance is a natural phenomenon, an outcome of evolution (Fedorka-Cray et al., 2005). Unavoidable increase of antimicrobial resistance (AMR) in companion animals continues to be studied worldwide. However, a limited number of studies have been observed focusing on the resistance profiles of *Klebsiella pneumoniae* isolates in budgerigars and parrots belonging to the Psittaciformes family, which are considered among the most common and popular pet bird species in close contact with humans (Rueanghiran et al., 2019; Sigirci et al., 2020; Yame et al., 2017).

Considering these reasons, it was aimed to determine the presence of *K. pneumoniae* in the faeces of healthy and unhealthy budgerigars and parrots from home care centers, petshops, and veterinary clinics in Istanbul and to determine their antibiotic resistance profiles.

MATERIALS AND METHODS

Samples: Between April 2018 and January 2019, a total of 96 faecal samples from 54 budgerigars and 42 parrots were collected by visiting 9 sales focus, Faculty of Veterinary Medicine Animal Hospital and also 12 home care centers, in Istanbul. The samples were stored at +4 ° C until reaching the laboratory and were cultivated the same day.

Culture: The samples were cultured in Triptic Soy Broth (TSB; Merck, 1.05459)and incubated at 37 °C for 24 h. After incubation, a loopful of each culture was subcultured onto MacConkey agar (HiMedia, M081) with and without 1 mg/L cefotaxime (HiMedia), and incubated at 37 °C for 24 h. A presumptive colony was randomly selected and subcultured. Isolates were identified by routine conventional methods as *K. pneumoniae* (Krieg & Holt, 2005) and were confirmed with API 20E system (BioMérieux).

***Antibiotic Susceptibility Tests*:** The antibiotic susceptibility tests were performed according to the Kirby-Bauer method recommended by the Clinical Laboratory Standards Institute (CLSI) to select the optimal antimicrobial agent for treatment. Isolates were tested for antibiotic susceptibility to 15 different antimicrobials from 8 distinct classes: amikacin (30 µg), amoxicillin-clavulanic acid (30 µg), ampicillin-sulbactam (20 µg), aztreonam (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), kanamycin (30 µg), levofloxacin (5 µg), nalidixic acid (30 µg), norfloxacin (10 µg), ofloxacin (10 µg), streptomycin (10 µg), sulfamethoxazole / trimethoprim (1.25/23.75 µg) and tetracycline (30 µg) (CLSI, 2014; EUCAST, 2015). Also, Extended-spectrum beta-lactamase (ESBL) production, carbapenem, acquired AmpC screening and confirmation tests were applied to the identified isolates to phenotypically determine beta-lactam resistance. The results were based on CLSI breakpoints (CLSI, 2018). For quality control, *E. coli* (ATCC 25922) and *K. pneumoniae* (ATCC 4352) were used. Beta-lactam, aminoglycoside, fluoroquinolone, tetracycline, chloramphenicol and sulphonamide groups were used to determine the multidrug resistance (MDR) defined as resistance at least three different antimicrobial classes (de Jong et al., 2018).

RESULTS

K. pneumoniae was isolated from 2 (3.7%) of 54 budgerigars and 1 (2.3%) of 42 parrots and in total from 3 (3.1%) of 96 faecal samples. All isolates were detected from different petshops and clinically healthy birds. Antibiotic susceptibility results for all isolates are shown in Table 1.

No resistance was found in any of the isolates as a result of screening and confirming tests for ESBL, carbapenem, and acquired-AmpC resistance to determine phenotypic antibiotic resistance of the isolates. Multidrug resistance was detected in only one isolate from budgerigar.

Table 1. Antibiotic susceptibility results for the isolates

Sources	OF	CIP	LEV	NOR	NA	AK	GE	ST	KA	TE	KL	SXT	AMC	SAM	AZ
Budgerigar	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	S
Parrot	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Budgerigar	S	S	S	S	R	S	S	S	S	S	**R**	S	S	S	S

Of: Ofloxacin; Cip: Ciprofloxacin; Lev: Levofloxacin; Nor: Norfloxacin; Na: Nalidixic acid; AK: Amikacin; Ge: Gentamicin; Strep: Streptomycin; Ka: Kanamycin; Tet: Tetracycline; Kl: Chloramphenicol; SXT: Sulfamethoxazole-Trimethoprim; AMC: Amoxicillin-Clavulanic acid; SAM: Ampicillin sulbactam; AZ: Aztreonam, S: Susceptible, R: Resistant

DISCUSSION

Although many articles are investigating the presence and antibiotic profiles of Enterobacteriaceae conducted on cage birds, the data about *K. pneumoniae* is scant. (Horn et al., 2015; Rueanghiran et al., 2019; Sigirci et al., 2019; Steger et al., 2020). Most studies focused on *K. pneumonia*, have been associated with companion animals, howbeit caged birds have been included in the category of companion animals in recent years.

A limited number of studies have paid attention to the presence of *K. pneumonia* from companion birds. Machado et al. (2015) reported that 6 of 79 samples obtained by cloacal swab method isolated *K. pneumoniae*. Yame (2017) reported 19 strains of *Klebsiella* spp. isolated from respiratory secretions of 46 diseased psittacines, 16 (16/19) were identified as *K. pneumoniae*, and three (3/19) were identified as *K. oxytoca*. It was reported that 3 strains of *K. pneumoniae* isolated from 100 parakeets, 23 parrots, 12 canaries, 12 Indian nightingales, 2 European goldfinches (Sigirci et al., 2019). Further, Rueanghiran et al. (2019) show that a total of 80/376 psittacine cases (21%) was diagnosed to have a respiratory problem and *K. pneumoniae* 7 isolates (8%) were obtained from 53 respiratory cases. Steger et al. (2020) was reported that 86 *K. pneumoniae* isolates from 811 birds of 20 zoological orders (mostly Psittaciformes 61.8 % and Passeriformes 14.5 % and from alive patients or pathological examinations) were found. Ahmed et al. (2021) emphasized that 17.6 % of the pet birds and 12.9 % of the human contacts were positive for *K. pneumonia*. Unlike previous studies, *K. pneumoniae* isolation rates were lower in the current study.

The authors pointed that *K. pneumonia* has become the most successful and modern pathogen by producing Extended Spectrum β-Lactamase (ESBL) and also shows high resistance to a broad spectrum of antibiotics including β-lactam antibiotics, fluoroquinolones, and aminoglycosides (Riwu et al., 2020). Davies et al. (2016) reported that the susceptibility profile of *K. pneumoniae* strains revealed a high resistance to ampicillin, nalidixic acid, sulphonamides and tetracycline. Further, Yame (2017) emphasized the antimicrobial susceptibility profile demonstrated high resistance to ampicillin (89.5%) and three strains of *K. pneumoniae* were positive for extended-spectrum beta-lactamase production. In another study conducted in our country, it was reported that the isolated *K. pneumoniae* species were all sensitive to the antibiotics tested and no resistance was observed (Sigirci et al., 2019).

In the current study, resistance was not detected in any of the isolates as a result of screening and confirming tests for ESBL, carbapenem and acquired-AmpC resistance to determine phenotypic antibiotic resistance. Besides, resistance to only chloramphenicol was detected in one of the two isolates taken from budgerigars, and it was observed that this isolate was susceptible to all other antimicrobials. However, the second isolate obtained from the budgerigar was resistant to all antimicrobials except aztreonam. Furthermore, it was observed that the only isolate detected from the parrot was susceptible to all the antimicrobials analysed. The reason for these differences in the findings between previous studies could be explained by the small sample sizes and the presence of geographical variants.

The complex hazard of AMR transmission from companion animals to humans has not been fully established. Consequently, studies on AMR and MDR, performed in various countries, unceasingly continue to provide more information. Unfortunately, data regarding MDR in companion birds are limited (Rueanghiran et al., 2019, Sigirci et al., 2020, Yame et al., 2017). According to the numerous reports, the MDR profiles of the *K. pneumoniae* strains reported between 25%- 57%, respectively (Ahmed et al., 2021, Ajayi & Egbebi, 2011, Davies et al., 2016, Rueanghiran et al., 2019). In the present study, MDR was noticed in only one isolate then this result is promising on its own.

As a result of this current study, the presence of antimicrobial resistance has been revealed in cage birds that have close relationships with humans. There are a limited number of studies on the antimicrobial resistance profiles of caged birds. Hence, monitoring pet birds as potential reservoirs of zoonotic bacterial pathogens is crucial to sustaining human health and it is necessary to develop routine analysis. Further studies are needed to evaluate risk factors, follow-up programs to prevent resistance. Because of the possibility that companion animals may act as reservoirs, the assessments must include commensal bacteria as well as pathogenic bacteria.

Especially the possibility that healthy animals contribute to the transmission of antibiotic resistance markers without being noticed makes the reports of antibiotic resistant isolates important.

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