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Evaluation of bacteria isolated from different animal species and antibiotic resistance in the veterinary diagnostic laboratory

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Abstract: Isolation of bacteria can be performed by taking correct and convenient samples, especially from the infection site. In addition, after isolation of the bacteria, the antimicrobial susceptibility test should be performed routinely to treat animals conveniently. For this purpose, 129 isolates were included in the current study from different animal origins and different examination materials in the culture collection of Atatürk University Faculty of Veterinary Medicine Department of Microbiology between 2020 and 2021. The isolates were identified by bacteriological methods and their antibiotic resistance was evaluated phenotypically. In the study, it was determined that bacteria belonging to the *Staphylococcus* genus (27.2%) were mostly involved in different infections. Overall results displayed those bacteria tested in this study were resistant to neomycin (100%), penicillin (76.74%), oxytetracycline (73.80%), and sulfamethoxazole-trimethoprim (61.25%) with a different rate, whereas they were susceptible to cephalosporin antibiotics (cefovecin %64.3 ceftiofur %80, and cefoxitin %81.8) used in the current study.
Keywords: Antimicrobial resistance, bacteria, fungi, retrospective study

Veteriner tanı laboratuvarında farkli hayvan türlerinden izole edilen bakterilerin ve antibiyotik direnç durumlarinin değerlendirilmesi

Özet: Özellikle enfeksiyon bölgesinden doğru ve uygun örnekler alınarak mikroorganizma izolasyonu yapılabilir. Ek olarak, bakterilerin izolasyonundan sonra, hayvanı uygun şekilde tedavi etmek için antimikrobiyal duyarlılık testi rutin olarak yapılmalıdır. Bu amaçla Atatürk Üniversitesi Veteriner Fakültesi Mikrobiyoloji Anabilim Dalı kültür koleksiyonunda, 2020-2021 yılları arasında farklı hayvan türleri ve farklı inceleme materyallerinden elde edilen 129 izolat mevcut çalışmaya dahil edildi. İzolatlar uygun yöntemlerle identifiye edilerek, fenotipik olarak antibiyotik dirençlilikleri değerlendirilmiştir. Çalışmada, en fazla *Staphylococcus* cinsine ait bakterilerin (%27,2) farklı infeksiyonlarda rol aldığı saptanmıştır. Genel sonuçlar, bu çalışmada test edilen bakterilerin farklı oranlarda neomisin (%100), penisilin (%76,74), oksitetrasiklin (%73,80) ve sülfametoksazol trimetoprim'e (%61,25) dirençli olduğunu, oysa ki bu çalışmada kullanılan sefalosporin antibiyotiklerine (sefovesin %64,3, seftiofur %80 ve sefoksitin %81,8) duyarlı olduklarını gösterdi.
Anahtar Sözcükler: Antimikrobiyal direnç, bakteri, mantar, retrospektif çalişma

Introduction

Infectious diseases caused by different microorganisms provoke various problems in animals, especially yield losses. Bacteria constitute most of these microorganisms. These bacterial infections are characterized by different clinical findings. Fungi, as well as bacteria, can cause infections in animals. To determine the treatment protocols of infections, it is necessary to determine the agent and, if the infection is caused by bacteria, these protocols should be arranged by using antibiotic susceptibility tests (EUCAST, 2017).

Bioactive substances that act on bacteria in different ways, preventing their development and growth or killing bacteria are called antibiotics. The ability of a bacterium to resist the lethal and inhibitory effect of antibiotics is called antibiotic resistance. Although this resistance can be found structurally in bacteria, it can also be acquired later (Denyer et al., 2004). Wrong choice of antibiotics as a result of misdiagnosis of physicians and widespread, unconscious, and continuous use of antibiotics play a role in the development of resistance in bacteria as well as take in place different mechanisms under developing antimicrobial resistances (Barnes et al., 2013).

Antibiotic resistance gained by bacteria is becoming a global threat. Because it limits the possibilities of drugs for the treatment of infections, increases treatment costs, and causes animal and loss of productivity. Adding antibiotics to animal feeds to accelerate growth and prevent disease formation creates a public health problem by causing the transfer of antibiotic-resistant bacteria found in animal foods to humans (Kilic, 2004). Various antibiotic susceptibility tests are performed in routine diagnostic laboratories to determine the antibiotic resistance status of the bacteria and to select the antibiotics to be used in the treatment more accurately (CLSI, 2017). In this study, bacteria isolated from different tissues and organs of different animal species and their antibiotic resistance status were evaluated.

Material and Method

In this research, 129 isolates belonging to the year 2020-2021, which were previously recorded in the culture collection of Ataturk University, Faculty of Veterinary Medicine, Department of Microbiology, were used. Microorganisms were isolated from different samples (skin lesion, urine, feces, blood, milk, tissue samples (lung, liver, spleen, heart, kidney), eye, ear, wound, joint, stomach content, mucous membranes, oral, nasal, pharyngeal, and rectal swabs) of various animal species (large ruminant, small ruminant, pet animals, poultry, horse, and rabbit) were recovered from -80 °C and *Gram* staining and biochemical tests were performed. Blood agar (Merck Cat No: 1.10886.0500, Germany), MacConkey agar

(Merck Cat No: 1.05465.0500, Germany), and Nutrient agar (Merck Cat No: 1.05450.0500, Germany) were used during the passage. Moreover, Mueller Hinton agar media (Merck Cat No: 1.05437.0500, Germany) was used for the antibiotic susceptibility test and Mycoplasma Agar Base (Oxoid Cat No: CM0401, United Kingdom) was used for the identification of *Mycoplasma* spp. catalase, oxidase, coagulase, biochemical tests (carbohydrate fermentation tests, motility test, urease activity, nitrate reduction test, hydrogen sulfide production test, ONPG test, gelatin hydrolysis test, etc.) were performed for the identification of bacterial agents (Quinn, 2004).

Disc diffusion technique was used to determine the antibiotic resistance profile of bacteria. The *Gram* characteristic of the isolates, as well as the animal and sample type from which the bacteria were isolated, were considered when selecting antibiotic discs. Inhibition zone diameters formed because of the test were measured and compared with the specified standards and antibiotics to which the identified bacteria were susceptible and resistant were determined (EUCAST, 2017).

Sabouraud Dextrose Agar (SDA) (Merck Cat No: 1.07315.0500, Germany) was used for mycological examination. After the isolates were inoculated on an SDA medium, after 21 days of incubation at 25 °C, fungal colonies were stained with Lactophenol cotton blue (Merck, Cat No: 113741, Germany), and fungal species were identified (Campbell et al., 2013). SDA containing 1.0% olive oil was used for suspected yeast isolates. After 7-10 days of incubation at 37 °C, the growing colonies were stained by the *Gram* staining method and identified by urease production and melanin production (Larone, 2002; Quinn, 2004). All obtained data were reported and recorded, and isolates were grouped according to animal species.

Results

Seven fungi and seven yeasts were identified among the 129 microorganisms in the culture collection, while 26.95% of the 115 bacteria were *Staphylococcus* spp., 33.91% were *Enterobacteriaceae*, 13.91% were *Pasteurellaceae*, 9.56% were *Actinomyces* sp., 7.82% were *Streptococcus* sp., 4.34% were *Enterococcus* sp., 1.73% were *Alcaligenes* sp. The bacteria membered of *Enterobacteriaceae* were isolated from cattle, calves, and poultry. In addition, most of *Staphylococcus* spp. were isolated from cats and dogs, whereas *Actinomycetaceae* was detected in sheep, goats, and lambs. When all animal species and isolates were evaluated, it was observed that the majority belong to the *Staphylococcus* spp. (27.2%), followed by the

families *Enterobacteriaceae* (25.5%) and *Pasteurellaceae* (14%). Figure 1 depicted the distribution of bacteria according to animal origins.

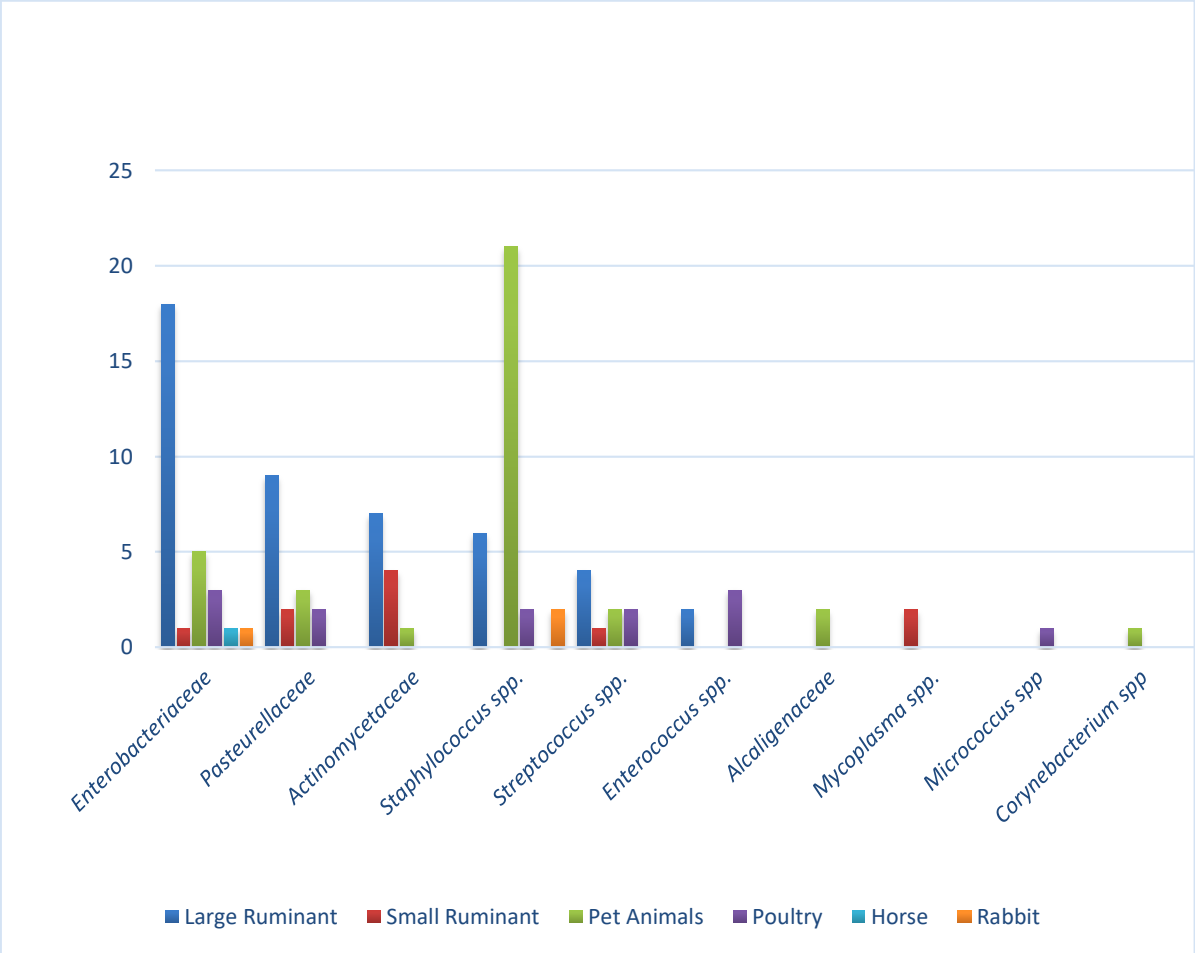

Figure 1: Distribution of identified bacterial families and general by animal origins

The origin of the isolates including bacteria and fungi were large ruminants (six abscesses, one skin lesion, six tissue samples, two wounds, one ear, one nasal, three fecal, and twelve joint swabs), horse (one skin lesion and abscess), small ruminants (nine tissue samples), pet animals (six skin lesion, three urine, one blood, four milk, one tissue sample, six eyes, four ears, two wounds, one oral, one nasal, one pharyngeal, and one discharge from the mucous membranes swabs), poultry (one tissue samples, one stomach contents, twelve fecal, one eye, and one laryngeal swab), and rabbit (one abscess and rectal swab). The distribution of bacterial families isolated from different sample types was shown in Table 1.

Table 1: Distribution of families of bacteria isolated from different sample groups (n)

	Enterobacteriaceae	*Pasteurellaceae*	*Actinomycetaceae*	*Staphylococcus* spp.	*Streptococcus* spp.	*Enterococcus* spp.	*Alcaligenaceae*	*Mycoplasma* spp.	*Micrococcus* spp.	*Corynebacterium* spp.
Abscess	5	0	3	3	2	1	0	0	0	0
Nasal swab	3	1	1	2	0	0	1	0	0	0
Skin lesion	1	0	0	2	0	0	0	0	0	0
Feces	7	0	0	1	2	3	0	0	0	0
Joint swab	4	6	2	2	2	0	0	0	0	0
Ear swab	0	0	0	5	0	0	0	0	0	0
Tissue samples	4	6	5	1	2	1	0	2	0	0
Rectal swab	1	0	0	0	0	0	0	0	0	0
Urine	1	0	0	1	0	0	1	0	0	0
Blood	0	0	0	0	0	0	0	0	0	1
Wound swab	2	1	0	2	0	0	0	0	0	0
Mucous membrane	0	0	0	0	1	0	0	0	0	0
Oral swab	0	0	0	1	0	0	0	0	0	0
Milk	0	0	0	4	0	0	0	0	0	0
Pharyngeal swab	1	1	0	0	0	0	0	0	0	0
Eye swab	0	0	0	6	0	0	0	0	1	0
Stomach contents	0	1	0	1	0	0	0	0	0	0

The seven isolated fungal agents were identified as *Aspergillus fumigatus* (n=2), *Blastomyces dermatitis* (n = 1), *Penicillium* spp. (n = 2), *Purpureocilium lilacium* (n = 1), and *Mucor* spp. (n = 1). All fungi were isolated from horse (n = 1), cattle (n = 2), and pet animals (n = 4) with skin lesions. A total of seven yeast were identified as *Macrorhabdus ornitogaster*

(85.72%) and *Candida* spp. (14.28%). Although *Macrorhabdus ornitogaster* was isolated from the fecal samples of different ornamental birds, *Candida* spp. was isolated from the laryngeal swab of the peacock.

When the antibiogram results of the bacteria whose identification was completed were evaluated, high resistance to (75%) trimethoprim-sulfamethoxazole was observed in bacteria belonging to the *Enterobacteriaceae* family, while low resistance to (20%) ceftiofur and (18.18%) cefoxitin was observed. *Pasteurellaceae* were found to be highly resistant to (35.29%) aminoglycoside group antibiotics, while low resistance to (8.33%) ampicillin-sulbactam was detected. *Staphylococcus* spp. were also found to be resistant to the (75%) tetracycline group, even though the low resistance to (8.69%) ceftiofur was found. *Actinomycetaceae* spp. were founded to be resistant to (100%) trimethoprim-sulfamethoxazole, (100%) tetracycline, (83.33%) enrofloxacin, and (72.72%) gentamicin, however, these bacteria were susceptible to ceftiofur. Gentamicin resistance was observed in 85.71% of bacteria belonging to the genus *Streptococcus* spp., while resistance to ceftiofur was detected at a rate of 14.28%. The distribution of antibiotic resistance bacterial families was represented in Table 2.

Table 2: Antibiotic resistance distribution of isolated bacterial families

Antibiotics*	E*nterobacteriaceae*		*Pasteurellaceae*		*Staphylococcus* spp.		*Actinomycetaceae*		*Streptococcus* spp.		*Enterococcus* spp.		*Alcaligenaceae*	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Gentamicin	9 (36)	16 (64)	5 (38.5)	8 (61.5)	9 (32.1)	19 (67.9)	8 (72.7)	3 (27.3)	6 (85.7)	1 (14.3)	3 (75)	1 (25)	0 (0)	2 (100)
Marbofloxacin	12 (48)	13 (52)	2 (15.4)	11 (84.6)	7 (25.9)	20 (74.1)	5 (45.5)	6 (54.5)	2 (40)	3 (60)	3 (75)	1 (25)	1 (50)	1 (50)
Enrofloxacin	12 (57.1)	9 (42.9)	4 (33.3)	8 (66.7)	5 (27.8)	13 (72.2)	5 (83.3)	1 (16.7)	1 (33.3)	2 (66.7)	3 (75)	1 (25)	1 (50)	1 (50)
Tetracycline	8 (100)	0 (0)	2 (50)	2 (50)	14 (66.7)	7 (33.3)	4 (100)	0 (0)	2 (66.7)	1 (33.3)	1 (100)	0 (0)	0 (0)	1 (100)
Ciprofloxacin	9 (45)	11 (55)	2 (20)	8 (80)	9 (34.6)	17 (65.4)	5 (55.6)	4 (44.4)	2 (40)	3 (60)	0 (0)	0 (0)	1 (50)	1 (50)
Amoxicillin Clavulanic Acid	13 (48.1)	14 (51.9)	2 (14.3)	12 (85.7)	5 (17.2)	24 (82.8)	2 (20)	8 (0)	4 (50)	4 (50)	2 (50)	2 (50)	0 (0)	2 (100)
Ampicillin Sulbactam	10 (40)	15 (60)	1 (8.3)	11 (91.7)	4 (17.4)	19 (82.6)	2 (18.2)	9 (81.8)	2 (28.6)	5 (71.4)	2 (50)	2 (50)	0 (0)	1 (100)
Trimethoprim-Sulfamethoxazole	18 (75)	6 (25)	3 (33.3)	6 (66.7)	11 (40.7)	16 (59.3)	8 (100)	0 (0)	4 (66.7)	2 (33.3)	4 (100)	0 (0)	1 (50)	1 (50)
Cefoxitin	4 (18.2)	18 (81.8)	1 (9.09)	10 (90.9)	5 (20)	20 (80)	1 (10)	9 (90)	0 (0)	4 (100)	2 (100)	0 (0)	1 (50)	1 (50)
Cefovecin	5 (35.7)	9 (64.3)	1 (20)	4 (80)	1 (9.1)	10 (90.9)	1 (20)	4 (80)	2 (66.7)	1 (33.3)	1 (50)	1 (50)	0 (0)	1 (100)
Ceftiofur	5 (20)	20 (80)	2 (15.38)	11 (84.6)	2 (8.7)	21 (91.3)	0 (0)	11 (100)	1 (14.3)	6 (85.7)	0 (0)	3 (100)	1 (50)	1 (50)
Tobramycin	3 (50)	3 (50)	0 (0)	3 (100)	4 (28.6)	10 (71.4)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)
Oxytetracycline	12 (80)	3 (20)	2 (28.57)	5 (71.4)	7 (100)	0 (0)	5 (71.4)	2 (28.6)	4 (100)	0 (0)	2 (100)	0 (0)	1 (100)	0 (0)
Neomycin	5 (100)	0 (0)	1 (100)	0 (0)	6 (100)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)
Spiramycin	3 (100)	0 (0)	0 (0)	1 (100)	0 (0)	3 (100)	1 (100)	0 (00)	0 (0)	0 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Penicillin	9 (100)	0 (0)	2 (66.7)	1 (33.3)	9 (90)	1 (10)	1 (25)	3 (75)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

*: The antibiotics were selected according to the sample types. Hence, some of the data missing for some isolates. R: Resistant, S: Susceptible

Discussion

The bacterial and fungal agents are routinely isolated in the diagnostic laboratory (Nocera et al., 2021; Kakooza et al., 2021; Jonker and Michel, 2021). Those studies have indicated not only single animal results but also antimicrobial resistance results of all samples from different origins. These reports are clearly essential to understanding and revealing antimicrobial resistance over the years. In this regard, the current study reports bacterial and fungal agent results isolated from different animal origins in the veterinary diagnostic laboratory between 2020 and 2021.

A study reported that the most common bacteria were coagulase-negative staphylococci (CNS) from ear samples of cats (Nocera et al., 2021). Similarly, CNS were isolated and identified from all ear samples in the current study. The antimicrobial resistance of CNS isolates was reported to be 64% against amoxicillin-clavulanic acid (Nocera et al., 2021). However, 17% of CNS in the current study was found to be resistant to amoxicillin-clavulanic acid. *Trueperella pyogenes* (*T. pyogenes*) were isolated from different clinical samples collected from cattle, sheep, goats, pigs, horses, dogs, and buffaloes (Ribeiro et al., 2015). The most common sample types of isolated *T. pyogenes* were reported as the mammary gland, abscess, and tissue samples of affected animals in the same study. In addition, those *T. pyogenes* isolates were highly resistant to (9.2%) tetracycline and (49.3%) trimethoprim-sulfamethoxazole (Ribeiro et al., 2015). It has been reported that the resistance rate of *T. pyogenes* isolated from uterine samples of cattle was 91.8% against trimethoprim-sulfamethoxazole (Adiguzel et al., 2021). Another study reported that *Escherichia coli* (*E. coli*) and *Klebsiella* spp. were isolated from the samples containing mastitis, wound, otitis, urinary tract, and respiratory tract samples from different animal origins. In the same study, *E. coli* was isolated from mastitis samples mostly, whereas *Klebsiella* spp. was isolated from wound swabs (Puvarajan et al., 2020). In contrast, *Pasteurella* spp. and *Staphylococcus* spp. isolated from wound swabs mostly, whereas *Staphylococcus* spp. was isolated from mastitis cases in the current study. In the same study, *Pseudomonas* spp. was isolated from urinary tract samples (Puvarajan et al., 2020), and even though *Alcaligenes* spp., methicillin-resistant *Staphylococcus felis*, and *E. coli* were isolated in the same samples in the current study. On the other hand, the antimicrobial resistance of *Staphylococcus* spp. isolated from tissue and joint samples were detected against (93.28%) tetracycline and (91.7%) penicillin (Puvarajan et al., 2020), which is similar to the current study results.

A study reported that 17 coagulase-positive staphylococci, two beta-hemolytic streptococci, 16 *Pseudomonas aeroginosa*, seven *Proteus mirabilis*, nine *Malassezia*

pachydermatis, and two *Candida* spp. were isolated from ear swab samples of dogs. In addition, they indicated that a 21.4% and 16.6% resistance rate was detected against chloramphenicol and gentamicin for all stains, respectively (Terziev & Urumova, 2018). Another study reported *Pasteurella* spp., *E. coli*, and *Proteus mirabilis* from 100 cats and 100 dogs' soral samples. Besides, the isolates were resistant to penicillin (11.53%) (Razali et al., 2020), in contrast to the current study result (33.3%).

Antimicrobial resistance is observed against almost all antibiotics in veterinary and human medicine (Hoang, et al., 2017). Recently, the increasing trend of antimicrobial resistance among bacteria due to their over and/or misuse of antibiotics for the treatment has been reported by investigators in previous studies (Srivastava et al., 2013; Nocera et al., 2021). Antimicrobial resistance is one of the most striking issues at the moment. Since antibiotic resistance spreads between bacteria, there will be an increase in bacterial-mediated diseases and clinical treatment failure, which is important for global public health (Adiguzel et al., 2021; Goulart et al., 2022; Baran et al., 2022). Similar to the previous report, moderately high antimicrobial resistance was detected in bacteria isolated in the current study.

Fungal agents were also reported in the previous study (Diren Sigirci et al., 2019). It has been reported that *Microsporum canis*, *Trichophyton* spp., *Microsporum gypseum, Trichophyton mentagrophytes, Microsporum nanum*, other *Microsporum* spp., and *Trichophyton tonsurans* were isolated from pet animals' skin lesion samples (Diren-Sigirci et al., 2019). However, *Aspergillus fumigatus*, *Penicillium* spp., and *Purpureocilium lilacium* were isolated from skin lesion samples collected from pet animals in the current study.

Conclusion

In summary, the current study emphasizes that the large, pet, and poultry animal samples were mostly submitted to the veterinary diagnostic laboratory between 2020 and 2021. An increasing trend of antimicrobial resistance was detected in the strains isolated from samples. These findings further emphasize that it is important to perform routine susceptibility testing in the veterinary diagnostic laboratory for the selection of appropriate antimicrobial therapy to prevent increasing antimicrobial resistance.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

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