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RESEARCH ARTICLE

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Respiratory viruses in the healthy middle ear and middle ear with otitis media with effusion

Bengul Durmaz[1] | Olkar Abdulmajed[1] | Rıza Durmaz[2] | Mehmet Koroglu[3] | Oguz Arı[4] | Serdal Celik[5] | Mahmut Tayyar Kalcioglu[5]

[1]Department of Medical Microbiology, Faculty of Medicine, Yuksek Ihtisas University, Ankara, Turkey

[2]Department of Medical Microbiology, Faculty of Medicine, Ankara Yıldırım Beyazıt University, Ankara, Turkey

[3]Department of Medical Microbiology, Faculty of Medicine, Sakarya University, Sakarya, Turkey

[4]Central Research and Application Center, Ankara Yildirim Beyazit University, Ankara, Turkey

[5]Department of Otolaryngology, Faculty of Medicine, Istanbul Medeniyet University, Istanbul, Turkey

Correspondence

Rıza Durmaz, Department of Clinical Microbiology, Faculty of Medicine, Ankara Yildirim Beyazit University, Ankara, Turkey.
Email: rdurmaz@ybu.edu.tr

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Abstract

To investigate the presence of respiratory viruses in the middle ear cavity of the individuals with a healthy middle ear and the children with otitis media with effusion (OME). A total of 72 middle ear samples were collected from 25 children with OME (Group 1) and 47 individuals with no middle ear disease (Group 2). Multiplex real-time polymerase chain reaction was used to investigate the presence of 20 different respiratory viruses. Virus results were compared with bacteriomes of the same populations. At least one respiratory virus was detected in 56% of the patients in Group 1 and 12.8% of the individuals in Group 2. The viral co-infection rate for Group 1 and 2 was 8% and 2.1%, respectively. In Group 1, adenovirus was the most frequently detected virus with a rate of 24%, either alone (16%) or concurrent with other viruses (8%), followed by influenza B (12%), rhinovirus, and bocavirus (8%) each. Parainfluenza 4, coronavirus OC43, and RSV A/B were detected in 4% of the sample each. In Group 2, rhinovirus was detected in two samples (4.3%) followed by adenovirus, coronavirus OC43, coronavirus E299, and coronavirus NL63 with a rate of 2.1% each. The detection rate of respiratory viruses was significantly higher in children aged 6 to 11 years. There was no positive association between virus and bacteria found in the middle ear cavity. The current study has provided comprehensive data indicating the presence of diverse respiratory viruses in the healthy middle ear cavity. Our results also suggest that respiratory viruses might have a contribution to OME pathogenesis.

KEYWORDS

middle ear cavity, otitis media with effusion, PCR, respiratory virus

1 | INTRODUCTION

Otitis media is a group of inflammatory diseases of the middle ear.[1] It is the second most common pediatric disease with a high rate of medical visits and antibiotic prescriptions following an upper respiratory infection.[2] Otitis media can be categorized as acute otitis media (AOM), chronic suppurative otitis media (CSOM), and otitis media with effusion (OME).[2,3] OMG is an accumulation of non-infected fluid in the middle ear cavity.[4] It is a multifactorial disorder; eustachian tube (ET) dysfunction, parental smoking, family history, increased number of siblings, and microbial, immunologic, genetic, and environmental factors might have a contribution to its pathogenesis.[5] It can lead to hearing loss, language, and developmental problems.[5,6] OME mainly affects children between the ages of 1 to 6.[4,5] Its point prevalence was reported as 20% among children aged 2 years.[5]

It is debated whether there is a microbiome in a healthy middle ear cavity that is related to the upper respiratory tract with the

ET.[7] As bacteria including *Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae*, and *Moraxella catarrhalis* and respiratory viruses have been identified from the middle ear samples collected from patients with otitis media, have also been detected from the upper respiratory tract (URT),[8–10] it is assumed that middle ear cavity might be colonized with the microorganisms coming from URT through ET.[9,11]

Multiplex polymerase chain reaction (PCR) showed the presence of diverse viruses in the samples collected from the middle ear cavity of the children with AOM.[9,12,13] In addition, next-generation sequencing technology identified diverse bacteriomes in the middle ear cavity of the patients with acute or chronic OM[9,14] and of the patients with OME,[15–18] and of the individuals with no middle ear diseases.[19–21] When compared to bacteria, there is a limited number of studies showing the viral genome in the microbiome of the middle ear cavity. PCR-based approaches revealed the presence of respiratory viruses including RSV, adenovirus, Influenza A and B viruses, coronaviruses, bocavirus, parainfluenza virus, enterovirus, and rhinovirus in the middle ear cavity of the patients with AOM,[9,10,22,23] OME,[24–26] and children with no middle ear disease.[24]

Although molecular approaches expanded our knowledge by providing more comprehensive data indicating the presence of bacteriome and virome in the middle ear cavity of patients having AOM, there is a limited number of studies on the composition of viruses in the middle ear cavity of patients with OME and the individuals with no middle ear disease. As far as we know, this is the second study carried out on middle ear samples collected from patients undergoing cochlear implantation. We were able to compare the virome of the middle ear cavity of the patients with OME and that of healthy individuals to discuss whether viruses contribute to the development of OME. Moreover, by comparing the middle ear virome with bacteriome of the same populations by reanalyzing of data available from our previous study, we highlight whether there is any interaction between bacteria and viruses for the colonization in the middle ear cavity.

2 | MATERIALS AND METHODS

2.1 | Samples

A total of 72 middle ear samples analyzed for bacteriome in our previous studies[15,21] were included in this study. Twenty-five of these samples were obtained from 25 children (between 1 and 9 years old) who had bi-lateral or unilateral persistent chronic OME for 6 months or longer (Group 1). The patients who had medical comorbidity, cleft lip and/or cleft palate, known craniofacial malformations, and otologic diseases other than OME were excluded from the study. Clinical and demographic characteristics of the patients with OME were detailed in Table 1. Forty-seven samples were taken from 47 individuals (38 children aged between 1 and 4 years, 9 adults aged between 18 and 62 years) with no middle ear disease, who underwent cochlear implantation (Group 2). The patient who had taken any antibiotics last month before the surgery were not included. All samples were kept at −32°C°C temperature until molecular study.

2.2 | Ethical considerations

The research protocols of the patients in Group 1 and Group 2 were approved by the University Clinical Research Ethical Committee with a protocol number of 2011/103 and 2018/0313, respectively. The protocols were performed in accordance with the Declaration of Helsinki.

2.3 | Nucleic acid extraction and amplification

All samples were vortexed for five seconds. Then, total nucleic acid was extracted from 400 µl of vortexed samples using the EZ1 Virus Mini Kit v2.0 (Qiagen) and Biorobot EZ1 equipment (Qiagen) following manufacturer's instruction (https://www.qiagen.com/us/resources/resourcedetail?id=ca13c92a-4b1b-4ced-9796-b0414a166803&lang=en).

A Fast track Diagnostic (FTD) respiratory pathogens 21 kit for the detection of 20 viruses and one bacterium (Fast Track Diagnostics) was used to test the presence of genomes of influenza virus A (IAV) and B (IBV), influenza A (H1N1) virus (swine-lineage), human parainfluenza viruses (HPIV-1, HPIV-2, HPIV-3, and HPIV-4), human coronaviruses (HCoV) NL63, 229E, OC43 and HKU1, human metapneumoviruses (HMPV-A and HMPV-B), human rhinovirus (HRV), human respiratory syncytial viruses (HRSV-A and HRSV-B), human adenovirus (HAdV), enterovirus (HEV), human parechovirus (HPeV), and human bocavirus (HBoV). Reverse transcription at 50°C for 15 min was performed to transcript targeted RNA to cDNA. Then, using the corresponding primers and double-labeled probe mixtures, five different multiplexes real-time PCR (RT-PCR) reactions in a total volume of 25 µl were done for each sample. The first reaction for IAV, IBV, IAV(H1N1) swl, and HRV; second reaction for HCoV NL63, 229E, OC43, and HKU1; third reaction for HPIV-2, HPIV-3, HPIV-4, and internal control (IC: equine arteritis virus); fourth reaction for HboV, HPIV-1, and HMPV-A and HMPV-B, and fifth reaction for HRSV-A and HRSV-B, HadV, EV, and HpeV. All multiplex real-time PCR reactions were done using on a Rotor-Gene 5plex HRM device (Qiagen). The amplification condition was as follows: enzyme activation at 94°C for 1 min following 40 cycles including 94°C for 8 s and 60°C for 1 min. Each run included a positive control (plasmid pool) for each tested virus, a negative control, and internal control (Equine arteritis virus, EAV). The results were evaluated following the kit's instructions. Before reporting the presence or absence of viral genome in the tested sample, positive results obtained from positive control and an internal control, and a negative result from negative control were recorded. When these criteria were met, the presence of specific viral genomes was detected by an increase in fluorescence observed from the relevant dual-labeled probe.

TABLE 1 Clinical and demographic characteristics of the patients with persistent chronic OME

Patient no	Age (years)	Gender	Uni or bi-lateral OME	Tympanogram type	Nature of effusion	Type of disease	Time of disease (months)
1	8	M	Unilateral	B	Mucous	OME	8
2	8	M	Bilateral	B	Mucous	OME	32
3	9	M	Bilateral	B	Mucous	OME	6
4	6	M	Unilateral	B	Mucous	OME	12
5	5	M	Bilateral	B	Mucous	OME	24
6	1	M	Bilateral	B	Mucous	OME	13
7	4	M	Bilateral	B	Mucous	OME	14
8	3	M	Bilateral	B	Mucous	OME	8
9	2	F	Bilateral	B	Mucous	OME	≥6
10	2	M	Bilateral	B	Mucous	OME	14
11	6	M	Bilateral	B	Mucous	OME	6
12	7	M	Unilateral	B	Mucous	OME	24
13	6	M	Bilateral	B	Mucous	OME	6
14	6	F	Bilateral	B	Mucous	OME	9
15	3	F	Bilateral	B	Mucous	OME	6
16	7	M	Bilateral	B	Mucous	OME	24
17	6	M	Bilateral	B	Mucous	OME	12
18	5	M	Bilateral	B	Mucous	OME	≥6
19	1	M	Unilateral	B	Mucous	OME	6
20	6	M	Unilateral	B	Mucous	OME	≥6
21	6	M	Unilateral	B	Mucous	OME	12
22	7	M	Bilateral	B	Mucous	OME	≥6
23	7	M	Bilateral	B	Mucous	OME	14
24	6	M	Bilateral	B	Mucous	OME	15
25	6	F	Unilateral	B	Mucous	OME	24

Abbreviation: OME, otitis media with effusion.

Any sample displaying an exponential amplification trace was considered as positive for one of the viruses targeted by the kit (https://doclib.siemens-healthineers.com/rest/v1/view?document-id=757096, accessed May 27, 2021).

2.4 | Statistical analysis

Chi-square test was used to analyze statistically significant differences for detected viruses between Group 1 and Group 2, between ages and genders. In addition, Fisher's exact test was used to find whether there are statistically significant correlations between viruses and the three main bacteria causing otitis media. For both tests, IBM SPSS (Version 26) software was utilized. A p value of ≤.05 was considered significant.

3 | RESULTS

Multiplex RT-PCR identified at least one virus in 14 (56%) of the 25 patients in Group 1 and 6 (12.8%) of the 47 individuals in Group 2. The difference between the total virus detection rates of these two groups was remarkable but not statistically significant ($p > .05$). No statistically significant difference was also observed between study groups for the distribution of any virus. The most prevalent virus in Group 1 was adenovirus with a rate of 24%, either alone (16%) or concurrent with other viruses (8%). The detection rate was 12% for influenza B and 8% for rhinovirus. Parainfluenza 4 and RSV A/B were detected in one sample (4%) each. Triple virus-infected samples included adenovirus + bocavirus + coronavirus OC43 ($n = 1$; 4%). The frequency of coexistence of adenovirus and bocavirus was 4%. Virus detection rate in Group 2 was 2.1% for adenovirus, coronavirus

TABLE 2 Viral detection in the middle ear samples of patients with OME and healthy middle ear

Viruses	No. (%) of middle ear samples of the patients with		*p* value
	OME ($n = 25$)	Healthy middle ear ($n = 47$)	
Adenovirus	4 (16)	1 (2.1)	.1875
Influenza A	1 (4)	0	.5
Influenza B	3 (12)	0	.1250
Coronavirus OC43	0	1 (2.1)	.5
Parainfluenza 4	1 (4)	0	.5
Bocavirus	0	0	1
Coronavirus E299	0	1 (2.1)	.5
Rhinovirus	2 (8)	2 (4.3)	.6875
Respiratory syncytial virus A/B	1 (4)	0	.5
Rhinovirus + coronavirus NL63	0	1 (2.1)	.5
Adeno + bocavirus + coronavirus OC43	1 (4)	0	.5
Adeno + bocavirus	1 (4)	0	.5
Total	14 (56)	6 (12.8)	>.05

TABLE 3 Age and gender distribution of the virus detected patients

	No. of patients	No. of the virus detected patients (%)	*p* value
Group 1: Patients with OME ($n = 25$)			
Age (years)			
0–2	3	1 (33)	.123
3–4	3	3 (100)	
5–11	19	10 (53)	
Gender			
Male	21	11 (52)	.396
Female	4	3 (75)	
Group 2: Patients with no middle ear disease ($n = 47$)			
Age (years)			
0–2	17	3 (18)	.243
3–4	21	3 (14)	
5–11	0	0 (0)	
≥18	9	0 (0)	
Gender			
Male	29	3 (10)	.446
Female	18	3 (16)	
Total ($n = 72$)			
Age (years)			
0–2	20	4 (20)	.019
3–4	24	6 (25)	
5–11	19	10 (53)	
≥18	9	0 (0)	
Gender			
Male	50	14 (28)	.949
Female	22	6 (27)	

OC43, coronavirus E299, and rhinovirus + coronavirus NL63 each. Sole rhinovirus was found in two individuals in group 2 (Table 2). Influenza A (H1N1), HPIV-1, HPIV-2, and HPIV-3, coronaviruses HKU1, HMPV-A and HMPV-B, and HPeV were not detected in any of the samples tested.

This study included 72 samples collected from male and female individuals at rates of 69.4% (50 samples), 30.6% (22 samples), respectively. The rate of virus-positive samples was 28% in males and 27% in females. Comparison of age groups showed that there were statistically significant differences between age groups. The highest detection rate (53%) was seen in children between the age of 5 and 11 years ($p < .05$). No virus was detected in the patient older than 17 years (Table 3).

To assess whether there is an association between virus and bacteriome on the side of colonization in the middle ear cavity, we compare the virus results with bacteriome in the same study populations. The bacteriome data of samples collected from the middle ear with OME[15] and healthy middle ear[21] were retrieved from the existing database established previously.

Associations between respiratory viruses and bacterial otopathogens, which are *S. pneumoniae*, *M. catarrhalis*, and *H. influenzae*, were analyzed on the 25 samples from patients with OME. Adenovirus (0% vs. 24%), influenza A (0% vs. 4%), influenza B (0% vs. 12%), parainfluenza 4 (4% vs. 0%), rhinovirus (4% vs. 4%), bocavirus (0% vs. 8%), and RSV A/B (0% vs. 4%) were detected in the *S. pneumoniae* positive ($n = 5$) and negative ($n = 20$) samples, respectively. Detection rates of these viruses in the *H. influenzae* positive ($n = 11$) and negative ($n = 14$) samples were as follows: 16% versus 8%, 0% versus 4%, 4% versus 8%, 0% versus 4%, 4% versus 0%, 8% versus 0%, 4% versus 0%, 4% versus 0%, respectively. Respiratory viruses were more frequently detected in the *M. catarrhalis* negative samples ($n = 20$) than positive samples ($n = 5$). Only adenovirus, influenza B, and rhinovirus were found in 4% of the five *M. catarrhalis* positive samples each. No statistically significant correlation was found for any bacterium-virus combination. In detail, of the 6 adenovirus positive samples, both adenovirus and *H. influenzae* were seen in 4 (16%), and adenovirus + *M. catarrhalis* in only one sample. The coexistence of adenovirus and *S. pneumoniae* was not found in any sample. Influenza virus A was detected in one sample without co-occurrence with any otopathogen. Influenza virus B was found in three samples, coexistence of influenza virus B and *H. influenzae*, and

influenza virus B and *M. catarrhalis* was 4% each. Parainfluenza 4 + *S. pneumoniae*, Rhinovirus + *M. catarrhalis* + *S. pneumoniae*, and Rhinovirus + H. *influenzae* were detected in one sample each. Rhinovirus was seen in two samples; dual infection with rhinovirus + *S. pneumoniae*, and rhinovirus + M. *catarrhalis* was observed in one sample each. Bocavirus was found just with *H. influenzae* in two samples. Dual infection with RSV A/B + *H. influenzae*, and coronavirus OC43 + *H. influenzae* was recorded in one sample each (Table 4).

In addition, the association between detection of viruses and the other two common bacteria, which were *H. aegyptius* and *Alloiococcus otitis*, in the patients with OME was analyzed. For the 15 *H. aegyptius* positive samples, the dual infection rate between *H. aegyptius* and adenovirus, bocavirus, rhinovirus, coronavirus OC43, and influenza B viruses was 20%, 13.3%, 13.3%, 6.7%, and 6.7%, respectively. Of the 16 *A. otitis* positive samples, three (18.8%) were also infected with adenovirus, one (6.3%) with coronavirus OC43, and one (6.3%) with rhinovirus.

Samples of the individuals undergoing cochlear implantation were also analyzed for the coexistence of virus and commonly detected bacteria. We did not detect any significant correlation between virus and bacteria found in the healthy middle ear. Rhinovirus, adenovirus, and coronaviruses (OC43, E299, NL63) were found in dual combination with bacteria in various ratios. Rhinovirus is most likely to be present as a co-infection with bacteria, followed by adenovirus and coronaviruses. For instance, dual infection with rhinovirus and *Propionibacterium acnes* was seen in 3 of the 47 *P. acnes* positive samples; adenovirus + *P. acnes* in one sample, coronavirus OC43, NL63, and E299 and *P. acnes* in one sample each (Table 5).

4 | DISCUSSION

Advances in molecular approaches have allowed us to identify the virome and bacteriome on/in different organs and tissues of humans.[27,28] A meta-transcriptomic analysis using the RNA-sequencing data revealed the presence of diverse viruses in several visceral organs.[27] However, there is only one study performed on a limited number of the sample ($n = 14$) indicating the presence of viruses in a healthy middle ear cavity.[24] The current study carried out on more samples ($n = 47$) from the individuals with no middle ear disease by using a multiplex RT-PCR kit, which was able to detect more diverse viruses than that used in the previous study (20 vs. 9 respiratory viruses, respectively) provided more comprehensive data to expand knowledge indicating the presence of virus in the middle ear cavity without the disease.

Multiplex RT-PCR test showed that 12.8% of the 47 healthy middle ears were colonized with five different viruses, including rhinovirus, adenovirus, coronavirus OC43, NL63, and E299. Rhinovirus had the highest detection rate (6.4%); other viruses were represented with one sample each. A previous study performed on 14 middle ear washes of patients undergoing cochlear implantation detected the virus in only three samples, two had enterovirus

TABLE 4 Association between viruses and the three otopathogenic bacteria detected in the 25 middle ear samples with OME

			S. pneumoniae			*H. influenzae*			*M. catarrhalis*		
			Present	Absent		Present	Absent		Present	Absent	
Total detected virus	Virus	Status	*n* (%)	*n* (%)	*p* value	*n* (%)	*n* (%)	*P* value	*n* (%)	*n* (%)	*p* value
6	Adenovirus	Absent	5 (20)	14 (56)	.2887	7 (28)	12 (48)	.35	4 (16)	15 (60)	1
		Present	0	6 (24)		4 (16)	2 (8)		1 (4)	5 (20)	
1	Influenza virus A	Absent	5 (20)	19 (76)	1	11 (44)	13 (52)	1	5 (20)	19 (76)	1
		Present	0	1 (4)		0	1 (4)		0	1 (4)	
3	Influenza virus B	Absent	5 (20)	17 (68)	1	10 (40)	12 (48)	1	4 (16)	18 (72)	.5043
		Present	0	3 (12)		1 (4)	2 (8)		1 (4)	2 (8)	
1	Parainfluenza	Absent	4 (16)	20 (80)	.2	11 (44)	13 (52)	1	5 (20)	19 (76)	1
		Present	1 (4)	0		0	1 (4)		0	1 (4)	
2	Rhinovirus	Absent	4 (16)	19 (76)	.3667	10 (40)	14 (56)	.44	4 (16)	19 (76)	.3667
		Present	1 (4)	1 (4)		1 (4)	0		1 (4)	1 (4)	
2	Bocavirus	Absent	5 (20)	18 (72)	1	9 (36)	14 (56)	.1833	5 (20)	18 (72)	1
		Present	0	2 (8)		2 (8)	0		0	2 (8)	
1	RSV A/B	Absent	5 (20)	19 (76)	1	10 (40)	14 (56)	.44	5 (20)	19 (76)	1
		Present	0	1 (4)		1 (4)	0		0	1 (4)	
1	Coronavirus OC43	Absent	5 (20)	19 (76)	1	10 (40)	14 (56)	.44	5 (20)	19 (76)	1
		Present	0	1 (4)		1 (4)	0		0	1 (4)	

Note: Percentages in Table were calculated by dividing the number of each parameter to total number of the sample ($n = 25$).

TABLE 5 Coexistence of viruses and bacteria commonly detected in the 47 healthy middle ears

Viruses	*P. acnes* (*n* = 47)	*A. otitis* (*n* = 23)	*T. otitidis* (*n* = 16)	*C. tuberculostearicum* (*n* = 24)	*R. solanacearum* (*n* = 44)	*R. mannitolilytica* (*n* = 37)	*R. insidiosa* (*n* = 31)	*P. puraquae* (*n* = 22)	*H. aegyptius* (*n* = 7)
Influenza B	0	0	0	0	0	0	0	0	0
Coronavirus OC43	1	0	0	1	1	1	1	1	0
Parainfluenza 4	0	0	0	0	0	0	0	0	0
Bocavirus	0	0	0	0	0	0	0	0	0
Adenovirus	1	1	1	1	1	0	0	0	0
Influenza A	0	0	0	0	0	0	0	0	0
Rhinovirus	3	2	1	0	2	2	1	0	2
RSV A/B	0	0	0	0	0	0	0	0	0
Coronavirus NL63	1	0	0	0	1	0	0	0	1
Coronavirus E299	1	1	0	0	1	1	0	0	0

Note: Propionibacterium acnes, Alloiococcus otitis, Truchella otitidis, Corynebacterium tuberculostearicum, Ralstonia solanocearum, Pelomonas puraquae.

(14.2%), and one had rhinovirus (7.1%).[24] To our knowledge, these two studies are the only studies that figures out the presence of viral genome in healthy middle ear cavity. Although we were not able to prove whether these PCR positive results indicate the presence of replicative virus in the samples, the presence of viral genome in the healthy middle ear cavity of some individuals can be considered as an indication that those individuals might be more likely to get otitis media. As indicated previously, respiratory viruses can result in inflammation generating host immune and inflammatory responses that lead to accumulation of fluid in the middle, increased middle ear pressure, and signs and symptoms of AOM developed.[12] As detected viruses in the healthy middle ear were like those identified in the upper respiratory tract,[9,10,29] like the suggestion indicating that bacteriome in the middle ear originated from the respiratory tract,[20] it can be suggested that viruses in the middle ear might be translocated from respiratory tract through the ET. On the other hand, as some previous studies showed the dissimilarity between the microbiome of the adenoid and the middle ear samples and these results made doubtful the microbial translocation theory,[16,18] viruses detected in healthy middle ear cavity might be present there as a resident of the ear virome. Further study, including samples collected from the respiratory tract and healthy middle ear cavity, is needed to clarify this theory. Using metagenomics sequencing and software for analysis of the virome can be more effective approaches by providing comprehensive data for the prevalence and diversity of viruses in a healthy middle ear cavity.

In comparison with the healthy middle ear, more diverse viruses with a high frequency were found in the middle ear samples of the patients having OME. More than half (56%) of the 25 children with chronic OME had at least one respiratory virus. Eight different respiratory viruses were identified either alone or with a combination. Adenovirus was the predominant virus found in a total of six samples (24%), either alone (16%) or in combination with other viruses (8%), followed by influenza B (12%), rhinovirus (8%), and bocavirus (8%). A PCR-based study performed to investigate influenza A, influenza B, adenovirus, and bocavirus on the 42 middle ear fluid samples from the 21 children having OME revealed that 11.9% of patients infected with viruses. Influenza A was the only virus reported.[26] In parallel to our results, a multiplex RT-PCR-based study used to investigate nine respiratory viruses (rhinovirus, influenza virus, picornavirus, syncytial respiratory virus, metapneumovirus, coronavirus, enterovirus, adenovirus, and bocavirus) revealed the presence of at least one virus in 51.3% of the 37 children with OME.[24] In that study, enterovirus was the predominant virus identified in 35.1% of children; other viruses were human metapneumonia virus (13.5%), respiratory virus (5.4%), and bocavirus (2.7%). Another PCR-based study carried out on 100 children to analyze the association of rhinovirus, RSV, and coronavirus infections with OME detected viral RNA in the 30 middle ear samples. Rhinovirus RNA was reported in 19 (63.3%) samples, followed by RSV RNA (8%) and coronavirus (3%).[25] Due to differences between the used PCR kit contents and goals of the studies, there was no concordance between the results indicating the predominance of respiratory viruses detected in the patients with

OME.[24–26] To our results, adenovirus, followed by influenza B, rhinovirus, and bocaviruses were more likely to cause OME. As OME is a multifactorial process,[5] along with the presence of diverse respiratory viruses in the middle ear of the patients with OME, as was discussed previously,[12] viral load can be another factor that might influence the development of OME. As the multiplex RT-PCR method used in the current study was not a quantitative approach, we were not able to compare the viral loads in the samples of our study population. It was also indicated that the frequent viral URT infections are another risk factor for the development of OME.[30,31] Together with previous study and our data, it can be suggested that there might be a causal relationship between the respiratory viruses and OME pathogenesis. Nevertheless, as the presence of viral nucleic acid in the middle ear may not indicate virus replication, further studies, which should be carried out on more samples using virus isolation techniques, are needed to support this suggestion.

As for analysis in terms of the virus detection rates between age and gender groups, it was found that children between the ages of 5 to 11 had a significantly high positivity rate. No virus was detected in the middle ear samples of adults. Although the number of samples collected from an adult was limited, our results were in constant with the previous studies, which identified respiratory viruses in the middle ear cavity of the pediatric patients with AOM[9,10] and with OME.[16,24] Consistently with a previous study indicating a similar virus detection rate for male (53%) and female (47%) of the children,[32] the virus detection rates were similar between female and male for our study group (27.3% vs. 28%). From these results, it can be concluded that being male or female gender may not have a contribution of the respiratory virus related OME development. On the other hand, the high detection rate of respiratory viruses in the children having also a high OME incidence indicates that there is a possible relation between respiratory virus related OME and being in the child age.

This is the second study that compares the presence of respiratory viruses and bacteriome including otopathogens in the middle ear cavity of the patients having OME and healthy middle ear. In our study, RSV, bocavirus, and coronaviruses were detected frequently with *H. influenzae*, parainfluenza virus with *S. pneumoniae*, adenovirus and influenza B with *H. influenzae* and *M. catarrhalis*, rhinovirus with *S. pneumoniae* and *H. influenzae*. Although the study population was different from ours, a previous study carried out on children with AOM showed that RSV was found frequently with *H. influenzae* (48%), parainfluenza virus with *M. catarrhalis* (60%), and influenza viruses with *S. pneumoniae* (100%).[32] Mainly, in the current study a negative interaction was detected between viruses and bacterial species identified in Group 1 and 2. A remarkable positive association was only recorded between adenovirus and *H. influenzae*. These findings indicate that there is mainly a negative association between bacteria and viruses concerning colonization in the middle ear cavity. Like our results, a previous study indicated that a positive association was only seen between *S. pneumoniae* and adenovirus in hypertrophic adenoids of patients with OME. In that study, it was also noted that no significant association was observed between virus and bacteria in the adenoid or middle ear cavity.[24] These results suggest that there is not a synergistic interaction between virus and bacteria in OME pathogenesis.

5 | CONCLUSIONS

The current study carried out on a considerably high number of samples confirmed the presence of a viral genome in the middle ear cavity without the disease. Detection of a more diverse and high prevalence of viruses in the middle ear cavity of the patients with OME than those with no middle ear disease might be considered that there is a causal relationship between respiratory viruses and OME pathogenesis. Further studies are needed regarding the clinical roles of each virus. The no remarkable association observed between viruses and bacteria in the middle ear cavity suggests there is no or a limited interaction between virus and bacteria on OME pathogenesis.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Bengul Durmaz: *management and coordination responsibility for the research activity planning and execution; critical review of the manuscript*. Ulker Abdulmajet: *conducting a research and investigation process; writing the initial draft, evaluation of the experimental data*. Rıza Durmaz: *Formulation and evaluation of research goals and aims; interpretation of data, preparation of the manuscript, critical review of the manuscript, and final approval of the version to be published*. Oguz Ari: *writing the initial draft, analyses, and interpretation of the experimental data*. Mehmet Koroglu: *performing experiments, analyses results, and review of the manuscript*. Serdal Celik and Mahmut Tayyar Kalcioglu: *acquisition of patient data; selection of the study groups, collection, storage, and sending of samples to the laboratory; interpretation of results and review of the manuscript*.

ORCID

Bengul Durmaz https://orcid.org/0000-0002-4519-8730
Olkar Abdulmajed https://orcid.org/0000-0002-6420-6185
Rıza Durmaz https://orcid.org/0000-0001-6561-778X
Mehmet Koroglu https://orcid.org/0000-0001-8101-1104
Oguz Arı https://orcid.org/0000-0003-4930-5035
Serdal Celik https://orcid.org/0000-0001-8469-1547
Mahmut Tayyar Kalcioglu https://orcid.org/0000-0002-6803-5467

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