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Microorganisms isolated from thermoplastic masks and storage racks in head and neck cancer patients with radiation dermatitis

Özlem Koca[1✉], Timur Koca[2], Rahmi Atil Aksoy[2] & Aylin Fidan Korcum[2]

Healthcare-associated infections (HAIs) remain a critical public health issue, as they contribute to prolonged treatment duration, increased healthcare costs, and heightened risks of morbidity and mortality. In head and neck cancer patients undergoing radiotherapy, thermoplastic masks (TMs), which come into direct contact with the skin, represent a potential vector for infection. Additionally, the storage racks where these masks are kept may also facilitate microorganism transmission. Our study aimed to isolate and identify microorganisms from infective skin lesions secondary to radiation dermatitis in head and neck cancer patients, as well as from the TMs and storage racks, and to evaluate the antibiotic resistance profiles of the isolated microorganisms. The study included 71 locally advanced head and neck cancer patients who underwent radiotherapy between August 2022 and November 2023. Patients were monitored daily, and their skin evaluations were made according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Grade 2 and 3 radiodermatitis was observed in 29 of these 71 patients. Samples were collected using sterile swabs from the skin lesions on the head and neck area, the inner surfaces of the TM, and the storage rack from 29 patients. Samples were inoculated into enrichment and selective media. After the growing microorganisms were identified, antimicrobial susceptibility was evaluated using conventional methods and automated systems according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. At least one type of microorganism was isolated from the skin samples of infected patients, and double growth was detected in two patients. Among the samples, 2 were methicillin-resistant coagulase-negative *Staphylococcus* (MRCNS), 1 was methicillin-resistant *Staphylococcus aureus* (MRSA), 1 was *Candida albicans* (*C. albicans*), 15 were methicillin-sensitive coagulase-negative *Staphylococcus* (MSCNS), 1 was methicillin-sensitive *Staphylococcus aureus* (MSSA), 1 was *Bacillus* subspecies (*Bacillus* spp.) and 3 were *Corynebacterium diphtheriae* (*C. diphtheriae*). Coagulase-negative *Staphylococcus* strains were isolated from the skin of 14 of 19 patients with grade 2 radiation dermatitis, whereas CNS strains were isolated from only 2 of 10 patients with grade 3 radiation dermatitis. Among the gram-negative bacteria, 3 *Pseudomonas aeruginosa* (*P. aeruginosa*), 2 *Enterobacter cloacae* (*E. cloacae*), 1 *Klebsiella pneumoniae* (*K. pneumoniae*) and 1 *Moraxella catarrhalis* (*M. catarrhalis*) strain were isolated. Sixteen (55.1%) of the TMs used in 29 patients and 20 (68.9%) of the storage racks harbored microorganisms, including HAI agents and flora bacteria. Bacteria colonize TMs and storage racks where they are risk factors for secondary skin infections in radiation dermatitis lesions that develop on the skin of head and neck cancer patients. Decontamination procedures should be meticulously applied to surfaces such as TMs and their storage racks during the course of radiotherapy.

Keywords Radiation dermatitis, Colonization, Risk factors, Secondary skin infection, Thermoplastic mask

Abbreviations

Bacillus spp.	*Bacillus* subspecies
C. albicans	*Candida albicans*

[1]Department of Medical Microbiology, Antalya Training and Research Hospital, University of Health Sciences, Konyaaltı, Antalya, Turkey. [2]Department of Radiation Oncology, Akdeniz University School of Medicine, Antalya, Turkey. ✉email: drozlemkoca@gmail.com

C. diphtheriae	*Corynebacterium diphtheriae*
CNS strains	Coagulase-negative *staphylococcus* strains
CTCAE	Common terminology criteria for adverse events
E. cloacae	*Enterobacter cloacae*
EMB	Eosin methylene blue
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GNRs	gram-negative rods
Gy	Gray
HAI	Healthcare-associated infections
K. pneumoniae	*Klebsiella pneumoniae*
M. catarrhalis	*Moraxella catarrhalis*
MRCNS	Methicillin-resistant coagulase-negative *Staphylococcus*
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSCNS	Methicillin-sensitive coagulase-negative *Staphylococcus*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
P. aeruginosa	*Pseudomonas aeruginosa*
TMs	Thermoplastic masks
UV	Ultraviolet
WHO	The World Health Organization

Nosocomial infections negatively affect the results obtained from healthcare services. The World Health Organization estimates that healthcare-associated infections (HAIs) affect hundreds of millions of patients each year and cause major economic losses[1]. Many national health organizations continue to work to take precautions to reduce the incidence of hospital infections[2]. Radiation dermatitis is a frequently observed side effect of radiation therapy and can cause interruptions in patient treatment. This situation becomes even more complicated, especially in infected dermatitis patients, and treatment interruptions are decreasing the efficacy of radiotherapy. Hospital-acquired infections are thought to be an important source of infections in patients who develop radiation dermatitis. In many studies, HAI pathogens such as *S. aureus*, MRSA, *enterococci* and gram-negative rods (GNRs) have been isolated from accessories used by personnel, such as laboratory coats and ties; textile products such as sheets and curtains; stethoscopes; electronics; and other medical devices. It has also been determined that HAI pathogens can adhere to the surfaces of medical devices, survive and be transmitted through contact. Catheter-related bloodstream and urinary system infections, ventilator-associated pneumonia and skin infections are the main clinical conditions[3–5]. These devices include heat-molded thermoplastic devices used in radiotherapy[6]. Thermoplastic masks (TMs) are individually shaped with heat to ensure immobilization in patients with head and neck cancer[7]. TMs can become contaminated due to improper use by healthcare professionals, such as not using a barrier or due to exposure to environmental bacteria on open racks where they are stored between treatment fractions[8]. Additionally, the risk of HAI is increased in patients receiving radiation therapy due to the immunosuppressive nature of cancer treatments[9]. Radiation dermatitis is a common side effect of radiation therapy and increases the risk of infection by disrupting skin integrity and causing moderate to severe skin lesions in 85–95% of patients[10]. To reduce the risk of nosocomial infection, it is recommended that smooth and highly hydrophobic and hydrophilic surfaces be chosen to prevent bacterial adhesion[11,12]. In addition, disinfection and UV radiation applied to TMs significantly reduce the number of surface bacterial colonies and the incidence of HAI[13]. In the present study, traditional *TMs* consisting of synthetic polymers were used for patient immobilization. This study aims to enable clinicians' predictions by evaluating the isolation, antibiotic resistance rates and risk potentials of bacteria colonizing the lesions of head and neck cancer patients who develop radiation dermatitis, TMs and storage racks where they are stored.

Methods

The study was reviewed and approved by the institutional ethics board of the Akdeniz University School of Medicine (Decision number: KAEK-441, Date: 20.07.2022) and followed the ethical principles of the 1964 Declaration of Helsinki. All participants provided written informed consent. Seventy-one head and neck cancer patients who underwent radiotherapy at our institution were evaluated between August 2022 and November 2023. Radiation oncologists scored all incidences of acute radiation dermatitis daily during treatment utilizing the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0[14]. Patients with Grade 2 or Grade 3 radiation dermatitis in the neck region were included in this study. Forty-two patients with Grade 1 dermatitis who developed mild erythema and whose skin barrier was not damaged were excluded from the study. Grade 4 radiation dermatitis was not detected in any patient. Swab samples were taken from the skin lesions in the neck area, the inner surfaces of the TM, and the storage racks of a total of 29 patients who developed grade 2 or grade 3 radiation dermatitis.

A gram stain was performed on each sample and sent to the microbiology laboratory during routine culture procedures. Samples were inoculated onto 5% sheep blood agar (BA), chocolate agar (CA), Eosin Methylene Blue agar (EMB), and Saboraud agar (SA) (RTA, Turkey) and incubated at 37°C for 18–24 h. Our studies identified the growing microorganisms using conventional methods and Matrix Assisted Laser Desorption-Ionization Time-of-Flight Mass Spectrometer (MALDI-TOF MS) (Biomerieux, France). Antibiotic susceptibility tests were performed via the VITEK 2 (Biomerieux, France) automated system and disc diffusion method. Colonies were inoculated on Müller-Hinton agar (RTA, Turkey) at 0.5 McFarland dilution to detect antibiotic susceptibility. Antibiotic discs were placed and incubated at 37 °C for 18–24 h. Diameters of the non-growth zone around the antibiotic discs were measured and sensitivities were evaluated according to the EUCAST criteria[15]. The workflow is presented in Fig. 1. *Escherichia coli* ATCC 25,922, *Pseudomonas aeruginosa* ATCC 27,853, *Enterococcus faecalis*

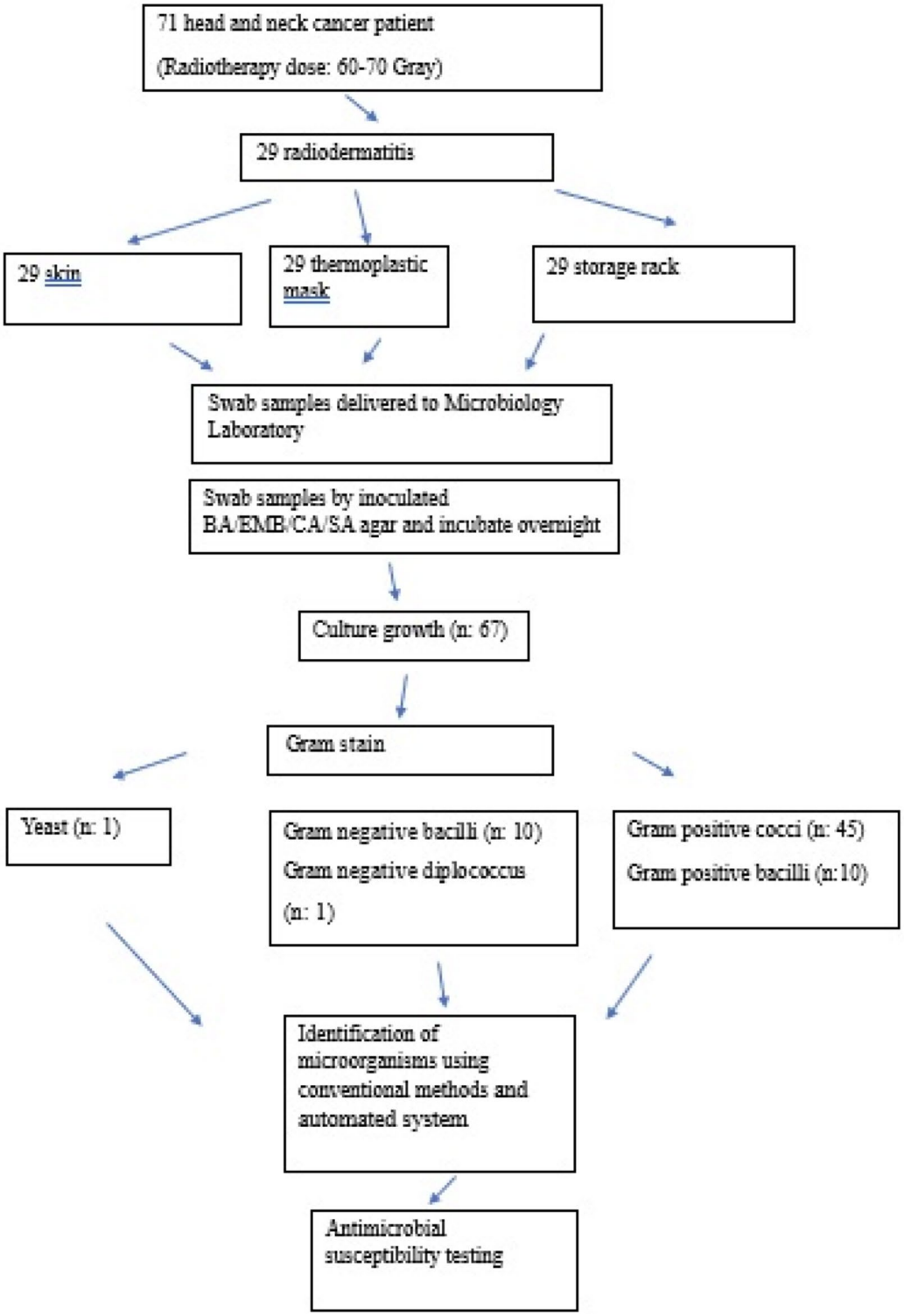


Fig. 1. Sample processing algorithm.

ATCC 29,212, *S. aureus* ATCC 29,213 and *Klebsiella pneumoniae* 700,603 (ESBL) control strains were used to evaluate the media and colonies.

Statistical analyses

Statistical analyses were performed utilizing IBM SPSS Statistics version 24.0 software (Armonk, NY: IBM Corp. 2016), and post hoc power analysis was conducted with the G-power tool. Descriptive statistics were calculated to present baseline characteristics. Categorical variables were analyzed utilizing the chi-square test, and p values

less than 0.05 were considered to indicate statistical significance. Post hoc power analysis showed that the total sample size ($N = 29$) was adequate to detect high effects ($f = 0.70$, $p \leq 0.05$, power = 0.84).

Results

The study included 29 patients, seven of whom were female and 22 of whom were male. The median age of the patients was 58 years (range, 19–79). The median total dose of radiotherapy administered was 70 Gy (Gy), with a range of 60–70 Gy. Most of these patients (82.7%) received concurrent chemotherapy with radiotherapy. Cisplatin was administered to 19 patients, cetuximab to three patients, and carboplatin-paclitaxel to two patients. Grade 2 radiation dermatitis was observed in 19 patients, while grade 3 radiation dermatitis was observed in 10 patients. Patient characteristics are shown in Table 1.

When the swab samples were evaluated, a single microorganism was detected in 27 patients, and more than one microorganism was found on the skin in two patients. While coagulase-negative *Staphylococcus* (CNS) and *M. catarrhalis* strains grew in one patient with more than one microorganism, *P. aeruginosa* and *C. albicans* growth were detected in the other patient. Additionally, simultaneous reproduction was detected in 16 (55.1%) TMs and 20 (68.9%) of the storage racks. HAI agents, such as gram-negative rods, MRCNS and MRSA, were isolated from the skin, TMs and storage racks. The data are presented in Table 2.

From the skin swab samples of the patients, 2 MRCNS strains, 1 MRSA strain, 1 *C. albicans* strain, 15 MSCNS strains, 1 MSSA strain, 1 *Bacillus* spp. strain and 3 *C. diphtheriae* strains were isolated. Among the gram-negative bacteria, 3 *P. aeruginosa*, 2 *E. cloacae*, 1 *K. pneumoniae* and 1 *M. catarrhalis* strain were isolated. The microorganisms isolated from the TMs included 2 MRCNS strains, 1 MRSA strain, 1 *Bacillus* spp., 2 *C. diphtheriae strains*, 1 *E. cloacae strain*, 8 MSCNS strains and 1 MSSA strain. The microorganisms isolated from

Characteristic	*N*	%
Age		
≤60 years	17	58.6
>60 years	12	41.4
Gender		
Female	7	24.1
Male	22	75.9
[1]ECOG-PS		
0–1	24	82.7
2	5	17.3
Diagnosis		
Larynx cancer	11	37.9
Oropharynx cancer	4	13.8
Oral cavity cancer	4	13.8
Hypopharynx cancer	3	10.3
Nasopharynx cancer	2	6.9
Others	5	17.3
Histopathology		
Squamous cell carcinoma	25	86.2
Others	4	13.8
Radiotherapy total dose		
60 [2]Gy	3	10.3
63 [2]Gy	1	3.4
66 [2]Gy	7	24.1
70 [2]Gy	18	62.2
Concurrent chemotherapy		
Yes	24	82.7
No	5	17.3
Chemotherapy regimen		
Cisplatin	19	79.1
Cetuximab	3	12.5
Carboplatin–paclitaxel	2	8.4
Radiation dermatitis		
Grade 2	19	65.5
Grade 3	10	34.5

Table 1. Patient characteristics. [1]ECOG-PS; Eastern Cooperative Oncology Group Performance Status. [2]Gy; Gray.

	Skin (n: 31)		Thermoplastic mask (n: 16)		Storage rack (n: 20)	
[1]MSCNS	15	(48.4)	8	(50.0)	12	(60.0)
[2]MRCNS	2	(6.5)	2	(12.5)	2	(10.0)
[3]MSSA	1	(3.2)	1	(6.25)	0	–
[4]MRSA	1	(3.2)	1	(6.25)	0	–
Bacillus spp.	1	(3.2)	1	(6.25)	2	(10.0)
C. diphtheriae	3	(9.7)	2	(12.5)	1	(5.0)
P. aeruginosa	3	(9.7)	0	–	1	(5.0)
E. cloacae	2	(6.5)	1	(6.25)	1	(5.0)
K. pneumoniae	1	(3.2)	0	–	1	(5.0)
M. catarrhalis	1	(3.2)	0	–	0	–
C. albicans	1	(3.2)	0	–	0	–

Table 2. Number of bacterial isolates by sampled area, n (%). [1]*MSCNS* methicillin-sensitive coagulase-negative *Staphylococcus*, [2]*MRCNS* methicillin-resistant coagulase-negative *Staphylococcus*, [3]*MSSA* methicilline sensitive *Staphylococcus aureus*, [4]*MRSA* methicillin resistant *Staphylococcus aureus*.

	[1]CNS Strains	Other	Total	*p* value
Grade 2	14 (73.7)	5 (26.3)	19	0.016
Grade 3	2 (20.0)	8 (80.0)	10	

Table 3. Isolated microorganisms from the skin of the patients with grade 2 and grade 3 radiation dermatitis, n (%). [1]*CNS* coagulase-negative *Staphylococcus*. *The patient in whom both CNS and *M. catarrhalis* strains were isolated was evaluated in the "other" group.

	Skin (n: 17)		Thermoplastic mask (n: 10)		Storage racks (n: 14)	
Cefoxitin	2	(11.8)	2	(20.0)	2	(14.3)
Ciprofloxacin	3	(17.6)	3	(30.0)	3	(21.4)
Erythromycin	3	(17.6)	3	(30.0)	6	(42.8)
Clindamycin	–		–		1	(7.1)
Fucidic asid	1	(5.8)	1	(10.0)	5	(35.7)
[1]SXT	2	(11.8)	1	(10.0)	2	(14.3)
Vancomycin	–		–		–	
Teicoplanin	–		–		–	
Linezolid	–		–		–	

Table 4. Antibiotic resistance rates for coagulase-negative *Staphylococcus*, n (%). [1]*SXT* Trimethoprim sulfamethoxazole.

the storage racks were 2 MRCNS, 2 *Bacillus* spp., 1 *C. diphtheriae*, 1 *P. aeruginosa*, 1 *E. cloacae*, 1 *K. pneumoniae* and 12 MSCNS.

The most common microorganisms isolated from the swab samples were CNS strains, which were detected on the skin of 17 patients, on the TM of 10 patients, and on the storage rack of 14 patients. Coagulase-negative *Staphylococcus* strains were isolated from the skin of 14 of 19 patients with grade 2 radiation dermatitis, whereas CNS strains were isolated from only two of 10 patients with grade 3 radiation dermatitis (Table 3). A statistically significant difference was observed between the Grade 2 and Grade 3 radiation dermatitis groups in terms of the isolation of CNS strains ($p = 0.016$). The patients in whom the CNS and *M. catarrhalis* strains were isolated were evaluated in the "other" group. The resistance rates of the CNS strains to antibiotics are shown in Table 4.

According to the antibiotic sensitivity tests, vancomycin, teicoplanin and linezolid resistance was not detected in *S. aureus* strains, and cefoxitin resistance was detected in two strains (Table 5). Carbapenem resistance was detected in 2 *P. aeruginosa* strains and 1 *K. pneumoniae* strain. No colistin resistance was detected in *P. aeruginosa*, *K. pneumoniae* or *E. cloacae* strains (Table 6).

Coagulase-negative staphylococcal strains were isolated from the skin and TM of 10 patients, from the skin, TM and storage racks of eight patients, and from the skin and storage racks of 4 patients. Moraxella catarrhalis

	Cefoxitin	Ciprofloxacin	Vancomycin	Teicoplanin	Linezolid
[1]MSSA (n: 2)	–	–	–	–	–
MRSA (n: 2)	2 (50%)	2 (50%)	–	–	–

Table 5. *Staphylococcus aureus* antibiotic resistance rates, n (%). *[1]MSSA* methicilline sensitive *Staphylococcus aureus*, *MRSA* methicillin resistant *Staphylococcus aureus*.

	Pseudomonas aeruginosa (n: 4)		*Klebsiella pneumoniae* (n: 2)		*Enterobacter cloacae* (n: 4)	
[1]AMC	2	(50.0)	–		2	(50.0)
Cefazolin	2	(50.0)	–		1	(25.0)
Gentamicin	1	(25.0)	1	(50.0)	1	(25.0)
Amikacin	1	(25.0)	1	(50.0)	1	(25.0)
Ciprofloxacin	2	(50.0)	–		1	(25.0)
Levofloxacin	2	(50.0)	–		1	(25.0)
[2]TZP	2	(50.0)	–		1	(25.0)
Imipenem	2	(50.0)	–		1	(25.0)
Meropenem	1	(25.0)	–		1	(25.0)
Colistin	–		–		–	
[3]SXT	2	(50.0)	–		1	(25.0)
Tigecycline	–		1	(50.0)	1	(25.0)

Table 6. Gram-negative bacteria antibiotic resistance rates, n (%). *[1]AMC* amoxicillin/clavulanic acid, *[2]TZP* piperasilin–tazobaktam, *[3]SXT* trimethoprim sulfamethoxazole.

and yeast colonies were isolated only from the skin. The growth distributions of other microorganisms on the surfaces are shown in Fig. 2.

Discussion

Nosocomial infection agents are most commonly found in the oral mucosa, followed by the skin and respiratory system. TMs are at risk of skin infection in patients with head and neck tumors, such as nasopharyngeal carcinoma, who receive radiotherapy[13]. To date, many studies have been conducted on the contamination of many frequently used surfaces in hospitals by various microorganisms and their causative agents[3]. It has been shown that biomaterial polymers used in head and neck surgery increase bacterial adhesion and support colonization[6]. CNS, S. aureus, Bacillus spp., and Viridans streptococci, along with several GNRs, were isolated from thermolabile plastic splints used in burn patients[10]. It has been shown that masks contain many different types of bacteria during radiotherapy. *Staphylococcus aureus*, *Bacillus* spp., CNS, and gram-negative rods are frequently isolated microorganisms[3,8]. In a study, the upper respiratory tract pathogens *Streptococcus pneumoniae*, *Haemophilus influenzae* and *P. aeruginosa* were isolated from the surfaces of TMs in a composite biomaterial structure containing glass granules, and it was noted that these factors have different binding abilities[6]. *Pseudomonas aeruginosa*, *K. pneumoniae* and Enterobacter sp. It has been shown in various studies that strains cause invasive HAIs, especially in patients with suppressed immune systems, by settling on the inner surfaces of catheters and other permanent devices via their biofilm-forming properties[16–19]. In our study, CNS strains were the most common microorganisms isolated from skin swab samples of patients who developed secondary infection on the basis of grade 2 radiation dermatitis. In patients with Grade 3 radiation dermatitis, the microorganisms isolated from skin samples were CNS agents and HAI agents, such as MRSA, MRCNS and GNR. High-touch surfaces such as storage racks, furniture surfaces and mobile medical equipment are among the most common sources of pathogenic microorganisms that cause nosocomial infections and play important roles in the chain of transmission[20–24]. In our study, 20 (68.9%) of the storage racks harbored microorganisms, including HAI agents and flora bacteria.

The transfer of microorganisms from a contaminated mask surface is related to adhesion strength and migration from contact points to the skin. Therefore, the transfer of some bacteria, such as those from the CNS, which is the skin flora, from the patient's skin to the mask surface should also be considered. Improper use of protective equipment by healthcare workers and open storage racks also increases the transmission of CNS strains[8]. In a study involving three separate radiotherapy centers, CNS strains, which are members of the flora, were among the most frequently isolated strains from TMs[3]. In our study, CNS strains were the most common microorganisms isolated from the swab samples taken from the skin, TM and storage racks and were found to be compatible with the literature.

In our study, the *C. albicans* strain was isolated from the skin swab sample of only one patient and could not be detected from the surface. However, in the study of Estvil et al., 84 *C. albicans* strains were isolated from

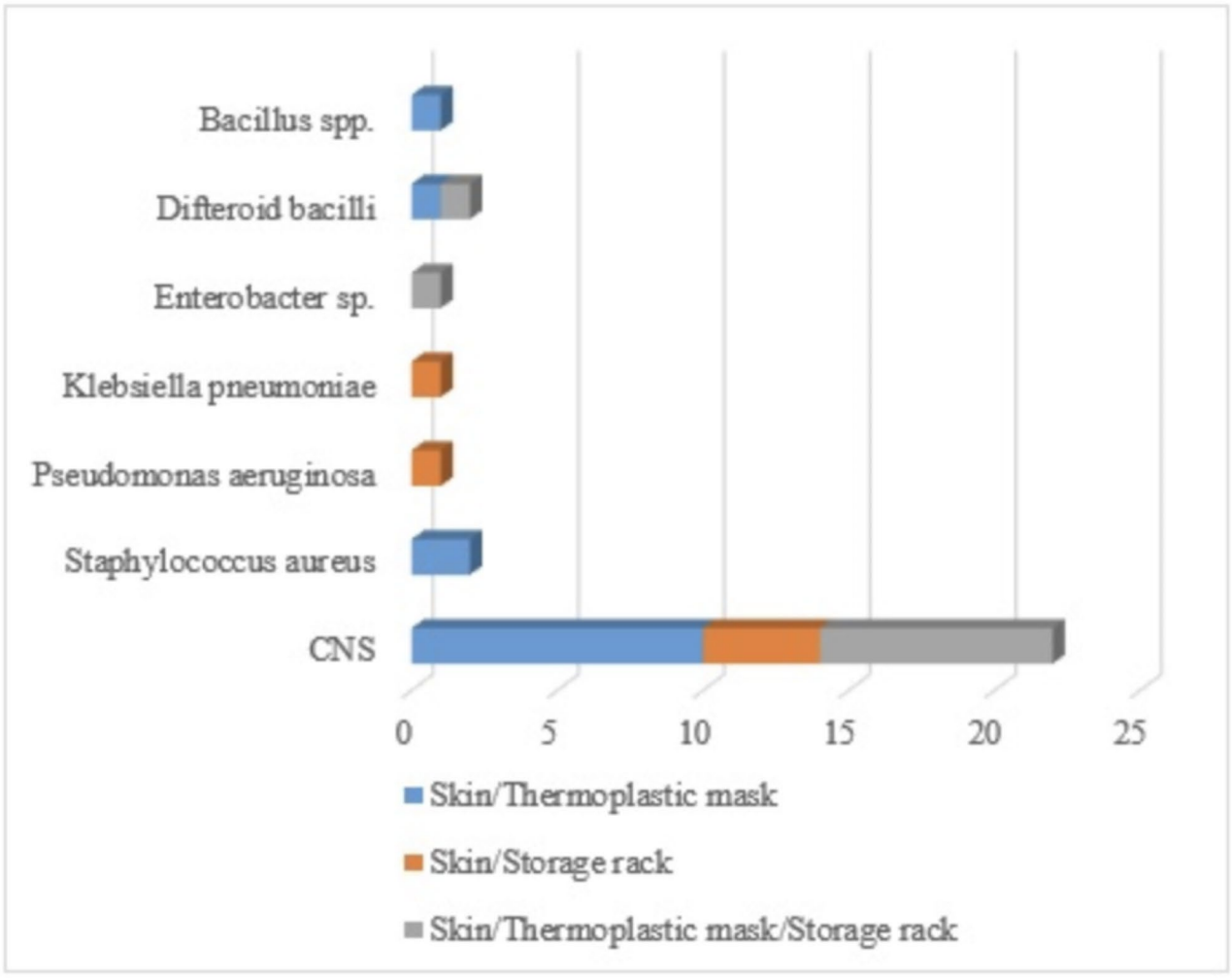


Fig. 2. Microorganisms isolated from sample areas.

TMs made of polyvinyl chloride (PVC), polyurethane and Teflon, and all of them were found to form biofilms on surfaces but to have different binding abilities. For this reason, they reported that *C. albicans* strains are the most common fungal pathogens in intensive care units and pose a significant risk for HAI[25]. It was thought that the reason why our study had different results regarding yeast colonization in masks was that the material of the TMs consisted of synthetic polymers. Similarly, some studies isolated a small number of C. albicans strains[10,26].

In our study, four *Bacillus* spp. strains were isolated from the same patient with Grade 2 lesions, one from the skin, the other from the TM and 2 from the storage racks. In their study, Ravine et al. also isolated two *Bacillus* species from masks belonging to four different patients. The authors also examined the adhesion properties and life spans of the bacillus strains. The authors noted that *Bacillus* species can adhere via spore structures, survive in non-nutrient environments for four weeks, infect patients wearing contaminated masks, and may cause infection depending on the number of spores[27].

While numerous studies have explored this subject, to our knowledge, this is the first investigation focused on the isolation and identification of microorganisms from infected skin lesions, thermoplastic masks (TMs), and storage racks associated with radiation dermatitis in patients with head and neck cancer. This study demonstrated that the storage racks for patient thermoplastic masks serve as critical reservoirs for contamination. Additionally, the antibiotic resistance profiles of the isolated microorganisms are evaluated. However, it is important to acknowledge the limitations of our study. A notable limitation is the lack of assessment regarding the life spans of the microorganisms and their capacity for surface adhesion. Nonetheless, regardless of type, thermoplastic medical and/or tissue engineering devices are often prone to microbial colonization, causing patient infections associated with medical biofilm formation[11].

Conclusion

TMs are commonly used for immobilization in head and neck cancer patients undergoing radiotherapy. Our study identified that bacterial colonization of TMs and the storage racks where they are kept presents a potential risk for secondary skin infections in these immunocompromised patients. These findings from our hospital highlight the importance of addressing surface-to-surface and surface-to-patient contamination. To mitigate this risk, rigorous decontamination protocols should be implemented for both individualized TMs and their storage racks throughout radiotherapy sessions.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

Ö.K.: Conducted experimental procedures, Planned the research methodology, Conceptualization, Data Collection, Investigation, Analysis and Writing Original Draft. T.K.: Conceptualization, Analysis, Supervision, Writing, Review & Editing. R.A.A.: Conceptualization, Supervision, Review & Editing. A.F.K.: Methodology, Writing, Review & Editing. All authors read and approved the article.

Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
The study was reviewed and approved by the institutional ethics board of the Akdeniz University School of Medicine (Decision number: KAEK-441, Date: 20.07.2022) and followed the ethical principles of the 1964 Declaration of Helsinki. All participants provided written informed consent.

Additional information
Correspondence and requests for materials should be addressed to Ö.K.

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