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ORIGINAL ARTICLE

Identification of human-dependent routes of pathogen's transmission in a tertiary care hospital

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ABSTRACT

Objectives: The purpose of the study was to validate the risk of patients' exposure to pathogenic flora carried on hands of students, visitors, and patients themselves, analyzing its density and genera and to compare them with the microflora of healthcare workers (HCWs).

Patients and methods: Between May and June 2018, five groups of participants were included. Each group consisted of eight individuals. Palmar skin imprints were obtained from dominant hands of doctors, nurses, students, visitors, and patients in orthopedics ward. Imprints were incubated at 37°C under aerobic conditions, and colony-forming units (CFU) on each plate were counted after 24, 48, and 72 h. Microorganisms were identified.

Results: Hands of doctors were colonized more often by Gram - positive non-spore-forming rods bacteria than hands of nurses ($p<0.05$). A higher number of *Staphylococcus epidermidis* CFUs was observed on doctors' than on nurses' hands ($p<0.05$), whereas *Staphylococcus hominis* was isolated from doctor's and patients' imprints, but was not from nurses' and students' imprints ($p<0.05$). Micrococcus luteus colonized patients' hands more often than students' ($p<0.05$), visitors' hands than doctors' ($p<0.05$), students' than nurses' ($p<0.05$), visitors' than nurses' ($p<0.05$) and patients' hands ($p<0.05$). *Staphylococcus aureus (S. aureus)* was isolated only from one doctor and one nurse (203 and 10 CFUs/25 cm^2). Imprints taken from the hands of patients, students and visitors were *S. aureus*-free. No methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci, nor expanded spectrum beta-lactamase-positive or carbapenemase-positive rods were isolated. The number of Gram-negative rods was the highest on visitors' hands, significantly differing from the number on patient's, doctor's, nurse's, and student's hands. Spore-forming rods from genus of *Bacillus* were isolated from representatives of all tested groups. *Bacillus* cereus occurred more commonly on visitors' hands than doctors' hands ($p<0.05$).

Conclusion: Patients, students, and visitors may play the causal role in the spread of pathogenic bacteria, particularly spore-forming rods. Our study results confirm the effectiveness of educational activities, that is the hospital's hand hygiene program among HCWs, patients, and visitors. Hand hygiene procedures should be reviewed to put much more effort into reducing the impact of all studied groups on the transmission of infectious diseases.

Keywords: Hand hygiene, healthcare workers, patients, skin microbiota, students, visitors.

Hospital-acquired infections (HAIs) complicate surgical procedures bringing the risk of severe disturbances. They are among the most crucial factors determining patient safety in the peri- and postoperative period capable to squander the final treatment result.[1]

Susceptibility to HAI increases with prolonged hospitalization, invasive procedures, comorbid diseases and their medication, advanced age, malnutrition, obesity, and currently running infections. When caused by pathogens of high virulence, and previously treated with antibiotics, it increases their resistance.[2] It is also well known that the number of HAI increases with poor compliance to antimicrobial procedures, overcrowding, poor hygiene, and sanitation, mainly when caused by pathogens of high virulence.[3] Shortage or even lack

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of effective methods of pathogen eradication enforces us to focus on prevention and control.

The hand hygiene (HH) program is crucial to control the spread of infection. Hospital staff is obliged to practice World Health Organization's (WHO) five indications for HH to reduce the risk of HAI, which is before touching a patient, before performing clean/aseptic procedure, after body fluid exposure risk, after touching a patient, and after touching patient's surroundings.[4] Visitors to hospitalized patients should be also considered as a pathway of microbial spread as they connect the microbiological environment of the hospital with their own home and work environment. Patients themselves may undertake risky social behaviors, e.g., direct interpersonal contacts and exchange of their properties, thereby opening routes to the transmission of infection. Teaching hospitals are exposed to pathogens that may be transmitted by students and young doctors having courses or internships at departments, possessing the capability to exchange microbial flora between them. As a result, they may serve as transmitters of HAIs, as well.

All these groups should be treated as a potential source of HAI infection, and their meaning should be updated, as their representatives have the possibility of exposing hospitalized patients to opportunistic or pathogenic microorganisms transmitted on the skin, shoes, or clothes.[5]

In the present study, we hypothesized that pathogenic bacteria were not present on the palm skin of students, patients and visitors and their resident and transient skin microflora did not differ from that of doctors and nurses, as the result within undertaken action according to hospital's HH program for healthcare workers (HCWs), patients and visitors. We, therefore, aimed to validate the risk of patients' exposure to pathogenic bacteria carriage of students, visitors, and patients themselves, analyzing its density and genera and to compare them with the microflora of HCWs.

PATIENTS AND METHODS

This observational study was conducted at the Department of Orthopaedic Surgery and Traumatology, Medical University of Warsaw, Poland, located at the humid, continental climate zone. The samples were taken in May and June 2018 during late springtime. Palmar skin imprints were obtained from dominant hands using Count-Tact® plates (25 cm^2) (bioMerieux, Marcy l'Etoile, France) from eight randomly selected participants among doctors, nurses, medical students, patients and visiting them relatives. Imprints were taken at the midday (±10 min) from previously non-informed participants. The palm of the dominant hand was chosen, as it is predisposed to become contaminated in consequence of frequent contacts with the environment, domestic and professional, including working equipment, furniture, and items of everyday use. It also serves for interpersonal contacts. Moreover, it is the palm of the dominant hand that usually keeps toilet paper. Thus, its skin perfectly reflects the owner's microbial environment. Orthopedic surgeons participated in the study on the non-operating (ambulatory) day to exclude the influence of hand washing and disinfection before the imprint's collection. Wound dressing nurses were excluded from the study, as they disinfected their hands more frequent than other nurses, even a few times per hour. The students taking practical classes at the ward took part in the study. Patients were hospitalized for at least three days, before the samples were taken. They were in the postoperative period. Patients after trauma and interventions to the dominant upper extremities were excluded from the study. Visitors were those visiting their relatives at the department at the midday.

Palms were pressed for 10 sec on Count-Tact® plates with a force of 5,0 N. Imprints were incubated at 37°C under aerobic conditions, and colony forming units (CFU) on each plate were counted after 24, 48, and 72 h. Identification of isolates was determined by the Vitek MS Matrix-Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry (Vitek, bioMerieux, Marcy l'Etoile, France), according to the manufacturer's instructions. Gram staining technique was applied to differentiate microorganisms and to determine their morphology. Bacterial resistance was evaluated according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.[6] The occurrence of resistance mechanisms on isolates was investigated (methicillin-resistant *Staphylococcus aureus*. [MRSA] isolates, glycopeptide resistance for Enterococcus spp., expanded spectrum beta-lactamase [ESBL], metallo-beta-carbapenemase (MBL), serine carbapenemase (KPC) and screening for carbapenemase oxa production for Gram-negative rods) according to local guidelines.

Statistical Analysis

Statistical analysis was performed using the Statistica version 13.3 software (StatSoft, Kraków, Poland). Data were presented in a number of CFU per plate (25 cm^2) with mean and standard deviation (SD) values. The Kruskal-Wallis test was used to compare the differences in species diversity between

TABLE I

The list of bacterial species isolated from palm microflora of each group and frequency of bacterial species occurrence (per 25 cm^2 plate)

	Doctors (n=8)	Nurses (n=8)	Students (n=8)	Patients (n=8)	Visitors (n=8)
	Total bacterial load (%)				
Bacillus					
B. altitudinis	0	0	0	0	0.1
B. cereus	0.1	1.8	7.4	0.7	0.5
B. circulans	0.1	0.2	0	0	0
B. clausi	0	0	0.1	0	0
B. licheniformis	1.6	0.8	0.4	1.8	4.8
B. megatherium	0.1	0.3	0.1	0	0
B. pumilus	0.1	0	0.1	0.8	0
B. simplex	0.2	0.2	0.1	0	0
B. subtilis	0.1	0.4	0.2	0	0.8
Brevibacillus					
B. brevis	0	0	0.1	0	0
Lysinibacillus					
L. sphaericus	0.1	0	0	0	0
Enterococcus					
E. faecalis	0	1.7	0	0.1	0
Kocuria					
K. rhizopus	12.3	0.4	0.1	0.4	0.2
Lactococcus					
L. lactis	0	32.0	0	0	0
Micrococcus					
M. luteus	0.8	0.4	16.4	0	18.8
M. roseus	0.1	0	0	0	0.1
Staphylococcus					
S. aureus	5.0	0.3	0	0	0
S. capitis	18.7	0.9	5.1	0	0
S. epidermidis	29.8	21.9	17.6	18.1	31.1
S. haemolyticus	12.4	23.2	1.6	14.2	0.9
S. hominis	16.8	4.3	6.9	62.6	22.5
S. pasteuri	0.1	0	6.8	0.1	1.1
S. simulans	0	0	0	0.2	0
S. vitulinis	0.1	0	0	0	0
S. warnerii	0	4.9	27.4	0.1	3.7
Corynebacterium					
C. falsenii	1.2	0	0	0	0
C. tuberculostearicum	0	0	0	0.3	0
C. mucifaciens	0	0	0	0.6	0.2
Acinetobacter					
A. baumannii	0	0	0	0.3	0
A. junii	0.1	6.8	0	0	14.4
A. lwoffii	0.1	0	0.2	0	0.6
A. ursungii	0.1	0	8.4	0	0
Pandoraea					
P. pnomenusa	0	0	0	0	0.2
Xanthomonas					
X. axonopodis	0	0	0.8	0	0
Pantoea					
P. dispersa	0	0	0.1	0	0
Enterobacter					
E. cloacae	0	0	0.1	0	0
Total	100% in each group				

the studied groups. A *p* value of <0.05 was considered statistically significant.

RESULTS

A detailed description of the occurrence of individual bacterial species in a given study groups is presented in Table I and the number of CFU per plate (25 cm[2]) of selected of microorganisms is presented in Table II. No MRSA isolates, glycopeptide-resistant Enterococcus spp., neither ESBL-positive, metallo-beta-carbapenemase-positive, serine carbapenemase-positive nor carbapenemase oxa-positive Gram-negative rods were isolated.

The majority of isolates constituted Gram-positive cocci. Their concentration on doctors' hands significantly exceeded those on nurses', students', and visitors' hands. Coagulase-negative Staphylococci formed the majority of all isolates. *Staphylococcus aureus (S. aureus)* was isolated only from one doctor and one nurse (203 and 10 CFUs/25 cm[2]). Imprints taken from the hands of patients, students and visitors were *S. aureus* free. *Enterococcus faecalis (E. faecalis)*, belonging to fecal flora was detected on one nurse's hands and one patient only. The *S. aureus* isolate was not MRSA and *E. faecalis* was not vancomycin-resistant enterococci (VRE).

Gram-negative rods were isolated from palms of three doctors, two patients, two nurses, two visitors, and five students. The number of Gram-negative rods was the highest on visitors' hands, significantly differing from the number on patient's, doctor's, nurse's, and student's hands (Table II).

TABLE II
Comparison between the number of isolates from studied groups at p<0.05
(Kruskal-Wallis' one way analysis variance)

	Nurses *(p)*	Students *(p)*	Patients *(p)*	Visitors *(p)*
Doctors	0.074 (All)	0.462 (All)	0.834 (All)	0.674 (All)
	0.046 (GP)	0.401 (GP)	0.834 (GP)	0.401 (GP)
	0.074 (S)	0.248 (S)	0.916 (S)	0.345 (S)
	0.011 (Sh)	**0.014** (Sh)	0.674 (Bc)	**0.049** MLF)
	0.031 (Se)	0.189 (Bc)	0.600 (GN)	**0.036** (Bc)
	0.916 (Bc)	0.600 (GN)		0.916 (Bc)
	0.958 (GN)			0.753 (GN)
Nurses		0.294 (All)	0.115 (All)	0.156 (All)
		0.345 (GP)	0.929 (GP)	0.172 (GP)
		0.189 (S)	0.046 (S)	0.318 (S)
		0.011 (ML)	**0.011** (Sh)	**0.014** (ML)
		0.248 (Bc)	0.916 (ML)	1.000 (B)
		0.753 (GN)	0.495 (GN)	0.637 (GN)
Students			0.248 (All)	0.294 (All)
			0.208 (GP)	0.294 (GP)
			0.115 (S)	0.916 (S)
			0.011 (Sh)	**0.014** (ML)
			0.156 (ML)	0.270 (B)
			0.270 (GN)	0.372 (GN)
Patients				0.600 (All)
				0.529 (GP)
				0.172 (S)
				0.004 (ML)
				0.916 (Bc)
				0.753 (GN)

The letter in brackets show compared species/bacterial group: (A) - All isolates, (GP) - Gram-positive isolates (without spore forming rods), (S) - *Staphylococcus spp.*, (Sh) - *S. hominis*, (Se) - *S. epidermidis*, (ML) - *M. luteus*, (B) - *Bacillus spp.*, (Bc) - *B. cereus*, (GN) - Gram-negatives rods.

TABLE III
The number of CFU per plate (25 cm²) of selected of microorganisms

	Isolates (CFU)			
	Total	Gram-positive (Without spore-forming)	Gram-positive spore forming rods	Gram-negative rods
Groups	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Doctors	507±523	568±531	11±19	1±1
Nurses	190±173	177±182	6±6	13±32
Students	231±166	209±139	19±36	22±49
Patients	431±294	412±294	11±24	1±3
Visitors	327±198	277±150	20±45	49±129

CFU: Colony forming unit; SD: Standard deviation.

Acinetobacter spp., Pandoraea spp., Xanthomonas spp., Pantoea spp., Enterobacter spp. isolates were not neither ESBL nor carbapenemase producers.

Spore-forming rods from genus *Bacillus*, were isolated from hands of six doctors, five patients, six nurses, six visitors, and seven students. Hands of doctors were colonized more often by Gram-positive non-spore-forming rods bacteria than hands of nurses ($p<0.05$).

Coagulase-negative Staphylococci constituted the most numerous groups among the isolates. However, there were differences in species diversity between the studied groups. The higher number of *Staphylococcus epidermidis (S. epidermidis)* CFUs was observed on doctors' than on nurses' hands ($p<0.05$), whereas *Staphylococcus hominis (S. hominis)* was isolated from doctor's imprints, but was not from nurses' and students' hands ($p<0.05$). A higher number of coagulase-negative *Staphylococci* CFU was observed from imprints taken from patient's hands than from nurses' hands ($p<0.05$). The *S. hominis* was most frequently occurring species on patients' palms than on nurses' palms ($p<0.05$). *Micrococcus luteus (M. luteus)* colonized patients' hands more often than students' ($p<0.05$), visitors' hands than doctors' ($p<0.05$), students' than nurses' ($p<0.05$), visitors' than nurses' ($p<0.05$) and patients' hands ($p<0.05$). *Bacillus cereus (B. cereus)* was more commonly found on visitors' hands than doctors' hands ($p<0.05$).

The comparison between the number of isolates from studied groups is presented in Table III.

DISCUSSION

Medical personnel are believed to be among the most important vectors of pathogens transmission. It involves students, patients, and visitors who are in hospital on a regular or occasional basis. To validate the risk of patients' exposure to pathogenic bacteria, we analyzed the quantity of aerobic microbiota composition of HCWs' dominant hand, as well as medical students, patients, and visitors in orthopedic ward. Imprints collected at the midday reflected normal skin flora but also environmental and occupational. The highest risk of hand's contamination occurs at the most crowded areas that have the highest bacterial concentrations: shops, working places, public transportation, and toilets, waiting rooms, etc. We chose the midday for taking samples to lose the effects of morning hand washing, which controls the skin microbiome. At least 4 to 5 h-long intervals should minimalize its influence on the hand's microbiome, pointing out to the flora residing on hands during the daytime. In our studies, representatives of normal human skin flora were most frequently obtained. *Staphylococcus aureus* was isolated from two medical staff. Gram-negative and Gram-positive spore-forming rods most frequently colonized student's hands which proves that students may serve as a vector of HAIs. Despite the emphasis on HH in medical staff, the control of HH in students, patients and visitors is poor. Their potential in the spread of HAI should not be ignored. In this study, doctors and patients had the highest concentration of microorganisms on their palms, twice as other groups. The presence of pathogenic flora transmitted due to interpersonal contacts from foci of infection, as well as a lack of proper HH, may have an impact on HAI transmission. The hand's skin microbiome is more variable and less stable than any other in-between the same organism, as it consists of resident and transient microorganisms.[7] Human skin is regularly colonized by microorganisms, aerobic and anaerobic, at concentration ranging from more than 10^6 on the scalp, 5×10^5 in the axilla, 4×10^4 on the abdomen, 10^4 on the forearm (CFUs/cm^2). Fingertip microbiome consists of up to 300 CFUs/cm^2. In HCWs, total bacterial counts should be reduced due to repeated HH procedures, but close contact with patients has an influence on the hands' microbiome. Regularly used medical equipment is not germ-free, either. Both facts may explain why medical professionals' fingertips contain 10^4 to 10^6 CFU/cm^2 of microorganisms.[8]

The transient and resident skin flora varies among individuals, being stable for the particular one.[9] An average of more than 150 species may be found on the palm. Normal microflora protects the host from the invasion of pathogenic strains, individuals possessing lower microbial diversity are more likely to harbor pathogenic microorganisms, such as MRSA, *Enterococcus spp.* and *Candida albicans.* Analyzing the hand's skin microbiota diversity, bacteria are the most prevalent microorganisms (>80% relative abundance), whereas viruses and fungi are presented in less than 5% each.[10]

The composition of the microorganisms that make up the skin microbiota is related to interactions between species.[11,12] Resident flora when transmitted into sterile body cavities, eyes, or non-intact skin may cause an infection. The transient microbiota is more amenable to removal by routine HH rather than a resident one. Healthcare workers regularly acquire them as consequence of direct contacts with patients and environment.[9] In our study, *S. aureus* accounted for 5% of the cultured microflora in doctors, and 0.2% in nurses. Our results show that patients, medical students, and visitors carry resident and transient skin microflora. In addition, students can transfer pathogens between patients, departments, and hospitals.[13] The WHO's five indications for HH and transmission-based precautions dedicated to HCWs should properly be abided by medical staff, patients, visitors, and students,[14] particularly nowadays, among novel coronavirus 2019 disease (COVID-19) pandemic.[15] Messages enabling patients understand and learn can be included in information leaflets and in posters displayed at the facility entrance and in waiting areas.[4] The unrestricted access to soap, water, sinks and alcohol-based hand rub (AHR) stations to all, should be available so handwashing followed by AHR would be performed at least: before entering the patient's room and immediately after leaving it.[16] Unfortunately, HH is widely ignored by medical staff, enforcing installation control devices including visual and acoustic reminders, or electronic.[17] Visitor HH is an evidence-based strategy to reduce pathogen transmission.[18] Social pressure highly influences visitor's compliance to HH rules.[19] Visitors being in the company are more likely to use AHR than being alone - a reduction of HH compliance from 44% at the main hospital's entrance to 4.1% at the departments, and only 2.7% at patient's room.[20] Furthermore, visitors are 5.28-times more prone to use AHR, when dispensers are located in the middle of the lobby and demonstrably labelled with landmarks and barriers.[19] The AHR use is 1.35-times more likely in the afternoon than morning, and by younger people than the elder. To increase the AHR usage, dispensers should be installed in exposed/public, not private areas. According to Birnbach et al.,[8] 64% of visitors disobeyed WHO's five indications for HH. Moreover, 42.8% of those who disobeyed them reported that they obeyed the HH. A total of 26% of visitors who disobey HH rules carry Gram-negative rods, and MRSA. Visitors obeying HH rules did not carry pathogenic flora on their hands and their hand normal microbial load was 0.9 CFU per cm^2, while the rate of disobeying was 89.3 CFU per cm^2 among visitors.[8]

The AHRs applied for HH alters human and environmental microbiota composition.[21] Although it is a well-known fact, we are reminded of this truth only when we have a patient infected with *Clostridioides difficile (C. difficile)*, in an individual case or during an outbreak. In everyday practice, HCWs pay no attention to the change of microflora, e.g., contamination of hands with spores or spore-forming rods.

The effectiveness of AHR solutions including ethanol and isopropanol, chlorhexidine, iodine povidone, and octenidine dihydrochloride is not permanent.[22] Pathogens may become tolerant to them. According to Pidot et al.,[23] *Enterococcus faecium* isolates became 10-times more tolerant to alcohol after their five years-lasting usages, becoming resistant even to standard 70% isopropanol due to mutation in genes responsible for carbohydrate uptake and metabolism. Silver nanoparticles-based gel hand wash is very promising, but still requires validation.[24]

The novelty of our study was to determine the quantitative microflora composition of hands of five groups interacting with each other in orthopedic ward. We concentrated on aerobic bacteria. The presence of *S. aureus* was found in one doctor and one nurse, and, while *Enterobacter cloacae (E. cloacae)* was found on the hand of the student and *Acinetobacter baumannii (A. baumannii)* of the patient, but these species accounted for only 5%, 0.2%, 0.1% and 0.3% of total CFU cultured in each group, respectively. The *E. cloacae* is a component of human fecal microflora and *A. baumannii* occurs naturally in water and soil, as well as in contaminated hospital environment or colonized patient/staff. The presence of Gram-negative rods in the skin microflora is a result of the HH neglect. In the study of Tang et al.,[25] the following results were obtained: *A. baumannii* constituted 15% of the bacteria on HCWs' hands, *Pseudomonas spp.* 9%, and *E. cloacae* 9%. The study by Domínguez-Navarrete et al.[26] revealed the presence of pathogenic bacteria on the hands of preclinical medicine students. A total of 60.6% of students were carriers of *S. aureus*, 3% *Pseudomonas aeruginosa*, 3% *Enterobacter*, and 18.1% *Candida spp.*[27]

Ssemogerere et al.[27] cultured fingertip imprints of ICU physicians, non-ICU physicians and non-clinicians, and isolated: *Acinetobacter spp.* from 34.4% samples, *Citrobacter spp.* 21.9%, *Pseudomonas spp.* 21.9%, *Klebsiella spp.* 3. 1%, *Serratia spp.* 3.1%, *Enterobacter spp.* 3.1%. Among carbapenemase producers, they observed 44.4% *Acinetobacter spp.* isolates 22.2% *Pseudomonas spp.*, 22.2% *Citrobacter spp.* and 11% *Klebsiella spp.* A total of 10.7% of *Acinetobacter spp.* and *Klebsiella spp.* and *Enterobacter spp.* were ESBL. Our Gram-negative rods isolates did not express neither ESBL nor were carbapenemase producers.

The differences we found concerned the quantity or quality of microorganisms that belong to the normal microflora of the hand skin (mostly coagulase-negative *Staphylococci*) and the probable environmental contaminant of the genus *Bacillus*. Our results confirmed the research hypotheses. We found no significant differences in the pathogenic transition flora, which was isolated in single cases, which may prove the effectiveness of educational activities, that is the hospital's HH program among HCWs, patients and visitors. Although the sample size of eight subjects per group is small and it is not representative of the entire multidisciplinary university hospital staff and is the limitation of the study. The results are preliminary, but can give a microbiological insight into HH compliance. While we were planning this experiment, we did not expect abundance of spore-forming rods, which do not belong to normal skin flora. Currently, it is relevant to investigate *C. difficile* hand contamination on HCW role in asymptomatic patients. Alcohol in AHR lacks activity against bacterial spores,[28] but it is effective in killing the vegetative cell (non-spore form) of *C. difficile* which may be present in higher numbers than the spores.[4]

In conclusion, as given in the medical literature, patients, students, and visitors may play the causal role in the spread of pathogenic bacteria, particularly spore-forming rods, their hands may carry pathogenic microflora. The results of our study demonstrate general compliance with the hospital's HH program for HCWs, patients and visitors, introduced years ago. Furthermore, HH is one of the topics covered during microbiology course, as well as during other courses, including clinical ones. Nevertheless, continuous training of staff in HH, as well as patients and visitors, and students, and particularly in monitoring compliance with them, should be maintained.

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Ethics Committee Approval: Investigations were anonymous. Investigations were approved by the Ethical Committee of the Warsaw Medical University (AKBE/158/2020). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: The subjects gave informed consent to participate. The patients and/or their families were informed that data from the case would be submitted for publication and gave their consent.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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