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Nine years of microbiological air monitoring in the operating theatres of a university hospital in Southern Italy

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Abstract

Introduction. *Surgical site infections are among the most frequent Health Care Associated Infections with severe impact on mortality and high economic costs; the role of air microbiological contamination in surgical site infections was amply discussed in the scientific literature, highlighting differences in air contamination rates between different ventilation systems, number of people present and door opening rates.*
Materials and methods. *The aim of our study was to monitor the presence of bacterial air contamination in operating theaters and its relationship with number of people and type of airflow over a period of nine years (January 2010-November 2018) at Messina's University Hospital. The Rho of Spearman test was used to evaluate differences in microbial contamination between empty and working theaters. The impact of the number of people on colony-forming unit values was assessed by performing a stepwise multiple regression analysis. The differences between the results recorded over the nine-year study period were evaluated using the variance analysis. Software R was used for the statistical assessment.*
Results. *Air samples were taken in each operating theater over nine years. A total of 1,425 samples were collected with a positivity rate of 37.3%. The median bacterial contamination rate was 30 cfu/m^3 in empty theaters, while this rate was significantly higher ($P < 0.001$) in working theaters, where it reached 85 cfu/m^3. A statistically significant difference was identified between laminar and turbulent airflow systems, with higher bacterial contamination rates with the latter ($p<0.001$); in these cases, the value of bacterial contamination (microbial count) was higher in the presence of a higher number of individuals in the operating theater ($p<0.001$).*
Discussion and conclusion. *Our study demonstrates the complexity of the Operating Theatres environment, in which a good ventilation system is, indeed, only one prerequisite for clean air, but other factors, such as the behavior of healthcare professionals and environmental conditions, can influenced the bacterial count.*

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Introduction

Surgical site infections (SSIs) are the most frequent Health Care Associated Infections (HCAIs), together with high and low respiratory infections, with incidence rates between 1.2% and 5.2% in developed countries (1, 2). SSIs unfortunately lead to high rates of morbidity and mortality and involve a high social and economic impact (3-4).

Many factors have been identified as contributing to their occurrence, both patient-related and also practice-related. One of the most important factors today considered is level of environmental microbial contamination in the operating theatres (OTs) that plays a significant role in the transmission of microorganisms, including multidrug-resistant organisms (5-7).

The role of air microbiological contamination was amply discussed in literature, highlighting differences in air contamination rates between different ventilation systems, number of people present and the door opening rates. In fact, opening the OTs' doors can change the dynamics of the unidirectional airflow pattern and increase air turbulence, thus facilitating a faster spread of airborne organisms (8-12).

Furthermore, bacterial contamination within an OT depends on the effectiveness of the controlled contamination ventilation and conditioning system (CCVCS), its periodic maintenance, the accuracy of OTs' sanitization procedures as well as the behaviors of health care workers (HCWs). Therefore, in this respect, it is important that the hospital hygiene office provides for careful monitoring and that infection prevention guidelines are adequately implemented (13).

A study by the Medical Research Council showed a correlation between microbial air contamination and SSI incidence in prosthetic joint surgery (14); furthermore, in a recent study, the microorganisms more frequently detected (around 50% of isolates) were *S. epidermidis* (21.8% of isolates), *S. hominis* (6.3%), *S. xylosus* (3.6%), *S. warneri* (2.8%) and, among hyphomycetes, the *Cladosporium* genus. *S. aureus*, *Enterobacteriaceae, Enterococci, Pseudomonas spp*. were never detected and also in other studies the role of environment is underlined (15-17).

The aim of our study was to monitor the presence of bacterial air contamination in OTs and some hospital settings and to detect the relationship of bacterial count (cfu/m^3) with number of people and type of airflow (18-25).

Materials and methods

Microbial monitoring was performed in 29 conventionally-ventilated OTs located in 10 operating suites.

Our facility includes various buildings with a different number of OTs, namely General Surgery (3 OTs), Plastic Surgery (1 OT), Otolaringology (1 OT), Ophtalmology (1 OT), Urology (2 OTs), Thoracic and Vascular Surgery (2 OTs), Gynecology and Obstetrics (6 OTs), Pediatric Surgery (3 OTs), Cardiology Intensive Care Unit (2 OTs, both with turbulent airflow), Orthopedic Surgery (2 OTs), Neurosurgery (2 OTs), Maxillofacial Surgery (1 OT, with laminar airflow) and Neuroradiology (1 OT). Three other important settings, i.e. the antiblastic drug unit (1 OT) and the transfusion medicine ward (1 OT), that require aseptic environment, were tested. In all OTs, air was supplied by a ventilation system designed to provide 15 air changes per hour. The system was equipped with high-efficiency particulate air filters, which can remove particles >0.3 mm diameter with an efficiency of 99.97%. Air pressure in the OTs was 5 Pa (Pascal) higher than in adjacent rooms.

Sanitization practices were implemented in every step of the process (environmental sanitization, reconditioning of surgical instruments, preparation of patients, antibiotic prophylaxis, behavioural norms) and didn't change during the study period.

Sanitization activities were performed by trained personnel at the beginning and at the end of the working day and between each surgical operation. In addition, weekly disinfection and quarterly reconditioning procedures (including dust suction and subsequent washing of the grilles installed on the air intake and return ports) were carried out according to the guidelines (26).

Microbiological sampling

Active air sampling was carried out according to UNI EN ISO 13098, 2002: for the evaluation of microbial contamination in OTs air, expressed as cfu/m^3, the sampling was carried out with a semi-automatic sampler (SAS Super100, Sampler Air System, PBI) containing a Plate Count agar (PCA) that sucked a volume of 180 L/min for 200 seconds. The operator in charge of sampling was wearing proper operating-room clothing, after getting his hands washed and wearing gloves. During sample collection, the operator stood back and still or, if possible, remained outside the room. Sampling was performed at a height of one meter from the floor and within a radius of about one meter from the surgical wound. Sampling started at the beginning of all surgical operations by taking the first sample at the stage of the surgical incision. The subsequent samples were taken in the next phases every 5 to 10 minutes depending on the duration of the intervention. All samples were taken immediately to the laboratory. Samples were considered positive according to the parameters suggested by the "guidelines on standards of safety and occupational health in the operating department" of the Italian Agency for prevention, occupational health and safety (ISPESL) which admits for OTs "at rest" an air contamination limit ≤ 35 cfu/m^3 and for OTs "in use" values ≤ 180 cfu/m3 (turbulent flow) and ≤ 20 cfu/m^3 (laminar airflow) (27-28).

We carried out sampling at intervals of 6 months (2 samplings/year as indicated by ISPESL guidelines) or on the request of the health management or the operating units, in empty OTs every time while for those in use, only during surgical operations; in 2012 a renovation of a building didn't allow the sampling in the following operative units: General Surgery (3 OTs), Plastic Surgery (1 OT), Ophthalmology (1 OT), Otorhinolaryngology (1 OT) and Urology (2 OTs). Also, in neuroradiology the samples were carried out by December 2015, because the OTs didn't exist before this date. In transfusional medicine the sampling was carried out monthly by September 2017 to November 2018, as indicates in "Guide to the preparation, use and quality assurance of blood components"(29).

Moreover, at the moment of each visit, the "power on" of the CCVCS and conditioning system and the correct application of guidelines in OTs was monitored, evaluating the presence of irregularities regarding the opening of the doors, the number of people present in the OTs, and the activation of the ventilation systems, etc.

Statistical analysis

A descriptive statistical analysis was performed to obtain mean, standard deviation, standard error of the mean, median and percentiles. The non-parametric correlation (Rho of Spearman) was used to evaluate differences in microbial contamination between empty and working OTs and the impact of the number of people on cfu values with turbulent and laminar airflow ventilation systems. Differences between the results recorded over the nine-year study period were evaluated using variance analysis. P values of <0.05 were considered

to indicate significance. Software R was used for statistical assessment (30).

Results

Air samples were taken for each OT at rest ("unused") and full ("in use") over nine years, with a total of 1,425 samples with a positivity rate of 33.96% (484/1425). The total number of samples in unused OTs was 790 (55.44%) with a positive rate of 31.6% and in the used OTs it was 635 (44.56%) with a contamination of 24.2%. See Table 1 and Figure1.

In OTs "at rest" the median bacterial contamination rate was 30 cfu/m^3, while this rate was significantly higher ($P < 0.001$) in OTs "in use", where it reached 85 cfu/m^3. Values for bacterial contamination varied widely, reaching 260 cfu/ m^3 in unused OTs and 500 cfu/ m^3 in OTs in use. The overall air compliance (cfu/ m^3) and overall number of people per operating theater is reported in Table 2.

In the wards of the antiblastic drug unit and the transfusion medicine department we found an overall compliance of 91.43% and 100% respectively, with a median bacterial contamination value of 25 cfu/ m^3 in the first one and 5 cfu/ m^3 in second one. We also obtained values under 35 ufc/m^3 for both wards in OTs "at rest" and higher values in the antiblastic drug unit in OTs "in use" (probably this might depend on a series of variables such as an occasional malfunctioning of CCVCS system, a higher number of people in the unit, etc.).

As regards the median total bacterial count (TBC) of surgery OTs we found a high variability: the lowest TBC equal to 20 cfu/m^3 was observed in orthopedic OTs, where low levels of microbial contamination are required; low values were also found in maxillofacial surgery (median=30 cfu/m^3. The highest median TBC was detected for general surgery (75 cfu/m^3) and in thoracic and vascular surgery (60 cfu/m^3).

Table 3 shows mean, median and QRI for bacterial contamination expressed in cfu/m3 for all OTs.

Bacterial contamination decreased over the nine-year period. The lowest value was detected in 2017, while the highest value was measured in 2012 and 2013, despite cfu/ m^3 values were not statistically significant ($p>0.05$) in all OTs (Table 4).

Only in 13 cases problems were observed with CCVCS that had not been switched on before the beginning of surgical operations and we found a bacterial count over the limit with a mean of 58.33 cfu/m^3 in OTs "at rest".

In 4 cases OTs "at rest" had the door open before the start of the controls and we found a bacterial count over the limit with an average of 42.5 cfu/m^3.

We intended to verify whether the number of people in the surgery room could impact on cfu values: from data obtained with the nonparametric correlation (Rho of Spearman) the "count" variable showed a significant correlation with the "number of people" ($r = 0.469$; $p <0.001$) and "OT" ($r = 0.450$; $p <0.001$). The correlation between two variables can be influenced by the presence of a third variable and its interaction with the two significant ones. Therefore, in our model all the quantitative and categorical variables were inserted as explanatory variables, leading to the following expression:

$$\text{Count} = \beta 0 + \beta 1 \times \text{operating units} + \beta 2 \times \text{number of people} + \beta 3 \times \text{type of air ventilation} + \beta 4 \times O + \varepsilon$$

On the basis of the ANOVA ($F_{4.341} = 26.405$; $P <0.001$) the null hypothesis was rejected (equality of the repressors). The coefficient of multiple determination R2 of the model (0.236) proved that the variables of the operating units, number of people present, laminar/turbulent airflow and OT could explain only 24% of the count variability (ufc). Results were summarized in Table 5.

Table 1 - Total number of samples divided by operative units and OTs

		OT1		OT2		OT3		OT4		OT5		OT6	
		at rest	in use	at rest	in use	at rest	in use	at rest	in use	at rest	in use	at rest	in use
Antiblastic drug unit	55	28	27	-	-	-	-	-	-	-	-	-	-
Cardiology Intensive Care Unit	50	18	8	18	6	-	-	-	-	-	-	-	-
Maxillofacial surgery*	55	28	27	-	-	-	-	-	-	-	-	-	-
Neurosurgery*	110	30	25	30	25	-	-	-	-	-	-	-	-
Pediatric surgery	115	30	28	30	27	-	-	-	-	-	-	-	-
Otorhynolaringology	60	30	30	-	-	-	-	-	-	-	-	-	-
Ophthalmology	80	40	40	-	-	-	-	-	-	-	-	-	-
Plastic surgery	80	40	40	-	-	-	-	-	-	-	-	-	-
Thoracic surgery	120	30	30	30	30	-	-	-	-	-	-	-	-
Urology	45	18	5	18	4	-	-	-	-	-	-	-	-
Gynaecology	305	31	25	31	22	31	20	31	18	31	16	31	18
General surgery	235	40	39	40	38	40	38	-	-	-	-	-	-
Orthopedics*	80	20	20	20	20	-	-	-	-	-	-	-	-
Neuroradiology°	20	16	4	-	-	-	-	-	-	-	-	-	-
Trasfusional medicine^	15	15	-	-	-	-	-	-	-	-	-	-	-

* Laminar airflow

° the sampling started in December 2015, because the OTs did not exist before this date; it should also be noted that sampling in the OT "in use" was conducted only during emergency surgical operations.

^ the sampling was carried out monthly from September 2017 to November 2018 (as indicated in the "Guide to the preparation, use and quality assurance of blood components").

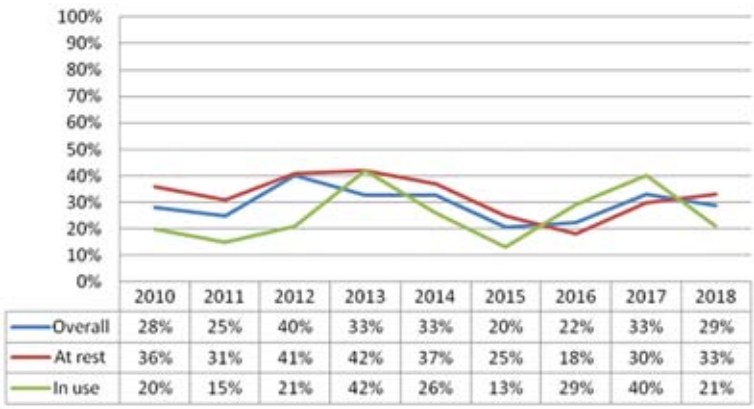

Fig. 1 - Trend of the positivity rate (OTs overall, at rest and in use)

Table 2 - Mean, maximum, minimum, SD, 1st quartile, 3rd quartile, QRI and median of overall air compliance, overall number of people, OTs "at rest" and "in use"(both laminar and turbulent airflow)

	Overall number of people in OTs "in use"	Overall bacterial count	At rest OTs	In use OTs	Laminar airflow and at rest OTs	Laminar airflow and in use OTs	Turbulent airflow and at rest OTs	Turbulent airflow and in use OTs
Mean	6.89	67.06	42.96	109.51	39.32	83.65	43.85	115.31
Minimum	2	1	0	5	5	10	0.00	5.00
Maximum	20.00	500	260	500	225	220	260.00	500.00
DS	3.53	70.88	44.83	87.48	39.45	63.33	46.12	92.15
1st quartile	5.00	20	15	50	15	28.75	15.00	55.00
3rd quartile	8.00	90	55	155	52.5	117.5	55.00	158.75
QRI	3.00	70	40	105	37.5	88.75	40.00	103.75
Median	7.00	45	30	85	22.5	67.5	30.00	90.00

Table 3 - Mean, Median and quartiles of total bacterial count in the different operating suites during surgery. Values measured by active sampling (colony-forming units/m^3)

		Overall OTs			At rest OTs			In use OTs		
OTs	N(total)	Mean	Median	QRI	Mean	Median	QRI	Mean	Median	QRI
Antiblastic drug unit	55	54.38	25	27.5	20.00	15	25	88.13	30	33.75
Cardiology Intensive Care Unit	50	54.09	30	35	51.50	30	13.75	80.00	60	20
Maxillofacial surgery*	55	65.38	30	55	54.38	27.5	28.75	83.00	60	95
Neuro surgery*	110	57.08	50	56.25	38.57	20	45	83.00	75	57.5
Pediatric surgery	115	83.00	50	90	50.00	30	35	153.13	142.5	112.5
Otorynolaringology	60	83.46	35	100	42.50	22.5	27.5	149.00	120	57.5
Ophthalmology	80	47.75	30	42.5	24.17	25	26.25	83.13	85	115
Plastic surgery	80	53.82	25	30	45.88	20	35	106.43	50	50
Thoracic surgery	120	75.48	60	72.5	36.58	30	37.5	137.08	130	117.5
Urology	45	46.50	50	47.5	42.78	35	50	80.00	60	20
Gynecology	305	64.86	47.5	56.25	43.82	35	35	115.95	100	95
General surgery	235	92.25	75	100	73.89	55	87.5	112.92	95	85
Ortopedics*	80	47.62	20	35	32.33	20	32.5	85.83	70	118.75
Neuroradiology	20	76.00	50	50	42.50	37.5	31.25	210.00	210	0
Trasfusional medicine	15	8.33	5	5	8.33	5	5	32.50	32.5	27.5

* Laminar airflow

Discussion and conclusion

The role of air as a carrier of surgical site infections is controversial, however, nowadays, adequate contamination prevention measures are recommended for OTs (27, 31-33).

To date, only a few studies have focused on the long-term evaluation of microbial air quality in OTs. Our study is one of few studies that investigate microbial air contamination in OTs employing standardized methods, providing a global evaluation of air quality over a long period of time, in both empty and "in use" OTs and using active sampling to assess bacterial contamination. It should also be taken into account that many of these studies had been conducted many years ago

Table 4 - Yearly variation in mean bacterial contamination in at rest and in use OTs. Values measured by active [colony-forming units (cfu)/m3] sampling

Year	Number	Mean	SD
2010	140	70.07	79.23
2011	140	61.72	56.72
2012	65	75.2	74.3
2013	181	74.2	73.1
2014	181	72.08	79.2
2015	171	57.72	58.36
2016	187	56.72	57.28
2017	200	54.7	52.3
2018	160	60.08	61.25

(11, 19, 34-40). Both passive and active sampling methods can be used for general monitoring of air contamination, such as routine surveillance programs; however, in some studies the researchers declared to have found a higher microorganism count with the active sampling methods. Accordingly, we decided to use this type of method in our research (41).

We found a higher variability in air microbiological contamination in the evaluated period with peaks of positive rate in some years and a maximum value in 2012 both for empty and "in use" OTs and in 2013 for the latter. In the unused OTs this depended on the ventilation system and its maintenance, number of people present and how often doors are opened. In fact, over the study period, we found problems with the air ventilation systems, which were not switched on before the beginning of surgical operations (11, 12, 19, 35-43).

Another reason that we found for non-compliance was the excessive number of people in the "in use" OTs. In fact, the number of people present in the OTs affects air contamination significantly, especially in presence of a turbulent airflow. In contrast, the same variable did not appear to affect air cleanliness in the operating field in OTs with laminar airflow systems. Our facility is a university hospital and this could explain the entrance of personnel in training less attentive to best practices, who could contribute widely to the environmental contamination; similar results about traffic in "in use" OTs are described by other authors (44).

These behaviours increased the risk of accidental contamination of sterile areas

Table 5 - Multiple regression analysis of the impact of number of people present on colony forming unit values with turbulent and laminar airflow ventilation systems. Variable dependent was colony forming unit values.

	Not standardized coefficents		t	p_{value}	Confidence interval 95%	
	B	Standard error			Lower limit	Upper limit
(Costant)	31.188	9.967	3.129	0.002	11.584	50.793
Operating unit	-0.119	0.939	-0.127	0.899	-1.966	1.728
Number of present	5.834	1.712	3.407	0.001	2.465	9.202
airflow/turbolent	15.292	8.622	1.774	0.077	-1.666	32.250
OT	26.802	13.660	1.962	0.051	-0.066	53.670

and instruments and, furthermore, the frequent opening of doors compromises the performance of CCVCS system in controlling environmental contamination (45-48).

This demonstrates the importance of monitoring air quality and the behavior of HCWs as well as the need for local cooperation between the engineering department and the hygiene unit in order to achieve an effective monitoring of OTs and to solve problems with the ventilation systems (11, 19).

Another reason of variability during the period of study of the microbial count could be the change of the cleaning staff, confirming the international literature. In fact, despite the presence of a standard protocol in our facility, it is well known how these procedures are employees-dependent: this is why trained operators and effective sanitization methods are needed to try to break down microbial contamination as much as possible (49).

Moreover, the results of our study show a better air quality with laminar airflow than turbulent systems, thus confirming the findings of others authors (35, 42, 43).

We found higher variability in air microbial contamination between the different OTs, with lower values in the antiblastic drug unit, in the trasfusional medicine department and in the OTs with laminar airflow systems; higher values were detected in others operating units and these data reflect international literature. As previously indicated, this depends on the type of airflow, laminar or turbulent, available in the OTs and on the type of surgery performed. Furthermore, it can correlate with the different opinions and attitudes towards safety and infection prevention, that can vary on the basis of the surgical specialty, as the staff tends to adopt different behaviors accordingly (50).

Also, another reason of variability between the OTs was the position of the OTs; we previously highlighted how the distribution of the OTs was not homogenous and so the CCVCS systems were different (structural characteristics, type of airflow, etc.). This could explain only in part the variability but other factors previously described also contributed too (51).

Furthermore, we did not analyze many important confounding factors, such as choice of clothing regimen, timing of prophylactic antibiotics, hypothermia and the sampling point inside the OTs (14, 50-52). Moreover, though we did not personally investigate the influence of door opening on air microbiological count, many studies demonstrated the role of this variable (9).

Over the nine-year period, the decrease in bacterial contamination in both unused and used OTs demonstrates the usefulness of microbiological monitoring for air quality improvement and the importance of staff education and application of prevention guidelines (19, 25, 52, 53).

Another limit of our study is the variability of behavior of the HCWs, that in some cases didn't wear a surgical hood tucked into the smock and covering all hair. In some cases, the door of the OTs was open before our arrival in the OTs. Similar critical behaviors emerged from Italian data on SSI control measures (54).

Some studies underline that the role of air contamination appears to be fundamental for microbial contamination of the surgical wound, and consequently for surgical site infection. In fact, the exogenous airborne microorganisms can fall directly into the surgical wound or they can be transferred to the wound from other contaminated surfaces by the hands of the HCWs (19, 25).

Our study demonstrates the complexity of the OT environment, in fact the ventilation system is only one prerequisite for clean air. An OT should be viewed as a complex system with interactions between patients, HCWs and environmental conditions.

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Riassunto

Nove anni di sorveglianza microbiologica dell'aria nelle sale operatorie di un ospedale universitario nell'Italia Meridionale

Introduzione. L'infezione del sito chirurgico è una delle infezioni associate all'assistenza sanitaria più frequenti, con un alto impatto sulla mortalità ed elevati costi economici; il ruolo della contaminazione microbica dell'aria nella possibile insorgenza delle infezioni del sito chirurgico è stato ampiamente discusso in letteratura, documentando differenze nei tassi di contaminazione dell'aria in base ai diversi sistemi di ventilazione, al numero di persone presenti e ai tassi di apertura delle porte nelle sale operatorie.

Materiali e metodi. L'obiettivo del nostro studio è stato quello di monitorare presso l'Ospedale Universitario di Messina la contaminazione microbiologica dell'aria nelle sale operatorie e valutarne la correlazione con il numero di persone presenti e la tipologia del flusso d'aria nell'arco di nove anni (gennaio 2010-novembre 2018). La correlazione non parametrica (Rho di Spermann) è stata utilizzata per valutare le differenze di contaminazione microbica tra sala operatorie a vuoto ed in attività. L'impatto del numero di persone sulla conta microbica è stato valutato eseguendo un'analisi di regressione multipla. Le differenze tra i risultati registrati nel periodo di studio di nove anni sono state valutate utilizzando l'analisi della varianza. Il software R è stato utilizzato per la valutazione statistica.

Risultati. Nei nove anni sono stati prelevati campioni di aria in ogni sala operatoria, per un totale di 1,425 campioni, con un tasso di positività del 37,3%. Nelle sale operatorie a vuoto il tasso medio di contaminazione batterica dell'aria era di 30 cfu/m^3, mentre era significativamente (P <0,001) più elevato nelle sale in attività, dove raggiungeva 85 cfu/m^3. È stata identificata una differenza statisticamente significativa tra i tipi di ventilazione presenti (flusso d'aria laminare e turbolento), con tassi di contaminazione batterica più elevati (p <0,001) con quest'ultimo; infine, il valore della contaminazione batterica aumentava in presenza di un numero maggiore di individui in sala operatoria (p <0,001).

Discussione e conclusioni. Il nostro studio conferma la complessità dell'ambiente della sala operatoria, in cui il sistema di ventilazione è, infatti, solo uno dei prerequisiti per una corretta gestione del rischio clinico in sala operatoria ed altri fattori, come il comportamento degli operatori sanitari e le condizioni ambientali presenti, possono influenzare la conta microbica.

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