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Assessment of Microbial Load in Regional Hospitals in Albania

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Abstract

BACKGROUND: Contaminated hospital indoor environments can expose patients to microorganisms and different infections.

AIM: The aimed study was to assess the microbial load in hospital facilities inside Albania Regional Hospitals during the period 2017–2019.

MATERIALS AND METHODS: A cross-sectional study was conducted during the period 2017–2019 for the assessment of microbial contamination in operating rooms, resuscitation, and delivery rooms in 12 regional hospitals in Albania. One thousand and three hundred microbiological specimens were collected from air and surfaces using 5% sheep blood agar (Oxoid, UK) and processed at IPH microbiology laboratory following the standard bacteriological procedures. Data were analyzed using the Statistical Software Package for the Social Sciences version 23.

RESULTS: Out of the total number of samples, 1148 (88.3%) were collected from surfaces and 152 (11.7%) were from the air. Bacterial growth was identified in 314 (24.2%) out of 1300 samples (95% CI 21.89–26.62). From the total site samples processed during the study period, bacterial growth showed 282 (89.8%) samples from surfaces and 32 (10.2%) air samples. There was found a significant association p = 0.035. Regarding the sampling place collection, the largest number were collected in operating rooms (60.3%) followed by emergency rooms (28.2%), intensive care units (7.7%), and maternity units (3.8%). Gram-negative isolates were predominant at 235 (74.8%), while the Gram-positive were at 60 (19.1%). *Escherichia coli* was the most frequent bacterial isolate (50%), followed by *Pseudomonas aeruginosa* (23.6%), *Staphylococcus aureus* (19.1%), and *Klebsiella pneumoniae* (1.3%). Furthermore, we found a fungal agent such as *Aspergillus* in 19 (6.1%) samples. The isolated bacteria's overall drug resistance profile revealed that 66.8% of Gram-positive bacteria were resistant to two or more antimicrobial drugs tested.

CONCLUSION: This study revealed that the surface and air and air within different wards of the hospitals studied were contaminated with different types of bacteria. Bacterial loads on the surface and air exceeded normal limits. In addition, the study pointed out high levels of antimicrobial resistance to the drugs commonly prescribed for isolates. Therefore, intervention strategies need to be strengthened to expand infection prevention practices in hospitals. Continuous monitoring and monitoring of in-hospital pathogen types and susceptibility patterns should be performed on a very regular basis.

Introduction

Contaminated hospital indoor environments can expose patients to microorganisms and different infections. The hospital's indoor air may contain a large number of disease-causing agents brought in by patients, staff, students, visitors, ventilation, or the outside. Hospitalized patients are at a higher risk of infection due to confined spaces, crowdedness, and poor infection prevention practices, which can accumulate and create favorable conditions for the growth and multiplication of microorganisms (Ashuro *et al*., 2022) [1]. Healthcare-associated infections are the most frequent and severe complication acquired in health-care settings with a high impact in terms of morbidity, mortality, and costs (Genovese *et al*., 2020) [2]. Assessments of hospital hygiene indicate that routine cleaning and disinfection may not be performed efficiently and may not be sufficient to eradicate nosocomial pathogens (Weber *et al*., 2013) [3]. Many bacteria could be implicated in these infections, especially multidrug resistance bacteria (Magiorakos *et al*., 2012) [4], which had the capability to efficiently spread from patient to patient and to easily acquire antibiotic resistance determinants (Mirande *et al*. 2018) [5]. Many studies reported that lack of air quality control in the operating theaters was one of the most important causative factors of surgical infections (Otter *et al*., 2011; Andersson *et al*., 2012; and Hailemariam *et al*., 2016) [6], [7], [8].

In our country, the establishment of the Nosocomial Infection Control system started in 2010. The first challenge was the establishment of a vertical system of control and prevention of nosocomial infections from IPH in Regional Hospitals. IPH – Sector for Control and Prevention of Nosocomial Infections is the focal point of this system and at the same time, the Control and Prevention Units of these nosologies were established in the Regional Hospitals. The establishment of control units for hospital infections during the years 2012–2014 has continued with the creation and structural consolidation in relation to the organizational chart defined by the ministry

of health and the ongoing application of the control protocol nosocomial infections. Monitoring microbial contamination in hospital facilities, surgical rooms, resuscitation rooms, and delivery rooms has been one of the objectives of the assessment of nosocomial infections by the Institute of Public Health for more than a decade. Furthermore, the control of nosocomial infections has been consolidated in 12 regional hospitals and this control has been extended to non-public clinics and hospitals with beds. The aimed study was to assess the microbial load in hospital facilities inside Albania Regional Hospitals during the period 2017–2019.

Materials and Methods

A cross-sectional study was conducted during the period 2017–2019 for the assessment of microbial contamination in operating rooms, resuscitation, and delivery rooms in 12 regional hospitals in Albania. One thousand and three hundred microbiological specimens were collected from air and surfaces using 5% sheep blood agar (Oxoid, UK) and processed at IPH microbiology laboratory following the standard bacteriological procedures. Pure isolates were tested against the recommended antibiotics using Kirby–Bauer disk diffusion methods, and the susceptibility profile was determined based on (CLSI, 2020) [9]. The air samples were collected 2 times per day: in the morning between 10 am and 11 am and in the afternoon between 1 pm and 2 pm, taking into consideration the high crowding in these time intervals. The standard protocol was applied for the sample collection using the settle plate or passive air sampling method following the 1/1/1 schedule (on 90 mm diameter sterile Petri dishes containing 5% sheep's blood agar left on the air for 1 h, 1 meter above the floor, and 1 meter away from the wall) (Pasquarella *et al*., 2016) [10].

Sterile gloves, surgical masks, and protective gowns were used during the air sampling procedure, to prevent contamination of the agar plates which were checked visually for any bacterial growth before the use. Similarly, sterile cotton swabs moistened with sterile normal saline were used to collect surface samples on 1 cm by 1 cm·area/cm^2/surfaces such as the floor, walls, equipment, instruments, operation tables, sink, light switch, chairs, beds, patient cloths, door/locker handlers, trolley, stretchers, sinks/faucets, intravenous stands, and oxygen cylinder (Dancer, 2004) [11].

All types of samples were transported to the IPH microbiology laboratory within 30 min for microbiological analysis after being labeled accordingly. Both air and surface samples were inoculated on blood agar plates and incubated at 37°C for 18–24 h. The standard microbiological procedures were applied for the identification of the isolates.

Colony characteristics, Gram reaction, and conventional different biochemical tests were used to identify the isolates (Cheesbrough, 2000) [12]. The microbial concentration of air was expressed as interims of colony-forming units (CFUs) using a colony counter, and the results were expressed in cfu/dm^2/h as described previously (Andersson *et al*., 2012) [7]. Similarly, the swab culture result was expressed in CFUs using colony counter, and results were expressed in cfu/cm^2 (Dancer, 2004) [11].

Kirby–Bauer agar disk diffusion method was used to determine the antimicrobial susceptibility profile of the isolates. The suspension of the identified test organism was prepared from similar colonies. McFarland 0.5 Barium sulfate solutions were used to determine the densities of suspension (Bauer *et al*., 1966) [13].

A sterile swab was dipped into the suspension of the isolate in broth and then speeded over the entire surface of the Muller–Hinton agar plate (Oxoid, Ltd.). Then, the antibiotic disks were placed on the surface of inoculated agar and incubated at 37°C for 18–24 h. The diameters of the growth inhibition of disks were measured and interpreted as per the CLSI guideline (CLSI, 2020) [9].

Data analysis

Data were analyzed using the Statistical Software Package for the Social Sciences (SPSS) version 23 (SPSS Inc., Chicago, IL, USA) for Windows. Descriptive statistic was used to present the data. Categorical variables were expressed as percentages and the Chi-square test was used to compare the proportions between variables. $p \leq 0.05$ was considered significant.

Results

In this study, about 1300 samples from the regional hospitals are processed for the evaluation of the microbial load. The majority of samples were collected in 2019 (44%), as shown in Table 1. Out of the total number of samples, 1148 (88.3%) were collected from surfaces and 152 (11.7%) were from the air. Bacterial growth was identified in 314 (24.2%) out of 1300 samples (95% CI 21.89–26.62). Of the total positive samples (314), the bacterial growth results in 282 (89.8%) positive samples from surfaces and 32 (10.2%) from the air samples. A significant association was found for the samples collection site, $p = 0.035$.

Regarding the sampling place collection, the largest number were collected in operating rooms

Table 1: Distribution of the samples during the periods of study

Variables	Frequency of total samples	Percentage of total samples	Frequency of positive samples	Percentage of positive samples	p-value
Years					<0.01
2017	493	38.0	142	45.2	
2018	235	18.0	75	23.9	
2019	572	44.0	97	30.9	
Site of samples collection					0.035
Surfaces samples	1148	88.3	282	89.8	
Air samples	152	11.7	32	10.2	
Sampling place					0.0001
Surgical/operating rooms	784	60.3	202	64.33	
Emergency rooms	366	28.2	55	17.52	
ICU	100	7.7	6	1.91	
Maternity units	50	3.8	51	16.24	

ICU: Intensive care unit.

(60.3%) followed by emergency rooms (28.2%), intesive care units (ICU) (7.7%), and maternity units (3.8%).

The distribution of positive isolates by year shows that the year 2017 had the largest percentage 142 (45.2%) as compared to 2018 with 75 (23.9%) and 2019 with 97 (30.9%) of positive cultures ($p < 0.01$) (Figure 1).

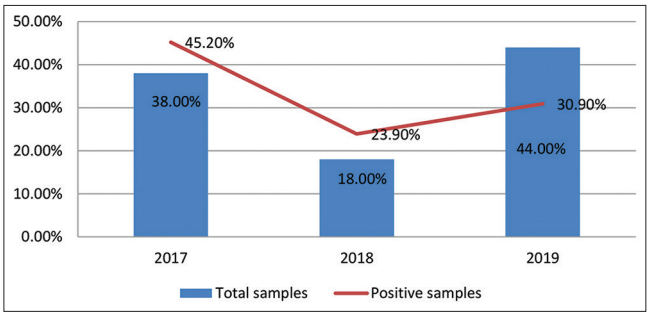

Figure 1: Positivity of samples during the periods of study

Regarding the distribution of total isolates from different wards/rooms, the highest bacterial growth for both surfaces and the air was recovered from the surgical wards/operation rooms at 202 (64.33%), followed by emergency rooms and maternity with 55 (17.52%) and 51 (16.24%), respectively. The least bacterial growth was found in ICUs, at 6 (1.9%) (Figure 2). There was found a significant association for the sampling place (wards/rooms), $p = 0.0001$.

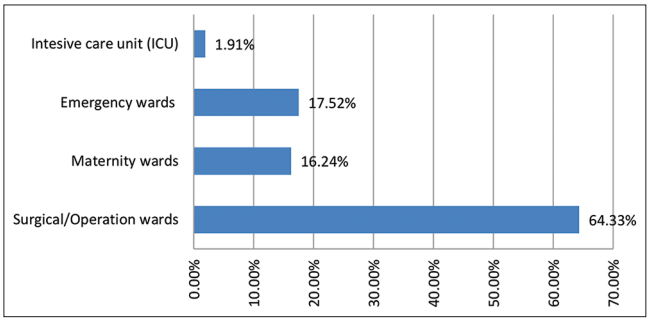

Figure 2: Distribution of positivity related to sampling place

Based on Gram stain, the Gram-negative isolates were predominant at 235 (74.8%), while the Gram-positive was at 60 (19.1%). We need to mention that from 314 positive samples, the mixed growth was reported on 42 (29.8%) samples in both surfaces and the air.

Table 2 shows the bacterial isolates identified during the study period. Overall, *Escherichia coli* was the most frequent bacterial isolate (50%) followed by *Pseudomonas aeruginosa* (23.6%), *Staphylococcus aureus* (19.1%), and *Klebsiella pneumoniae* (1.3%). Furthermore, we found a fungal agent such as *Aspergillus* in 19 (6.1%) samples.

Table 2: Microbial and fungal agents identified during the study period

Positive samples	n	%
Gram stain isolation		
Gram-negative	235	74.8
Gram-positive	60	19.1
Microorganism		
E. coli	157	50.0
P. aeruginosa	74	23.6
S. aureus	60	19.1
K. pneumonia	4	1.0
Fungal agent		
Aspergillus	19	6.1

The mean bacterial load in the air of the surgical ward was 624 cfu/dm^2, and in the maternity ward, 589 cfu/dm^2. The bacterial load of the operating room during the active time was at 93.2 cfu/dm^2. The mean aerobic colony count from surfaces was higher within hospitals than the permitted limits, at 5 cfu/cm^2, in terms of bacterial burden. In the analyzed wards, the mean total aerobic colony counts from all surfaces were 11 cfu/cm^2. It was in the surgical wards, where the greatest mean bacterial colony number was found (18 cfu/cm^2), followed by maternity and ICU at 15.2 cfu/cm^2 and 13.3 cfu/cm^2, respectively, and operating rooms had the lowest at 8.0 cfu/cm^2 (Table 3).

Table 3: The mean of wards bacterial colony number

Wards/rooms	Mean of the bacterial colony (cfu/cm^2)
Surgical wards	18 cfu/cm^2
Operating rooms	8.0 cfu/cm^2
Maternity wards	15.2 cfu/cm^2
ICU wards	13.3 cfu/cm^2

ICU: Intensive care unit.

Figure 3 shows the isolated strain for the periods of the study 2017–2019. During the year 2017, all isolated strains presented a higher number compared to the other 2 years 2018 and 2019. *E. coli* presented a higher difference number between other strains and also a difference between years. During the 2018 year, we show a decrease in the number of isolated strains compared to 2017 and 2019. In 2019, the number of isolated strains undergoes again a significant increase compared to 2018 (Figure 3).

The isolated bacteria's overall drug resistance profile revealed that 66.8% of bacteria were resistant to two or more antimicrobial drugs tested. Figure 4 shows the

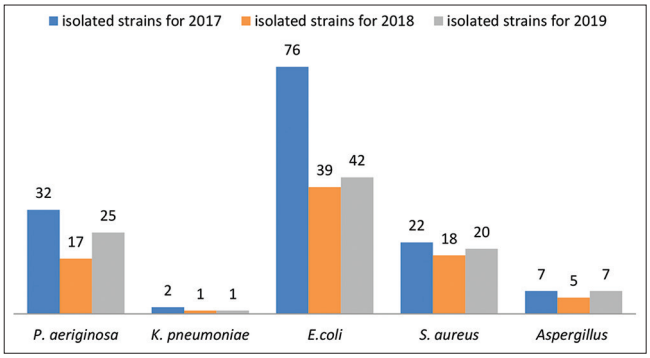

Figure 3: The isolated strains during the periods of study

antibiotic resistance of isolated strains. Every year, we see that the antibiotic resistance of isolated strains undergoes a significant increase reaching its peak in 2019.

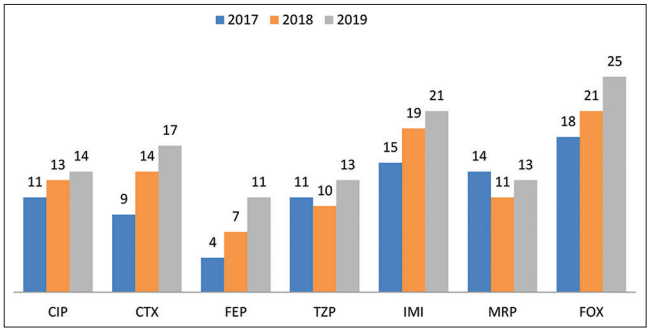

*Figure 4: The antibiotic resistance of isolated strains. *For Gram-negative: CIP: Ciprofloxacin, CTX: Cefotaxime, FEP: Cefepime, TZP: Piperacillin/tazobactam, IMI: Imipenem, MER: Meropenem, and for MRSA: Fox: Cefoxitin*

Discussion

When assessing microbial contamination, an important element is the location and the setting where the microbial load assessment is performed. Different studies had reported that air and hand contact surfaces of the health-care service units are contaminated by different pathogens which might serve as sources of infections. This study was carried out to gain an insight into the distribution, frequency, bacterial load, and antimicrobial susceptibility profile of pathogens in regional hospitals in Albania. Sample collection was performed in surgical wards, operating rooms, intensive care wards, and emergency wards. Regarding the site of sample collection, the largest number were collected in operating rooms (60.3%), followed by emergency rooms (28.2%), ICUs (7.7%), and maternity units (3.8%). Meantime, the aerobic culture results revealed that 24.6% (282/1148) surface samples and 21.1% (32/152) air samples were found contaminated by various bacterial pathogens. This finding is relatively lower than other similar studies done abroad that reported bacterial growth at 52.9% and 65.7%, respectively (Cabo *et al*., 2015) [14].

When we see the specific type of the isolates, *E. Coli* at (50%), *P. aeruginosa* (23.6%), *S. aureus* (19.1%), *Aspergillus flavus* (6.2%), and *K. pneumonia* (1.1%) were predominant.

All of these are known nosocomial pathogens, especially in surgical wards and operating rooms in hospital settings. This result was found to concur with one done in Mexico (Genet *et al*., 2012) [15]. The level of fungal contamination in the hospital environment can increase dramatically by various factors, including construction activities and air conditioning systems. Considering the standards set by the literature, CFUs of <100 CFU/m^3 and more than 1000 CFU/m^3 are the best limits for non-contaminated indoor environments (Rostami *et al*., 2017; Bozic *et al*., 2019) [16], [17].

The WHO guidelines suggested a limit of 50 CFU/m^3 for fungi in the hospital air (Mirzaei *et al*., 2014) [18]. However, the determined limits were above the WHO standard in most sampling sites of this study. The highest fungal density was observed in the derm ward) 110 CFU/m^3). The fungal contamination in the indoor air depends on various factors, such as the outdoor air entrance, meteorological parameters, the number of people and their activities, as well as efficiency of the ventilation system (Rostami *et al*., 2017) [16].

Based on the literature, the most common fungi isolated from the hospital air and causative factors of infections associated with the hospital settings were Cladosporium, *Penicillium*, and *Aspergillus* (Khan and Karuppayil, 2012; Cabo *et al*., 2015) [14], [19].

These species are resistant to water scarcity, which helps them to stay alive in different wards of the hospital for a long time. Therefore, their concentration is higher in hospital air. Ventilation systems were also introduced as one of the sources of fungal bioaerosol (Kim *et al*., 2014) [20]. In comparison to other selected wards, the surgical ward had the highest number of isolates recovered from air and surface samples, implying that the risk of getting nosocomial infections in this particular ward is higher.

About 32 (21.1%) of the total hospital air samples examined during the study period revealed bacterial growth. This meant that a large number of pathogenic germs may float around in the air. The mean bacterial load in the surgery ward, 624 cfu/dm^2, and the maternity ward, 589 cfu/dm^2, were both higher than the recommended levels (250–450 cfu/dm^2) (Fischer *et al*., 1972; Pasquarella *et al*., 2016) [10], [21].

The bacterial load in the operating room during the active time in this investigation was 93.2 cfu/dm^2, which is somewhat more than the acceptable standard limit.

Poor ventilation and cleaning standards, as well as the high and unrestricted number of people, could explain the reported high load of mean aerobic bacterial counts, particularly among medical/health

science students who were present in the hospital most of their time as part of their practical learning process. Similar findings were obtained in other countries' investigations (Fauci *et al*., 2017) [22]. Surface samples were taken in the surgical ward, maternity ward, ICU, and operating room yielded mean aerobic colony counts of 18 cfu/cm^2, 15.2 cfu/cm^2, and 13.3 cfu/cm^2, respectively, in the current investigation. This result exceeds Dancer's permissible limits, which stipulate that the average aerobic count from the bacteriological culture of surface samples should be <5 cfu/cm^2 (Dancer, 2004) [11].

Patients in the study hospital environment may face an elevated risk of infection as a result of the reported figure. Furthermore, the findings urge stakeholders to rigorously analyze and develop infection prevention methods, as well as to monitor the bacteriological quality of the hospital environment regularly. In addition, the findings of this study call for stakeholders to evaluate and strengthen the practice of infection prevention protocols strictly and to regularly monitor the bacteriological quality of the hospital environment. Infection control is of great importance in all wards of a hospital, especially the operating theater. In this regard, the UK and Switzerland set more stringent limits at 35 CFU/m^3 and 25 CFU/m^3 for bacterial density in operating theater ventilation systems, respectively (Dehghani *et al*., 2018) [23]. In general, various environmental factors, such as the service type, ventilation systems' performance, infectious waste management, staffs hand hygiene, surface disinfection methods, as well as air pollution control engineering can significantly affect the level of microorganisms in the hospital's air (Cabo *et al*., 2015) [14].

Numerous studies reported that lack of air quality control in the operating theaters was one of the most important causative factors of surgical infections (Totaro *et al*., 2019) [24]. Many studies reported that Gram-positive bacteria were more frequent than Gram-negative bacteria in the hospital's air (Liu *et al*., 2017; Banerjee *et al*., 2016) [25], [26].

The resistance of different infectious agents to different antibacterial agents poses a high risk of public health threats around the world, and urgent intervention is needed to contain the problem. Large amounts of antibiotics used in medical treatment as well as for livestock have led to the selection of pathogens resistant to multiple drugs (Wise *et al*., 1998) [27].

In this study, the authors tested the antimicrobial resistance profile of isolates to commonly prescribed drugs to highlight their current profile. Medically important bacteria continue to raise increasing concerns around the world about administrative decisions. Widespread use of drugs, especially over/inappropriate/use of antibiotics, lack of regularly updated guidelines for drug selection, and routine microbiology to test antimicrobial susceptibility profiles for common drugs. Lack of methods makes up a large proportion of antibiotic resistance. In this study, 66.8% of bacteria were resistant to more than one antibiotic tested. Very similar results were reported in another study (Fauci *et al*., 2017) [22].

Furthermore, this study found shortcomings or interruptions of the functional chain of control of nosocomial infections in terms of use of the specific disinfectants, managing the entry, and exit of staff but also of the patients and the family members who visit them. It must be emphasized that the identification of these pathogenic microorganisms suggests that the hospital infection control protocol has not been implemented strictly, and rigorously, but also organizational, infrastructural, and technical deficiencies were found as well.

Conclusion

This study revealed that the surface and air and air within different wards of the hospitals studied were contaminated with different types of bacteria. Bacterial loads on the surface and air exceeded normal limits. In addition, the study pointed out high levels of antimicrobial resistance to the drugs commonly prescribed for isolates. Therefore, intervention strategies need to be strengthened to expand infection prevention practices in hospitals. Continuous monitoring and monitoring of in-hospital pathogen types and susceptibility patterns should be performed on a very regular basis.

Ethical approval statement

The study protocol was submitted to the Ethics Committee of the Medicinal Faculty. Ethical approval was not required for this study.

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