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Review Article

Bacteriological Evaluation of Air, Surfaces of Critical Areas and Water, in Operating Rooms of Two Private Health Centers. Valencia, Carabobo 2018

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Abstract

Intrahospital infections (IIH) generate a considerable increase in morbidity and mortality, with the consequent increase in hospitalization costs. They are considered a controllable epidemic problem, but difficult to eradicate; however, through effective programs their incidence can be significantly reduced and morbidity and costs drastically reduced, optimizing the limited allocation of resources for health. In the research, samples of indoor environment were collected from two private centers located in the city of Valencia, Venezuela, samples such as water, air, surfaces. Whose samples have been processed according to Spanish environmental regulations and even through official gazettes and the standard method for drinking water and waste water. The results yielded alarming data, data that were quantified and identified microorganisms causing hospital-acquired infections, which are easily dispersed by hospital areas, affecting susceptible patients, non-susceptible, visitors and staff in general.

Keywords: Clinical Centers; Bacteriology; Hospital-Acquired Infections

Introduction

Over time, the concept of Hospital-acquired Infection (HHI) has changed; Initially, infection that appeared 48 hours after admission, during hospital stay and up to 72 hours after discharge and whose source was attributable to the hospital was included under that term. However, the Center for Disease Control (CDC) in Atlanta has now redefined the concept of IIH as any localized or systemic condition resulting from an adverse reaction to the presence of infectious agents or their toxins, without evidence that the infection was present or incubating at the time of admission to a hospital. that manifests clinically, or is discovered by direct observation during surgery, endoscopy and other diagnostic procedures or tests, or that is based on clinical judgment. This definition includes those infections that by their incubation period manifest after discharge from the patient, and are related to hospital procedures or activity, as well as those related to outpatient services [1].

In relation to this type of infections, various investigations have documented that IIH are an important cause of morbidity and mortality, in addition, they are considered endemic or epidemic diseases, as the case may be, affecting both developed countries and those that are developing, which are lacking resources; in this way IIH constitute an important public health problem worldwide, not only for clinical reasons but also for economic reasons [2].

IIH generates a considerable increase in morbidity and mortality, with the consequent increase in hospitalization costs. They are considered a controllable but difficult to eradicate epidemic problem; however, effective programmes can significantly

reduce their incidence and drastically reduce morbidity and costs, optimizing the limited allocation of resources for health [3].

In Venezuela, there is no periodic national registry of IIH, however, some private centers and hospitals publish their data in a timely manner, but their incidences are very variable, as there is no unification of criteria at the time of collecting the information [4]. According to experts from the Venezuelan Society of Infectiology, the incidence of IIH ranges between 3 and 17% in the world, it is acceptable that it does not exceed 5%, however, in Venezuela it is estimated that it can reach 25% or more, but there are no concrete figures in the country, because there is no statistical data, with which the indices can be compared [5].

In relation to the hospital environment, the transmission of diseases caused by microorganisms can be favored through work tools and contaminated surfaces. These are important factors, because they could condition the application of IIH; however, in recent decades the role of air in the transmission of microorganisms and other toxic substances which are harmful to health, which come not only from hospitalized patients, but also from staff and visitors to the facilities in question [6] has been demonstrated.

In this order of ideas, as air is a vehicle transporting microorganisms, the procedures to have clean air are of utmost importance, especially in critical areas such as operating rooms, where it is required to be an environment free of microorganisms. In order to determine the quality of the various hospital areas, it is necessary to carry out constant environmental monitoring, and thus provide relevant information to hospital areas on the appropriate climatic characteristics to avoid contamination [7].

Several studies exist regarding the evaluation of the hospital environment and air in spaces of surgical interventions, as described above, but, for the study of the water used in the sink of the operating room, these studies are really scarce, and it is considered where a potential source of infection of microorganisms can be found [8].

Finally, Venezuela does not have regulations with which we can compare reference values of quality of hospital environment, this applies to air and surface evaluations, which is why standards of Spanish origin are taken into account, such as the Technical Prevention Standards (NTP); because of this, research like this arises, where not only does it collaborate with the institution in which the project is going to be developed, but also a timely guide is being offered to avoid health problems gr birds, which significantly increase the operating expenses of these institutions [9-11]. Based on the above, the concern was established to develop this research, where it is intended to evaluate the bacteriological quality of air, surface of critical areas and sink water, in operating rooms of two private health centers in Valencia, Carabobo state.

Population and sample

The population for the present study was constituted by all the private health centers, located in the municipality of Valencia of the state of Carabobo, which had one or more operating rooms, in this sense, the population is considered as "the totality of a study phenomenon and includes all the units of analysis that integrate said phenomenon and that must be quantified for a certain study" [12].

The sample consisted of two private health centers located in the municipality of Valencia in the state of CaRabobo (Center A and Center B), each center had 2 operating rooms. The selection of the same was non-probabilistic and intentional, since, it was part of it, those centers whose Coordinators, Directors or Heads approved to participate in the investigation during 2018 [12].

Methodological procedure

Request for authorization to the health center

A verbal and written request was made to the health centers interested in the study to invite them to participate in the research work.

It contemplates a transcendental alliance (if analyzed from ethics), with profound cultural, administrative and personal implications, essential in the activity, where it was guaranteed that the individual (director of the health center), referred his intention to participate in the research after having understood the information that has been added to him (annex 1) [13].

Sampling

To detect and quantify the number of microorganisms present in the air, surfaces of critical areas and sink water, in the study operating rooms, the following sampling was carried out.

Air

Impaction

For the count of bacteria in air, an air sampler MAS 100 NT MERCK (NTP-203 and 299) was used, through which 100 liters of air per minute were sucked for each determination, which was left to impact on Petri dishes (plastic) with blood agar culture medium (BBL), MacConkey agar (BBL). The sampler was placed in strategic locations within each operating room which were: next to the main door, at the back of the operating room [14].

Plate sedimentation

The samples were taken by plate sedimentation in each center, for which a Petri dish (glass) with nutritive agar (BBL) was left for 15 minutes in the corridor area outside the operating rooms, and another in the hand washing area, so that the microorganisms that were in the environment sedimented on them. Subsequently, these plates were incubated at 37°C for 24 to 48 hours for bacterial growth and their respective identification. Sampling in the corridors was carried out with the intention of comparing microorganisms inside and outside the operating room [14,15].

Surfaces

For the study of the surfaces, the swab method described in the Peruvian technical guide for the microbiological analysis of surfaces in contact with food and beverages (461-2007 MINSA) was used, which consisted of rubbing with a sterile swab previously moistened in sterile saline, the area determined in the sampling. In this order of ideas, the sampling was carried out in critical surfaces of the operating rooms such as: work table, operating room lamp handle and surgery table [16].

Procedure [16]

- The swab was moistened in the sterile saline and lightly pressed into the tube wall with a rotational motion to remove the excess solution.
- For flat surfaces, with the swab inclined at an angle of 30 °, the surface d was rubbed 4 times bounded by a template of 100 cm^2, each in the opposite direction to the previous one, the swab was ensured on the entire surface.
- The swab was placed in the tube, with the sterile saline solution, discarding the distal end of the swab that was in contact with the analyst's fingers.
- For uneven surfaces, in the case of utensils, the same procedure was used.

Subsequently, isolation was performed in the laboratory; this was made in Petri dishes with blood agar medium (BBL), MacConkey agar (BBL). They were incubated in an oven at 37°C for 24 to 48 hours, then bacterial growth was examined and conventional biochemical tests were performed for bio typing [16].

Water from sinks in operating rooms

Water samples were taken from the sink of the operating rooms in sterile glass containers of 450 mL, with sodium thiosulfate (0.5 mL at 10%). Overall, a portable lighter was also used to avoid any type of pollution from the air. It is important to note that the sink faucet was flamed with the lighter, to avoid contamination and the water was allowed to flow for several seconds before taking the sample [17,18].

Temperature and humidity measurement

For the determination of temperature and humidity, a thermohydrometer (Mannix Model CMM 880) was used in the same areas where the samples were taken.

Transport of the sample

All air, surface and water samples were transported in a container and isothermal to the laboratory for analysis.

Microbiological processing.

For microbial identification, the conventional biochemical identification, macro and microscopic observation method was used.

Cultivation and isolation

Once in the laboratory, striation was carried out in plates with blood agar, MacConkey agar (BBL) and nutritive agar (BBL), for the primary isolation of microorganisms from samples from surfaces; these were then incubated in an oven at a temperature of 37°C for 24 to 48 hours, together with the environmental plates (by sedimentation) and those of air [14].

As for the water samples, a filtering with microfiltration membranes of 0.45 µm for total and fecal coli forms on MacConkey agar and 0.22 µm for Pseudomonas on cetrimide agar was

performed, simultaneously, the sample was determined mesophilic aerobic count by the plate pouring technique with nutritive agar [18].

These samples are incubated with the rest of the abovementioned samples at a temperature of 37°C for 24 to 48 hours [19]. Additionally, the water samples were performed a direct microscopic examination, to show the presence of parasitic forms [20].

G ram stain and direct observation of colonies.

At the end of the incubation time, the plates were observed to show bacterial development; Gram staining of the isolated colonies was performed in order to identify their morphology [14].

Biochemical tests

Biochemical tests were performed according to the type of microorganism isolated according to morphology, tintorial affinity and physiological characteristics. Later they were incubated at 37ºC for 24 hours, after this time the pruebas were read and developed to identify the microorganism with genus and species. Likewise, the isolated colonies were minced in nutritive agar (BBL) to preserve them and arrange them fresh for the realization of the susceptibility test to thymicrobial once the microorganism was known [14].

The biochemical tests used for identification in case of being a Gram negative bacillus were: triple sugar agar (TSI), lysine-iron agar (LIA), motility-indole-ornithine (MIO), urea, sulfur-indole-motility (SIM), citrate and malonate. For those bacteria that did not use lactose, the oxidase test was also performed, with growth at 42°C, oxidation/fermentation, and fresh motility. The biochemical tests for the identification in case of being a Gram positive coconut were: Catalase, coagulase, salted mannitol, DNAsa, bile-esculin, hyper salty broth and growth at 42°C. In the event that yeast has resulted in the isolation, the germ tube test was performed [14].

Antimicrobial susceptibility test

Once the phenotypic characteristics of the isolated microorganisms were carried out, the antimicrobial susceptibility pattern was evaluated, using the Kirby-Bauer methodology recommended by the CLSI 2019 (Clinical and Laboratory Standards Institute) [21].

A suspension of the strain to be studied in sterile Saline Solution was made until reaching a density equal to that of the 0.5 McFarland pattern, a sterile swab was impregnated with this suspension for subsequent sowing in three directions on Müeller-Hinton Agar and within the next 20 minutes up to 7 discs of antibiotics per plate were strategically located. The PSA reading was taken after 24 hours of incubation at 37°C, and the diameter of the inhibition halo obtained was compared with the CLSI 2019 table [21].

Evaluation of biocides

For the evaluation of the biocides used by the clinics for the sanitization of the operating rooms, a sample of the biocide used by the health center in the operating room was taken in a sterile urine collector; this was confronted in the laboratory against the microorganisms isolated from the center; these microorganisms were exposed to the biocide for 5, 10 and 15 minutes to later analyze the effects of the same, evaluating the microbiological growth [22].

Each microorganism was previously isolated and identified, then 4 test tubes were prepared, one with the biocide, with which a bacterial solution (0.5 Macfarland) and another 3 tubes with sterile nutritive broth were prepared, which were labeled as follows (5, 10 and 15 minutes). Once the first tube of the biocide with the bacterial suspension has been prepared; the time was timed; After the first 5 minutes, an aliquot of 0.3 mL of the suspension was taken and added to the tube labeled with “5 minutes” of nutritious broth. This process was repeated with the remaining tubes of 10 and 15 minutes [22].

Once the procedure was performed, the sealed tubes were incubated in a study at 37°C for 24 to 48 hours, then it was verified whether or not there was growth of the bacterial inoculated agent and thus evaluate the effects of the biocide [22].

Data analysis

The results obtained were presented in tables and graphs, showing absolute frequency of the variables, descriptive statistics were applied for subsequent analysis and related to the variables and objectives established. This was done with the Microsoft Excel computer program and the SPSS statistical program [2].

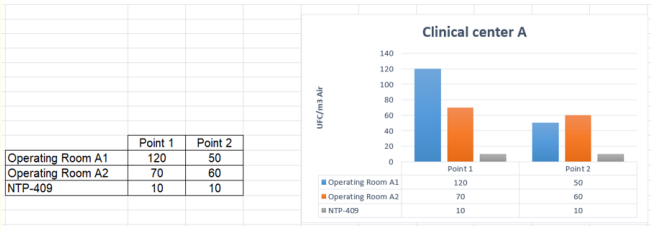

Figure 1: Determination of CFU/m3 of air in the operating room compared to NTP-409, in health center A.

Results

The Spanish Technical Prevention Standard 409 (NTP-409), establishes a microbial level lower than 10 CFU/m^3 of air in critical areas such as operating room [10]. In this order of ideas, for the two operating rooms of health center A, in the method of impaction, a statistically significant difference ($p < 0.05$) was obtained to be a higher amount of CFU/m^3, compared to the reference standard; while, in the sedimentation plates for the common area of the external part of the operating rooms, for the hand washing area, a reading of 7 CFU/m^3 was obtained, and for the aisle area 18 CFU/m^3.

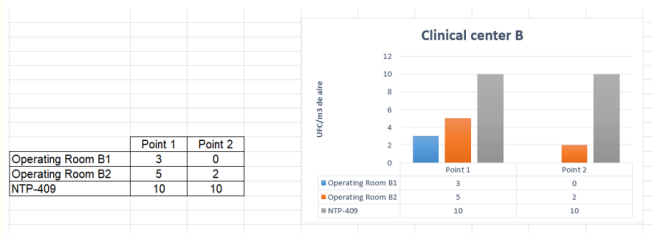

Figure 2: Determination of CFU/m3 of air in the operating room compared to NTP-409, in health center B.

In Health Center B, CFU/m3 were lower in both operating rooms compared to the Spanish non-MRA (10) ($p < 0.05$), even in operating room B1 there was no growth at point 2. In this order of ideas, in the common external areas of both operating rooms, by the sedimentation method, growth of 9 CFU/m3 was obtained in the washing area of men, and for the corridor area 35 CFU/m^3.

Regarding the temperature values, there were no significant differences ($p > 0.05$) in points 1 and 2 in all the operating rooms

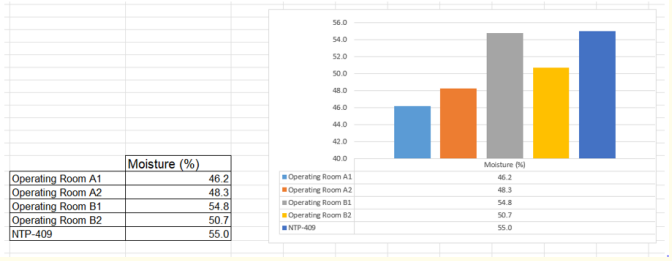

Figure 3: Temperature determination compared to NTP-409, in health centers A and B.

analyzed, therefore, a single average value is shown in each of the operating rooms. In this order of ideas, for temperature, all operating rooms showed values above the norm of up to 6.1 C ° (Operating Room A1).

Figure 4: Determination of relative humidity compared to NTP-409, in health centers A and B.

There were no significant differences ($p > 0.05$) in points 1 and 2 in all operating rooms analyzed for humidity, therefore, a single average value is shown in each of the operating rooms. The humidity was always equal to or lower, compared to the values of the Spanish reference standard, the greatest difference was observed in the A1 operating room obtaining a difference value of 8.8%.

For health center A, in operating room A1, growth was higher on all surfaces analyzed, compared to the other operating rooms of both centers. On the other hand, in health center B, operating room B1, on the lamp handle surface, showed greater bacterial growth in contrast to operating room B2, belonging to the same center.

Place	Operating Room 1	Operating Room 2	Operating Room 3	Operating Room 4
Camilla	++	+	-	+
Lamp handle	+++	+	++	+
Work table	++	-	+	-
Semi-quantitative assessment: (-) negative, (+) 1-49 CFU, (++) 50-100 CFU, (+++) >100 CFU				

Table 1: Qualitative bacterial growth on operating room surfaces of two private health centers. Valencia, Carabobo 2018.

Microorganisms	F	%
Staphylococcus aureus	4	57.13
Streptococcus spp	1	14.29
Bacillus spp	1	14.29
Enterococcus spp	1	14.29
Total	7	100.00

Table 2: Frequency of bacteria isolated in the air and operating room surfaces of two private health centers. Valencia, Carabobo 2018.

The microorganism present in the air most frequently, in all centers was Staphylococcus aureus, even it was also detected in the area of corridors and hand washing, of the two health centers.

In this order of ideas Staphylococcus aureus, it was also the only microorganism isolated on the different surfaces analyzed, from all the operating rooms in both health centers.

	Mesophilic aerobes CFU/mL	Total coliforms CFU/100mL	CFU/100mL fecal coliforms	*Pseudomonas aeruginosa* UFC/100mL	Molds and yeasts CFU/mL	Parasitic forms
Health Center A	$4,0 \times 10^4$	16	0	9	0	Abundant flagellates and ciliates
Health Center B	$2,8 \times 10^4$	9	0	5	0	Abundant flagellates and ciliates
G.O. 36,395	<100	0	0	0	0	0

Table 3: Evaluation of tap water in operating rooms of two private health centres. Valencia, Carabobo 2018.
CFU/mL: Colony forming units per milliliter of sample CFU/100 mL: Colony forming units per 100 milliliters of sample G.O. 36.395: Official Gazette "Sanitary Drinking Water Quality Standards".

Mesophilic aerobes were significantly higher in both health centers ($p < 0.05$), compared to the Venezuelan norm. On the other hand, parasitic forms such as flagellates and ciliates abundant in the microscopic analysis (>10x field) were determined.

The double-stranded quaternary ammonium used in health center A, had an effectiveness of 100% at 10 minutes compared to the strains isolated in the sampling, compared to bromine-based quaternary ammonium used in health center B, which had an effectiveness of 0%, however, the two biocides obtained an effectiveness of 100% at 15 minutes.

Center	Origin	Microorganism	5 minutes	10 minutes	15 minutes
Health Center A Double quaternary ammonium chain	Air	*Streptococcus* spp	C	S/C	S/C
	Air	*Staphylococcus aureus*	C	S/C	S/C
	Surface	*Staphylococcus aureus*	C	S/C	S/C
	Surface	*Staphylococcus aureus*	C	S/C	S/C
Health Center B Bromine-based quaternary ammonium	Air	*Bacillus* spp	C	C	S/C
	Air	*Staphylococcus aureus*	C	C	S/C
	Surface	*Staphylococcus aureus*	C	C	S/C
	Surface	*Staphylococcus aureus*	C	C	S/C

Table 4: Biocide efficiency: Microdilution as a function of time, in operating rooms of two private health centers. Valencia, Carabobo 2018.

The antibiotic that presented the highest frequency of resistance in the strains of S. aureus was erythromycin, as well as linezolid and clindamycin. Erythromycin also presented resistance in the studied strain of Streptococcus spp and finally Enterococcus spp presented resistance to rifampicin and linezolid.

Antibiotics	*S. aureus* (n = 13)			*Bacillus* spp (n = 1)		
	S	I	R	S	I	R
AMIKACIN	12	1	-	1	-	-
CEFTRIAXONE	-	-	-	1	-	-
CIPROFLOXACIN	13	-	-	1	-	-
CLINDAMYCin	4	6	3	-	-	1
ERYTHROMYCIN	-	2	11	-	-	-
GENTAMICIN	12	1	0	1	-	-
LEVOFLOXACINA	9	4	-	1	-	-
LINEZOLID	2	1	10	-	-	-
TEICOPLANIN	-	-	-	-	-	-
VANCOMYCIN*	8	3	2	1	-	-

Table 6: Antimicrobial susceptibility of species isolated in operating rooms of the private health center B. Valencia, Carabobo 2018.

The antibiotic that presented the highest frequency of resistance in the strains of S. aureus was erythromycin, as well as linezolid, also Bacillus spp presented resistance to clindamycin.

Discussion

In a clinical center, IIH is a serious health problem, representing a significant amount in terms of infectious diseases, increasing their mortality rates, costs and hospital extensions [2]. Therefore, it is a priority to maintain an environmental control where the ranges or reference values of all those factors that may favor bacterial proliferation can be maintained [7].

The Spanish Prevention Technical Standard 409 (NTP-409), establishes a microbial level lower than 10 CFU/m^3 of air in critical areas such as operating rooms; if we refer to graph 1 in both points of the clinical center (A) values higher than 50 CFU/m^3 were found being not acceptable because of the stimulated in the standard. These values are similar to those obtained by Izzeddin in 2017, where his results were between 50 and 60 CFU/m^3, highlighting that in the clinical center (B) the values of the counts were lower than 5 CFU/m3 complying with the established [14].

In another order of ideas, the temperature measurements of all operating rooms were found elevated in relation to what was established by the (NTP-409) similar to that evaluated by Izzedin N in 2011 where the temperature of the operating room studied exceeded the limit, this factor being favorable for bacterial growth.

It must be essential to maintain the air conditioning systems as well as keep the doors closed and thus reach adequate temperatures. In relation to the relative humidity in the clinical

center (A) the values obtained were in what was established by the standards, which favors bacterial control; However, in clinical center (B) the values were below the values. This could cause dryness in the mucous membranes of the patients [6].

In this study, a total of 12 surface samples were evaluated, presenting 9 of them bacterial growth in both clinical centers, similar to the study presented by Izzedin N 2017 where more than 50% of the surfaces evaluated will present n pollution which can be propitiated by the air as a vehicle of microorganisms or the breach of biosafety standards by the health team. Therefore, the use of the clothing required for this type of ambientes is recommended [14].

In relation to the isolated microorganisms, it was evident that Staphylococcus aureus prevails with a frequency of (66%) being this usual microbiota of the skin and ubiquitous in the environment. Obtaining a similarity with what was mentioned or in the work of Pérez G 2016 where a frequency of 64% of Staphylococcus spp was evidenced in clinical centers [7]. Garza and Zúñiga 2012 mention the clinical importance of this germ in the hospital environment adding that it can cause affections in those patients weakened either by injuries caused by surgery or to treatments for which they have been subjected that lower cellular immunity (bibliography to add http://www.scielo.org.mx/pdf/eq/v24n1/v24n1a2.pdf).

On the quality of water there is little that is known, about the quality in the clinical centers since there are no elements to decide what is the best method to determine whether or not there is microbiological contamination in a clinical center (https://www.redalyc.org/journal/2738/273849945004/html/) also the reference values of the G. O. 36.395 were utilizer: Official Gazette "Sanitary Standards of Drinking Water Quality" where in both centers the bacterial and parasitological load exceeded the established, similar to the results exposed Germán Oved 2016 where the bacterial load was high (https://www.redalyc.org/journal/2738/273849945004/html).

Other parameters studied but no less important, were biocides; in both clinical centers were used compounds of Quaternary Ammonium, presenting a bactericidal effect in a time 15 minutes. A study conducted by Ramos in 2011 evaluated 4 bacterial strains isolated from patients with IIH of which only one presented resistance against a quaternary ammonium compound, unlike in this study the strains were only exposed up to a time of 5 minutes of exposure being time a determining factor for a bactericidal effect (http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S1315-25562011000200009).

With respect to the study of the antibiogram for the strains of Staphylococcus aureus, presented resistance to Clindamycin levofloxacin, gentamicin, linezolid, erythromycin and vancomycin by the method of kirby Bauer, however vancomycin could not be verified by the minimum inhibitory concentration (MIC) which is recommended before stopping the health authorities, Tapia E 201 3 presented in his study resistance bacteria of S. aureus for vancomycin sterilized blankets (http://www.scielo.org.bo/pdf/rccm/v17n1/v17n1_a06.pdf).

Conclusions

Clinical center A presented high values of microorganisms present in the air greater than 10 CFU/m^3 allowed by NTP.409, however clinical center B presented values less than 5 CFU/m^3 complying with the standard, while water analysis in both clinical centers presented high bacterial and parasitic loads in the water, that is, they did not comply with the provisions of G.O. 36,395.

In both clinical centers, the most frequently identified microorganism was staphylococcus aureus, however Streptococcus spp and Enterococcus spp were also identified to a lesser extent in clinical center A and clinical center B Bacillus spp.

The antibiograms obtained in the different strains studied of S. aureus present aron resistance to Clindamycin levofloxacin, gentamicin, linezolid, erythromycin. Similarly Streptococcus spp presented resistance only to erythromycin, also Enterococcus spp present resistance to linezolid and rifampicin, finally Bacillus spp presented resistance only to clindamycin

In the effectiveness of the biocides, an efficiency was obtained in times greater than 10 minutes in clinical center A and greater than 15 minutes in clinical center B, using both centers composed of quaternary ammonium of different commercial brands.

Temperature measurements were greater than or equal to 18°C and relative humidity less than 50% in clinical center B, whose ranges should be: 15°C-18°C, and 50 and 70%, respectively.

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