https://doi.org/10.5272/jimab.2018244.2236

Journal of IMAB - *Annual Proceeding (Scientific Papers)*. 2018 Oct-Dec;24(4)

Journal of IMAB
ISSN: 1312-773X
https://www.journal-imab-bg.org

Original article

MICROBIOLOGICAL MONITORING OF HOSPITAL ENVIRONMENT IN REGION VARNA

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ABSTRACT

The hospital environment is widely contaminated with opportunistic and pathogenic microorganisms and is considered as a source of outbreaks of nosocomial infections which are a worldwide phenomenon. Thus, it is clear that monitoring of the hospital environment is an essential element in the control of nosocomial infections.

The purpose is to isolate and identify the microbes from hospital environment samples of different hospitals and healthcare services in the Region Varna and to analyze the risk that hospital environment poses in the region.

This study was conducted over a period of 5 years from January 2012 to December 2016. A total of 12,673 different types of samples were collected and delivered quickly to the Laboratory of Microbiology of Regional Health Inspectorate Varna, where analyses were performed.

We found bacteria in 816 of the tested objects and isolated a total of 29 bacterial species. The results show predominant Gram positive flora (71.81%), while Gram negatives represented only 28.19%.

Prevention of nosocomial infections is a responsibility of all health-care workers. Microbiology laboratories should play a significant role in the attempts to minimize the occurrence of nosocomial infection through accurate identification of responsible organisms, timely reporting of laboratory data and participation in hospital infection control.

Keywords: Nosocomial infections, Bacterial contamination, Hospital environment, Hospital-acquired infection

INTRODUCTION

Many microorganisms can colonize the hospital environment and can compose true ecological niches [1]. There is an increasing evidence suggesting that the environment may act as a reservoir for some of the pathogens causing hospital acquired infections [2]. According to World Health Organization (WHO) hospital acquired infections (HAIs), also known as nosocomial infections, are "…Infections acquired during hospital care, which are not present or incubating at admission. Infections occurring more than 48 hours after admission are usually considered nosocomial." [3]. Nosocomial infections are a worldwide phenomenon. It is estimated that at any time, over 1.4 million people in the world are suffering from infections acquired in treatment centres, with 80000 deaths annually [4]. The hospital environment is a reservoir of pathogenic microorganisms in a higher or lesser extent, regardless of the world region [5].In developed countries nosocomial infections affect 5% to 10% of all patients admitted to healthcare centres. In developing countries they impact on 25% of hospitalized patients [1].Thus, it is clear that monitoring of the hospital environment is an essential element in the control of nosocomial infections.

Furthermore, the widespread use of broad spectrum antibiotics has led to the acquisition of resistance to antibiotic agents, complicating the treatment of infections due to drug-resistant pathogens. Significant problem are nosocomial infections caused by multi-drug resistant organisms, which lead to increased morbidity and mortality from HAIs.

In the process of limiting the dissemination of antimicrobial resistance (AMR) and HAIs, it is necessary to consolidate the efforts at European and world level.

From May 11, 2013, the Regulation No. 3/2013 of the Ministry of Health on prevention and control of nosocomial infections has been implemented in Bulgaria [6]. The main objective of this standard is to limit the spread of HAI and AMR through the exercise of epidemiological surveillance, control and systematic implementation of preventive measures. According to the regulations all hospitals should have a Program for prevention and control of HAI sand for limiting AMR. The head of the health care facility must provide the necessary organizational, staff and financial resources to implement the programs. The implementation control is carried out by the Minister of Health, the Regional Health Inspectorates, the Medical Audit Executive Agency and the management bodies of the health care establishments.

The purpose of this study is to isolate and identify the microbes from hospital environmental samples of different hospitals and healthcare services in Region Varna and to analyze the risk that hospital environment poses in the region.

MATERIALS AND METHODS:

Period of study and materials:

This study was conducted over a period of 5 years from January 2012 to December 2016. A total of 12673different types of samples were collected from 14 hospitals and 38 Medical centers in Region Varna, Bulgaria. The samples were taken from surfaces, medical tools and specialized medical equipment, patients'objects and dishes, and laundry.All samples were delivered as quickly as possible to the Laboratory of Microbiology of Regional Health Inspectorate Varna, where analyses were performed.

Methods:

1. Sampling surfaces of objects and medical devices

The surface samples were taken one hour after disinfection from an area of 100 cm^2 with a sterile swabs moistened in sterile isotonic liquid. Each swab was inoculated on the surface of agar medium (blood agar and Endo agar) for48 hours.

2. Hand-swab samples from medical personnel

After preliminary disinfection with the currently used disinfectant, personnels' fingers and hands were rubbed with sterile swabs moistened with a sterile isotonic liquid. The collected samples were immediately inoculated on the surface of agar medium (blood agar and Endo agar).

3. Assessment of microbial number in disinfectant solutions

These samples were taken under aseptic conditions using sterile laminar flow cabinets and protective equipment (sterile laboratory coat, gloves and eye protection). Samples were collected in sterile containers and then diluted 1:10 (9 ml saline solution and 1 ml sample). After homogenization 0.2 ml of samples was spread on the whole surface of the agar medium (blood agar and Endo agar). The inoculated media were incubated at 36±1ºC for 48 h. After incubation the colony count was determined to define the microbial number.

4. Assessment of microbial number in dialysis fluids

These samples were also taken under aseptic conditions. Three fixed volumes (1 ml, 0.5ml and 0.1 ml) of dialysis fluids were poured onto sterile plate and were mixed with warm agar (40-50ºC). The samples were swirled to mix and were allowed to solidify. Plates were incubated at 36±1ºC for 48 h. The resulted colonies were counted and the microbial number was estimated.

5. Control of sterilized materials

Pieces of gauze, threads or cotton were placed aseptically in tubes with tryptone soya broth and liquid tioglycolate medium. The samples were incubated for 14 days at 36±1ºC.

Bacteria isolated from all types of samples were identified to genus or species by biochemical tests.

RESULTS:

During the present study, a total of 12673 samples were collected: 5541 samples from medical tools and specialized medical equipment, 5109 samples from surfaces, 603 – from hands, 1164 samples from disinfection solutions, 204 – from patients'objects and dishes and 53 samples – from laundry (Table 1).

Table 1. Number of samples collected

	2012		2013		2014		2015		2016		Total	
Sample type	**N samples**	(+) samples	**N samples**	(+) samples	**N samples**	(+) samples	**N samples**	(+) samples	**N samples**	(+) samples	**N samples**	(+) samples
Hands	**163**	23	**159**	21	**103**	15	**78**	22	**100**	24	**603**	105
Medicaltools	**955**	38	**1222**	32	**683**	47	**955**	51	**824**	53	**4639**	221
Specialized medical equipment, sterilized materials and dialysis fluids	**323**	12	**251**	12	**256**	11	**44**	5	**28**	9	**902**	49
Laundry	**1**	1	**14**	8	**3**	1	**24**	10	**10**	3	**52**	23
Patients' objects	**60**	9	**47**	12	**7**	5	**43**	11	**16**	9	**173**	46
Dishes			**1**	1	**5**	5	**12**	8	**13**	5	**31**	19
Surfaces	**1250**	83	**766**	58	**1280**	77	**874**	41	**939**	39	**5109**	298
Disinfectant Solutions	**272**	10	**256**	14	**238**	15	**210**	10	**188**	6	**1164**	55
Total	**3024**	176	**2716**	158	**2575**	176	**2240**	158	**2118**	148	**12673**	816

The highest proportion of positive results we found among samples of dishes (61.31%) and laundry (44.2%), followed by objects of patients (26.58%) and hands (17.4%). The lowest number of positive samples (0.54%) was detected in the group of specialized medical equipment.

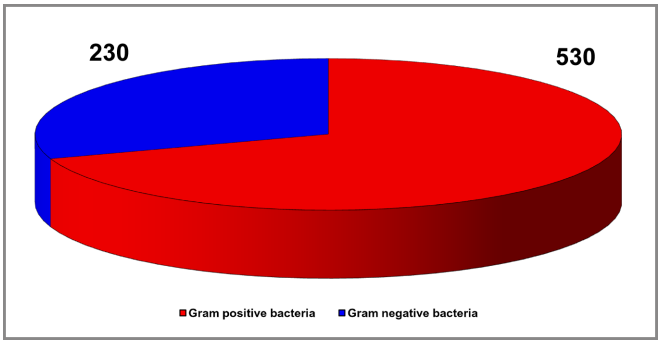
We found bacteria in 816 of the tested sites/objects and isolated a total of 29 bacterial species, presented in Table 2.

Table 2. Bacteria isolated from the tested sites/objects

	Bacteria	2012	2013	2014	2015	2016	Total
Gram +							
	Staphylococcusaureus	5	2	3	9	4	23
	Streptocccus - á	2	1	1		1	5
	Bacillusspp.	10	8	17	11	13	59
	Micrococcusluteus	2	7	13	8	7	37
	Enterococcusfaecalis				1	2	3
	Candida				1		1
	Non-diphtheriaeCorynebacteriumspp.				8	1	9
	Coagulase-NegativeStaphylococci	104	103	78	91	73	449
Gram-							
	Pseudomonasaeruginosa	5	1	17	5	2	30
	Pseudomonasputida				1	7	9
	Pseudomonasstutzeri			3	2	3	9
	Pseudomonasoryzihabitans			9			9
	Acinetobacterlwoffii	22	24	18	16	25	105
	Acinetobacterbaumannii	3	1	3			7
	Escherichiacoli	9	1	2			12
	Enterobactercloaceae	1	2			1	4
	Enterobacteraerogenes		3	3		1	7
	Pantoeaagglomerans	5		2		3	10
	Klebsiellaoxytoca	2		1			3
	Klebsiellapneumoniae	2			1		3
	Proteus mirabilis	1	1				2
	Sphingomonaspaucimobilis	2	3	1			6
	Citrobacter	1				1	2
	Alcaligenesfaecalis			5	3	2	10
	Alcaligenesodorans					1	1
	Moraxelladuplex					1	1
	Total	176	158	176	158	148	816

The results show predominant Gram-positive flora (71.81%), while Gram negatives represented only 28.19% (Fig. 1).

Fig. 1. Proportion of Gram positives and Gram-negatives species in the collected samples.



The analysis of bacterial strains isolated from the studied objectsshows a diversified microflora. The majority belongs to coagulase-negative *staphylococci* (55%), *Acinetobacter lwoffii* (13%), *Bacillusspp.* (7%), *Micrococcus luteus*(4.5%), *Pseudomonasaeruginosa* (3.7%), *Staphylococcusaureus* (2.8%), *Escherichiacoli* (1.5%), *Pantoeaagglomerans*(1.4%), *Alcaligenesfaecalis* (1.2%),*Non-diphtheriaeCorynebacteriumspp., Pseudomonas putida, Pseudomonas stutzeri, Pseudomonas oryzihabitans*(1.1%), and *Enterococcus faecalis, Acinetobacter baumannii, Enterobacter cloaceae, Enterobacter aerogenes, Klebsiella pneumonia, Sphingomonaspaucimobilis* (< 1%).

DISCUSSION:

Nosocomial infections occur worldwide and affect both developed and developing countries. They represent a major problem both for the patient and the public health(3). In our study we noted predominant prevalence of Gram-positive bacteria(71.81%), while Gram negatives represented only 28.19%. *Lemmen et al.* also reported a higher percentage of Gram-positive bacteria (24.7%) than Gram-negative bacteria (4.9%)[7], but the results of the study conducted by *OualiLalami et al.* showed predominance of Gram-negative bacteria(73.33%)[1].

Our results agree with the study of *Oumokhtaret al.* who have found prevalence of coagulase-negative *staphylococci* (47%), *Enterobacteriaceae* (39.2%), *Bacillus sp* (20%), *Enterobacter sp., Pantoea sp., Klebsiella sp., Escherichia coli, Pseudomonas sp., Stenotrophomonas sp., Enterococcus sp.*[8]. Nabila et al have found that the most frequently isolated germs were *Bacillus* (27%) and coagulase-negative *staphylococci* (26%) followed by *Staphylococcus aureus* (20%) and *Klebsiella pneumoniae* (16%) and finally *Pseudomonas aeruginosa* (5%) as well as *Enterobacter cloacae* (5%) and *Proteus Vulgaris* (1%) [9].

The results of our study and other studies show that the hospital environment is widely contaminated with opportunistic and pathogenic microorganisms and is considered as a source of outbreaks of nosocomial infections.We found that dishes and laundry are the most contaminated with microorganism, followed by objects of patients and hands. They could play important role in development of nosocomial infections as a reservoir of microorganisms that can be transmitted in hospitals by several routes: direct and indirect contact, airborne, common vehicle and vector borne[10]. The most frequent route of transmission is indirect contact through a health care worker or a medical kit [4]. The infected patient could contaminate a second individual during the process of treatment by touching an object, an instrument, or a surface [11].

CONCLUSION:

Nosocomial (hospital-acquired) infections continue to represent a major problemfor the public health. The results of our study showed that gram-positive bacteria are most frequently the etiologic agents, but a number of other pathogens are also playing a role. All HAIs are difficult to control. However, prevention of nosocomial infections is a responsibility of all health-care workers. Microbiology laboratories should play a significant role in the attempts to minimize the occurrence of nosocomial infection through accurate identification of responsible organisms, timely reporting of laboratory data and participation in hospital infection control.

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Please cite this article as: Nedelcheva G, Tsankova GS, Ermenlieva NM, Todorova TT, Tsankova DT. Microbiological Monitoring of Hospital Environment in Region Varna. *J of IMAB.* 2018 Oct-Dec;24(4):2236-2240.
DOI: https://doi.org/10.5272/jimab.2018244.2236

Received: 18/06/2018; Published online: 20/11/2018

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