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PREVALENCE OF GRAM-NEGATIVE BACILLI ISOLATED FROM THE EQUIPMENT AND SURFACES IN HOSPITAL WARDS OF GOLESTAN PROVINCE, NORTH OF IRAN

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Nosocomial infections are one of the most common causes of death in hospitals. This study aimed to determine the prevalence of gram-negative bacilli isolated from the equipment in hospital wards of the Golestan province, in the year 2015. In this cross-sectional study in 2015, 1980 samples from medical and nonmedical equipment and surfaces were collected from the wards of 13 teaching hospitals, in the Golestan province. Samples were inoculated into eosin methylene blue agar and blood agar culture media and isolated colonies were identified by standard biochemical tests. The obtained results were then analyzed using SPSS 22 software and χ^2 test. Among 1980 isolated samples, 601 samples (30.35%) were infected with gram-negative bacilli while *Enterobacter aerogenes* (37.27%) was responsible for most of the contaminations. The highest rate of infection was observed in the intensive care unit (33.1%), and the highest level of contamination in the medical equipment was associated with laryngoscope and its blade (10.48%), as well as ECG sensor and its monitoring connector (6.65%). Meanwhile, phone (6.32%) and patients' beds and linen (5.15%) had the highest level of contamination in the nonmedical equipment. Considering the high rates of gram-negative bacilli contamination in the hospital wards of the Golestan province, thorough hand washing as the main action for disinfection and sterilizing the equipment, as well as performing periodic cultivation alongside the use of standard guidelines for prevention and control of nosocomial infections, are recommended to reduce the level of contamination.

Keywords: gram-negative bacilli, nosocomial infection, hospital equipment

Introduction

Despite recent medical and technological advancements, microbial contamination in hospital settings and its consequent complications remain as a challenge in all countries. Infection prevention is an essential element of hospital management [1, 2], which requires identification of contaminations sources and attempts to effectively control the infection in hospital equipment [3]. Frequent sampling from hospital equipment along with their microbiological culture is among the most important factors for infection control in hospitals [4].

A recent study in the United States indicated that gram-negative bacilli are responsible for more than 30% of all hospital-acquired infections and Enterobacteriaceae family is the most common group of this category [5]. Majority of these are opportunistic bacteria that can lead to infection in immunocompromised patients [6]. Medical equipment and surgical instruments are associated with nosocomial infections, since pathogens can often live on surfaces for months and remain as continuous source of microorganisms' transmission in hospital settings. Gram-negative bacilli such as *Acinetobacter* spp., *Escherichia coli, Klebsiella, Pseudomonas aeruginosa,*

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Serratia marcescens, and *Shigella* can resist most antibiotics and survive for months on dry surfaces and wet environments as well as extreme environments compared to other bacteria. All the above factors are involved in their survival in hospitals' sinks, baths, hot water pipes, medical supplies and equipment, leading to nosocomial infection of patients [6, 7]. It is currently not possible to completely eliminate infections, but taking appropriate measures (to determine the contamination levels of hospital equipment, using the most appropriate methods of sterilization and effective antibiotic treatment) may reduce the rate of infections, costs, and the resulting morbidity and mortality [7, 8]. Given the importance of this issue and the lack of a comprehensive study in Golestan province, North-eastern Iran, this study aimed to determine the prevalence of gram-negative bacilli isolated from the equipment in hospital wards of the Golestan province, in the year 2015.

Materials and methods

Sample collection

In this cross-sectional study, 1980 samples from medical supplies and equipment (1502) and nonmedical surfaces (478 cases) were collected from 7 different wards (operating room, intensive care unit [ICU], cardiac care unit [CCU], surgical ward, pediatric ward, emergency, and internal medicine unit) of 13 teaching hospitals in the Golestan province (three hospitals in Gorgan, three in Gonbad, a hospital in Kordkoy, Bandar-Gaz, Bandar-Turkman, Aq-Qala, Aliabad, Minoodasht and Kalaleh) through census method. Contamination with gram-negative bacilli, 1–2 h after the sterilization of equipment, was the criteria for evaluating the contamination rates.

Isolation and identification

Samples from different medical and nonmedical surfaces that had contact with patients were collected using sterile swabs which had been dampened with Trypticase Soy Broth liquid medium. The samples were sent to the laboratory under sterile conditions, and after inoculation in eosin methylene blue agar and blood agar culture media for 24 to 48 h, they were incubated at 37 °C and examined for growth. Identification of contaminating agents was performed by gram staining and standard biochemical tests such as indole, methyl red, voges proskauer, citrate (IMViC), triple sugar iron (TSI), oxidative fermentative (OF), oxidase, catalase, ortho-nitrophenyl-β-galactoside (ONPG), urea agar, lysine, ornithine decarboxylase, phenylalanine deaminase, and arginine hydrolase [9, 6]. All culture media and consumables were purchased from Merck Co., Germany.

Statistical analysis

The obtained data were analyzed using SPSS software (version 22) and descriptive statistical tests. χ^2 analysis were performed to analyze the results and P value of less than 0.05 was considered as statistical significance level.

Results

Among 1980 samples, 601 samples (32.17%) were infected with gram-negative bacilli with the *Enterobacter aerogenes* (37.27%) as the most common cause of contamination *(Table 1)*. The highest and lowest rates of infection in hospital wards were observed in the ICU (33.1%) and internal medicine unit (25.71%), respectively (*Tables 2* and *3*). However, this difference was not statistically significant between the hospital wards ($P = 0.25$).

Table 1. Prevalence of gram-negative bacilli contamination in 13 hospitals of the Golestan province

Type of contamination	Number of contamination (%)
Enterobacter aerogenes	224 (37.27)
Serratia marcescens	103 (17.13)
Citrobacter spp.	85 (13.81)
Klebsiella spp.	83 (14.14)
Pseudomonas aeruginosa	55 (9.15)
Proteus mirabilis	27 (4.5)
Escherichia coli	18 (3)
Shigella sonnei	3 (0.5)
Providencia spp.	3 (0.5)
Total contamination	601 (30.35)

Among the examined equipment and surfaces, the highest level of contamination in medical equipment was observed in laryngoscope and its blade (10.48%) as well as ECG sensor and its monitoring connector (6.65%). Among nonmedical equipment, phone (6.32%) and patients' beds and bedsheet (5.15%) showed the highest levels of contamination, while no infection was reported regarding the dining table *(Table 4)*.

The percentage of contamination among the 13 investigated hospitals was ranging between 26.36 and 37.5%. Statistical analysis showed significant correlations between the type of gram-negative bacilli and hospital wards with the rate of contamination in equipment ($P < 0.05$).

Discussion

The development of nosocomial infections particularly antibiotic-resistant infections has become a major problem in hospitals. Surfaces and medical equipment are suitable for colonization of microorganisms. In this study, the infection rate among the 13 investigated hospitals varied

Table 2. Distribution of the participants according to the wards and gram-negative bacilli contamination

Ward	Contamination		Number of contamination (%)
	Not contaminated number (%)	Contaminated number (%)	
Surgical ward	214 (67.3)	104 (32.7)	318 (100)
Operating room	232 (70.5)	97 (29.5)	329 (100)
Intensive care unit (ICU)	198 (66.9)	98 (33.1)	296 (100)
Cardiac care unit (CCU)	167 (69.0)	75 (31.0)	242 (100)
Emergency	206 (70.8)	85 (29.2)	291 (100)
Neonatal and pediatric unit	154 (68.8)	70 (31.3)	224 (100)
Internal medicine unit	208 (74.3)	72 (25.7)	280 (100)

Table 3. Prevalence of gram-negative bacilli contamination in different hospital wards

Ward	The level and type of contamination									Number of contamination (%)
	E. aerogenes	*S. marcescens*	*Klebsiella spp.*	*Citrobacter spp.*	*P. aeruginosa*	*P. mirabilis*	*E. coli*	*Sh. sonnei*	*Providencia* spp.	
Surgical ward	40	22	18	16	6	1	1	0	0	104 (32.7)
Operating room	33	19	5	16	15	8	1	0	0	97 (29.5)
Intensive care unit (ICU)	44	17	15	14	5	0	2	1	0	98 (33.1)
Cardiac care unit (CCU)	28	4	17	18	1	1	4	1	1	75 (31.0)
Emergency	28	18	14	9	9	6	1	0	0	85 (29.2)
Neonatal and pediatric ward	25	14	6	4	9	6	3	1	2	70 (31.3)
Internal medicine unit	26	9	8	8	10	5	6	0	0	72 (25.7)

from 26.36% to 37.5%, which indicates an alarming level of gram-negative bacilli contamination. Thus, hospitals are required to adopt specific policies for the disinfection and cleaning of at risk areas for better efficiency to avoid additional costs. Studies for determination of gram-negative bacilli infections by Amanlou in Zabul, Tohidnia in Kermanshah, and Moniri in Kashan reported 30%, 31.6%, and 65.7% infection rate, respectively, which correspond with the alarming rates found in the present study [7, 10, 11]. However, Jalalvandi in Kermanshah (5%), Bell in the United States (8.8%), and Afshar Yavari in Orumiyeh (15.15%) reported low prevalences of gram-negative bacilli contamination in hospital equipment and surfaces [12–14].

Based on the obtained results, the ICU (33.1%) had the highest and the internal medicine unit (25.71%) had the lowest infection rate of gram-negative bacilli contamination which contradicts with other studies. In 2010, Aslani et al. reported the neonatal ward with the highest infection rate (27.7%) among 137 tested samples [8]. In a ten-year

Table 4. Prevalence of gram-negative bacilli contamination in terms of bacterial type and hospital equipment

Equipment/supplies	The level and type of contamination								
	E. aerogenes	*S. marcescens*	*Klebsiella* spp.	*Citrobacter* spp.	*P. aeruginosa*	*P. mirabilis*	*E. coli*	*Sh. sonnei*	*Providencia* spp.
Laryngoscope and blade	24	10	18	8	0	2	0	1	0
ECG sensors and its Monitoring connector	10	8	6	4	6	0	3	0	0
Suction	8	16	0	6	7	1	0	0	0
Telephone handset	17	4	4	1	2	5	5	0	0

Table 4. *(cont'd)*

Equipment/supplies	The level and type of contamination								
	E. aerogenes	*S. marcescens*	*Klebsiella* spp.	*Citrobacter* spp.	*P. aeruginosa*	*P. mirabilis*	*E. coli*	*Sh. sonnei*	*Providencia* spp.
Drugs' trolley	9	9	3	5	4	0	0	0	0
Patients' beds	8	0	12	1	7	0	3	0	0
Bedsheet	11	0	9	8	1	0	3	0	1
Dressing trolley	11	14	0	6	0	0	0	1	0
Oxygen mask	8	5	6	0	0	7	0	0	0
Gan	19	0	0	2	4	0	0	0	0
Infusion set	12	4	0	5	3	0	0	0	0
Patient clothing	3	7	0	9	4	0	0	0	0
Bagging	13	0	3	7	0	0	0	0	0
Anesthetic machine	1	4	0	1	0	0	0	0	0
Endotracheal tube	11	5	0	0	0	0	1	0	0
Ventilator	3	3	8	0	0	0	3	0	0
Bronchoscope	2	0	0	2	0	1	0	0	0
Surgical instruments	0	2	4	4	0	2	0	0	0
Intravenous (IV)	4	2	1	0	5	1	0	0	0
Endoscope	2	0	0	3	0	0	0	0	0
Neonatal incubator	2	3	0	0	0	3	0	0	0
Electroconvulsive	12	0	2	1	0	1	0	0	0
Sialic lights	3	1	1	2	0	3	0	0	0
Negatoscope	2	0	0	1	0	0	0	0	0
Cardiopulmonary resuscitation (CPR) Trolly	4	1	0	0	0	0	0	0	0
Gurney	4	1	0	0	5	0	0	0	0
Colonoscope	0	0	1	0	0	0	0	0	0
Nebulizers	1	0	0	2	2	0	0	0	0
Cystoscope	0	0	0	0	2	0	0	0	0
Cautery	3	0	3	2	0	1	0	0	0
Otoscope	2	0	1	0	2	0	0	0	0
Thermometer	0	0	0	0	0	0	0	0	1
Oxygen flow meter	3	1	0	1	0	0	0	0	0
Refrigerator door handle	4	0	0	0	0	0	0	0	0
Mounting sleeves	1	0	0	1	0	0	0	0	0
Manometer	2	1	0	1	1	0	0	0	0
Baby scales	1	1	0	0	0	0	0	0	0
Wardrobe cases	0	0	0	0	0	0	0	0	1
IV stand	0	1	0	2	0	0	0	0	0
Echocardiogram	1	0	1	0	0	0	0	1	0
Dining table	0	0	0	0	0	0	0	0	0
Numbers (%)	224 (37.27)	103 (17.13)	83 (13.81)	85 (14.14)	55 (9.15)	27 (4.5)	18 (3)	3 (0.5)	3 (0.5)

study in Mashhad, Ghenaat et al. reported a higher prevalence in the internal medicine unit (44.5%) [15]. These discrepancies may be due to a number of factors including the wards' conditions and performance of the staff. However, the results of the present study indicated higher prevalence of contamination in the surgical wards and operating rooms compared with the internal medicine unit, which further highlights the need to develop programs for prevention of infections in these wards. Nevertheless, infections in the internal medicine unit should not be neglected, since most hospitalized patients in this unit are susceptible to nosocomial infections (especially immunocompromised patients).

The highest contamination level in medical equipment was observed in the laryngoscope and its blade (10.48%), ECG sensor and its monitoring connector (6.65%), suction (6.32%), and dressing trolley (5.32%), respectively. The highest number of contamination for nonmedical equipment was associated with the phone (6.32%) and patients' beds and bedsheet (5.15%). In Jalalvandi et al.'s study, the suction device (28.8%) and dressing trolley (23.3%), and in Aslani et al.'s study, phone (18.2%), manometer (17.5%), refrigerator door (8%), and beds (7.2%) had the highest incidents of contamination [8, 12]. Suction contamination may be due to the higher tendency of microorganisms toward horizontal surfaces particularly the surface of equipment in the surgical ward and operating room. Also, the proper cleaning of this device may be sometimes neglected due to lack of attention to the connection between suction pipe and the suction fluid reservoir.

Hands have an important role in spread of contaminations in nonmedical equipment such as phone, refrigerator, and beds. Therefore, thoroughly washing of hands should be instructed and well monitored. Moreover, contact between the staff and patients during transferring should be limited and patients' rooms should be closed as much as possible [16]. The infection rate of gram-negative bacilli is variable in the previous studies. The highest infection among the gram-negative bacilli was related to *E. aerogenes* (37.27%), *S. marcescens* (17.13%), *Citrobacter* spp. (14.14%), *Klebsiella* spp. (13.81%), *P. aeruginosa* (9.15%), *Proteus mirabilis* (4.5%), *E. coli* (3%), *Shigella sonnei* (0.5%), and *Providencia* spp. (0.5%).

These results are consistent with the findings in studies by Jalalvandi and Moniri in which *Enterobacter* was found as the most common gram-negative bacillus [7, 12]. In a study, by Alemu et al. in Ethiopia, *Providencia* spp. (27.8%), *Citrobacter* spp. (19.9%), and *Enterobacter* spp. (16.9%) were reported as the most common gram-negative bacilli, respectively [17]. Ensayef et al. from Iraq reported *P. aeruginosa* (30.4%) as the most common gram-negative bacillus among 1216 tested samples [18]. Results from a study by Amanlou and colleagues revealed *Klebsiella* 47.2%, *P. aeruginosa* 27.7%, *E. coli* 19.4%, and *Serratia* 5.5% as the most prevalent gram-negative bacilli in surfaces of hospital equipment and operating rooms [10]. In a study by Afshar Yavari et al. on surgical wards' infection in the hospitals of Urmia, *Pseudomonas* (60%), *Klebsiella* (20%), *E. coli* (8%), *Enterobacter* (8%), and *Proteus* (4%) were found as the most prevalent causes of gram-negative infections, respectively [14]. Tohidnia et al. also observed Klebsiella as the main gram-negative bacillus in the infection of radiology equipment (60.8%) [11]. Furthermore, *E. coli* was responsible for most of the contaminations as assessed by Halil and colleagues [19].

Attempts to control or prevent hospital contamination are cost-effective, considering the health problems and expenses that can be caused by nosocomial infections. It is also clear that effective contamination control policies, even in short term, has several times higher cost efficiency. Finally, it is emphasized that regular meetings and sampling result presentation to the physicians and nurses, as well as annual symposiums along with general hospital infection control policies (accurate strategies for operating rooms, ICU and even CSR and kitchen), can be very useful in further reducing contamination and nosocomial infections.

Conclusion

Bacterial infection rates with gram-negative bacilli in nonmedical and medical equipment of hospitals in the Golestan province are alarming. Proper hospital management, careful handwashing, disinfection and sterilization of the equipment, performing periodic bacterial cultures from at-risk areas and equipment, and antibiogram tests to determine the most effective antibiotics are recommended to reduce the consequent morbidity, mortality, and costs of these infections.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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