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Prevalence of Environmental Gram-negative Bacilli in the Intensive Care Units of Hospitals from the City of Qom

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Background: The role of the hospital environment as a source of dissemination of pathogens is critical. Environmental surfaces in the Intensive Care Units (ICUs) are suitable for the growth of Gram-negative bacteria that normally circulate between the environment and patients and can cause outbreaks of nosocomial infections. In this study, the prevalence of Gram-negative bacilli in the environment of the ICUs and neonatal ICU (NICU) of hospitals in the city of Qom was evaluated.
Materials and Methods: During a 6 month period from November 2012 to April 2013, samples were collected from environmental surfaces of ICUs of four hospitals and NICU of one hospital located in the city of Qom. Sampling was done from equipment, fluids, and surfaces and identification was carried out based on culture and biochemical tests for Gram-negative bacilli.
Results: A total of 230 swab samples was collected and 50 colonies of Gram-negative bacilli were isolated from environmental surfaces. Overall, 64% of the isolates belonged to non-fermentative bacteria and 36% of the isolates belonged to Enterobacteriaceae family. Strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex accounted for the highest rates of environmental isolates. In addition, *Klebsiella pneumoniae* was isolated from NICU.
Conclusion: The high frequency of genus *Acinetobacter* among Gram negative bacteria isolated from environmental surfaces has a public health impact and *Acinetobacter* spp. should be considered in the infection control programs in hospitals. Isolation of *K. pneumoniae* should be regarded as a risk factor for fatal neonatal infections.

Keywords: Nosocomial infections, Environmental surfaces, Gram-negative bacteria

1. Background

Nosocomial infections are defined as the infections that appear after 48-72 hours in patients admitted to hospital. These infections are usually transferred through contaminated instruments and equipment, aerosols contaminated with infectious agents, or hospital staff (1-3). Several studies have identified major pathogens of nosocomial infections that can circulate between the patients and the environment and might persist in the environment for a long time and can easily be transferred to the hospital staff hands (2). More than 1.4 million people worldwide suffer from complications of nosocomial infections (4). The report of the World Health Organization (WHO) from 14 countries including 4 WHO regions showed that the average prevalence of nosocomial infections was 8.7% in the hospitalized patients. In this report, the highest frequency of nosocomial infections was recorded from hospitals in the Eastern Mediterranean and South-East Asia regions (11.8 and 10.0% respectively) (5). In addition, in studies that were performed in the hospitals around the world, the rate of nosocomial infection was ranged from 12% to 68% with the average of 32% (6-9). Long duration of hospitalization, use of a variety of maintenance and monitoring devices and use of vascular catheters cause the emergence of nosocomial infections in ICUs. These infections usually interfere with the functions of body organs (10). According to the reports of WHO in high-income countries, nearly 30% of patients in ICU are involved by at least one nosocomial infection. Also, in low- and middle-income countries the prevalence of intensive-care unit acquired

(ICU-acquired) infection is 2-3 times more than high-income countries (11). The risk of nosocomial infection in the ICU was 5-10 times greater than those acquired in general medical and surgical wards in the European region (12).

Among the bacteria that cause hospital infections, Gram-negative bacilli are primarily important. Environmental resources of hospitals such as toilets, surfaces, machineries, equipment, and injectable solutions are suitable settings for the growth of Gram-negative bacteria. These infections are important in aspects of challenges in management and nursing, emergence of drug resistance, and poor prognosis (13-15). Increased resistance of bacteria, especially Gram-negative bacteria to antibiotics is particularly evident in the isolates of ICUs and multi-drug resistant bacilli grow in ICUs and circulate between the environment and patients (16).

In previous studies that were performed in hospitals in the city of Qom, only clinical samples have been investigated despite the importance of environmental surfaces as an emission source of pathogens in these infections. Reports of these studies showed that the highest incidence of nosocomial infections occurred in the ICUs (17, 18).

2. Objectives

This study was performed to evaluate the most prevalent Gram-negative bacilli in the environmental surfaces of ICUs and NICUs in the hospitals of the city of Qom.

3. Materials and Methods

3.1. Sampling and data collection

During a 6 month period from November 2012 to April 2013, samples were collected from environmental surfaces of ICUs and NICUs in five different hospitals (Nekoee, Valieasr, Kamkar, Shahid Beheshti, and Izadi) located in the Qom city. Sampling was done from equipment including vital sign monitor, defibril

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lator, ventilator, electrocardiogram, and medical imaging devices, fluids and surfaces using a moistened sterile swab from a 10 cm^2 surface area or 1mL of liquids. Detailed information about each sample, including the name of hospital, date of sampling, type of surface or equipment/devices was collected in the special data forms.

3.2. Identification of bacterial isolates

Swabs were cultured on Blood agar medium. After incubation at 35°C for 48 hours, colonies were sub-cultured on MacConkey agar for the selection of Gram negative bacteria. Gram-staining of the colonies grown on the MacConkey agar was performed and well-isolated single colonies recovered from agar plate were inoculated into the Triple sugar iron (TSI) agar and the media were incubated at 35°C for 18-24 hours. The TSI test results were used for screening the Gram-negative bacilli; if the organism could not ferment glucose, then an alkaline-slant/alkaline-deep reaction would be observed that indicated nonfermenters; an acid-slant/alkaline-deep or acid-slant/acid-deep reaction was considered as a characteristic of *Enterobacteiaceae* family. Preliminary identification of bacteria was done based on colony characteristics of isolation media. Further, to differentiate species of *Enterobacteriaceae* family, phenotypic characteristics were determined using biochemical reactions, including methyl red (MR), Voges-Proskauer (VP) tests for sulfur reduction, Indole production and motility (SIM), Simmons Citrate Agar (Sc), and presence of lysine decarboxylase, urease, and oxidase. For identification of nonfermentatiing bacteria other characteristics, including growth at 44°C, oxidase test, urease, lysine decarboxylase, Oxidative-fermentative test (OF) with maltose, mannitol and dextrose, and DNase were measured.

4. Results

A total of 230 swab samples was collected and 50 colonies of Gram-negative bacilli were isolated from environmental surfaces. No colonies of Gram-negative bacilli were isolated from the hands of nurses and from the fluids. Overall, 86% of the isolated Gram-negative bacilli were recovered from environmental surfaces and 14% were recovered from medical equipment. In addition, 64% of the isolates belonged to nonfermentative bacteria and 36% of the isolates belonged to *Enterobacteriaceae* family. Frequency of species in accordance with ICU and NICU wards is summarized in Table 1.

Table 1. Frequency of isolated bacteria in ICU and NICU wards of the hospitals in the city of Qom

	Microorganisms	***Number***	**Percentage (%)**
ICU	*Acinetobacter baumannii* complex	15	30
	Pseudomonas aeruginosa	6	12
	Acinetobacter spp.	4	8
	Klebsiella pnuamoniae	3	6
	Enterobacter cloacae	3	6
	Citrobacter freundii	3	6
	Stenotrophomonas maltophilia	2	4
	Acinetobacter calcoaceticus	2	4
	Enterobacter spp.	2	4
	Pantoea agglomerans	2	4
	Enterobacter aerogenes	1	2
NICU	*Acinetobacter baumannii* complex	2	4
	Enterobacter cloacae	2	4
	Pseudomonas aeruginosa	1	2
	Klebsiella pnuamoniae	1	2
	Klebsiella oxytoca	1	2
	Total	50	100

5. Discussion

Nosocomial infections are a major source of morbidity and mortality in hospitalized patients. Increasing reports of nosocomial infections due to the drug resistant strains of bacteria have drawn much attention in the last decade. Several studies have shown a relation between hospital infections and various Gram-negative pathogens (17-19); Also, contaminated surfaces play an important role in the transmission of prevalent bacteria such as *A. baumannii* complex and *P. aeruginosa* (20). It is estimated that 20 to 40% of nosocomial infections occur through the transmission of infection from the hands of hospital staff to the patients (21).

In the current study, the most prevalent Gram-negative bacteria on the surfaces of hospitals were among the non-fermenting group. However, in the study of Fazeli and colleagues (2013), Gram-negative oxidase positive bacteria was the most frequent isolates, while 22.6% of the isolates belonged to the genus *Acinetobacter* (22). Recent evidences suggest that environmental contamination plays a role in the transmission of *Acinetobacter* spp. which remains in the environment for a long time and infections might occur through contaminated surfaces of hospital and staff hands (21). In this study, *A. baumannii* complex and *P. aeruginosa* were the most common bacteria that were isolated from ICUs. These two species of non-fermentative bacteria have been reported as the most prevalent causes of nosocomial infections in the clinical samples of the patients around the world (20, 23-25). Similar to this result, another study on environmental surfaces of ICUs in India showed that *P. aeruginosa* and *A. baumannii* complex was the most prevalent isolated bacteria (26). Also, in a study on water samples of ICU in a hospital in Germany, *P. aeruginosa* was recovered in 60 (42%) of 143 samples (20).

In this study the frequency of nonfermenting Gram-negative bacilli (64%) was more than that of *Enterobacteriaceae* (36%). In contrast, in some reports, the frequency of isolated bacteria in the family of *Enterobacteriaceae* was more than that of nonfermenting bacteria (17). In a study from Iran, the percentage of Gram-negative bacteria of the *Enterobacteriaceae* family was 9.8%, following by 3.9% of *Pseudomonas* species and 4.51% of other bacteria (27). Possible reason might be due to the difference in sampling surface (dry vs. moist), since moist surfaces are suitable for the growth of nonfermenting bacteria including *Pseudomonas* spp.

In this study in the evaluation of NICU, *Klebsiella pneumoniae* and *P. aeruginosa* were isolated from different environmental surfaces. In a study on medical equipment, beds and environmental surfaces of NICUs of two hospitals in India, *K. pneumoniae* was the most predominant organism. In addition, *P. aeruginosa* and *Citrobacter ferundii* were isolated. Overcrowding, poor ventilation and lack of detailed protocol of nursing were considered as possible reasons (28).

K. pneumoniae is medically the most important species of the genus *Klebsiella*. Isolation of *K. pneumoniae* from environmental surfaces in NICU is considered as an important risk factor for the neonatal infections, including septicemia, urinary tract infections, pneumonia, and soft tissue infections. It was shown that many outbreaks of *K. pneumoniae* infections in the NICUs have an environmental reservoir such as the hands of healthcare workers (29-31). Drug resistant *K. pneumoniae* was the most common isolate causing neonatal sepsis with a high mortality rate (32) and it was reported that 80% of the outbreaks (20/25) due to *K. pneumoniae* being involved the bloodstream and urinary tract infections, while 50% of these outbreaks occurred in NICU and person-to-person spread was the most common mode of transmission (33).

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6. Conclusion

The presence of these bacteria in ICU and NICU increases the risk of transmission to patients leading to nosocomial infections. Successful prevention of the nosocomial infections needs to investigate the sources of environmental contamination and practical ways to prevent the spread of bacteria. The high frequency of genus *Acinetobacter* among Gram negative bacteria isolated from environmental surfaces should be considered in control programs in hospitals. There is a need for the education of health care workers in the ICU wards on the importance of these bacteria and the modes of spread. Active surveillance of *K. penumoniae* in the NICUs is also important to prevent outbreaks.

Conflict of Interests

The authors declare they have no conflict of interests.

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Authors' Contributions

Farzaneh Mehraban collected the samples; Farzaneh Mehraban and Masoumeh Dolati conducted the laboratory experiments; Masoumeh Douraghi provided laboratory protocols. Mahmoud Nateghi Rostami designed and supervised the study, estabilished laboratory methods, and analysed the data.

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