

Full Length Research Paper

Frequency of isolated antibiotic resistant bacterial strains from nursing staff (Azzahra Hospital, Iran)

Shila Jalalpour*¹ and Abdol Ghaffar Ebadi²

¹Lecture of Microbiology, Islamic Azad University Shahreza Branch, Membership of Young Researchers Club, Islamic Republic of Iran..

²Department of Biological Sciences, Jouybar branch, Islamic Azad University, Jouybar, Iran.

Accepted 12 October, 2011

Nursing staff have important roles to play in nosocomial infections. Antibiotic resistance is the ability of a microorganism to withstand the effects of an antibiotic; it evolves naturally via natural selection through random mutation, but it could also be engineered. The purpose of this study was to survey the relative frequency of antibiotic resistant strains in isolated bacteria from nursing staff. The present study was performed at one of the tertiary care hospitals in Isfahan, Iran, during a 24 month period (2007 to 2009). Eighty bacterial isolates from nursing staff were studied. Samples were collected with finger print method. Standard microbiological methods were performed for the detection of bacterial species. Bacterial antibiogram pattern according to Clinical and Laboratory Standards Institute (CLSI) 2010, were used by disk diffusion method. The result showed that *Staphylococcus* spp. 28 (53%), *Bacillus* spp. 48 (60%) and *Enterobacteriaceae* 4 (5%) were constituents of the isolated bacteria from nursing staff. According to the antibiogram pattern, 17.5, 59.5, 69.5, 41, 70.5, 50, 78, 97.5 and 64% of *Staphylococcus* spp.; 28, 30.5, 59, 65, 65, 77.5, 98, 93.5 and 100% of *Bacillus* spp. and 25, 73, 6, 75, 65, 50%, 64, 15 and 95% of *Enterobacteriaceae* were sensitive to Penicillin, Cephotaxime, Clindamycin, Co-trimoxazol, Ampicillin, Erythromycin, Tetracycline, Vancomycin and Gentamicin respectively. The results showed a high frequency of antibiotic resistant strains on nursing staff. Establishing systems for monitoring antimicrobial resistance in hospitals and the community and linking these findings to resistance and disease surveillance data is fundamental to develop treatment guidelines accurately and to assess the effectiveness of interventions appropriately.

Key words: Antibiotic resistance, nosocomial infection, nursing staff.

INTRODUCTION

Nosocomial infections (NIs) remain a major global concern. Overall national prevalence rates have been described as ranging between 3.5 and 9.9% (Ducel et al., 2002; Jalalpour et al., 2007; Raymond and Aujard, 2000; Sehulster and Raymond, 2003). Hospital surfaces and nursing staff are the most prevalent sources of NIs (Jalalpour et al., 2007). Microorganisms are transmitted in hospitals through several routes; nursing staff are the most prevalent sources of the transmission of the bacteria in hospitals (Kampf and Kramer, 2004). The most important and frequent mode of transmission of

nosocomial infections is divided into two subgroups: Direct contact transmission and indirect contact transmission. Direct contact transmission involves a direct body surface to body surface contact and physical transfer of microorganisms between a susceptible host and an infected or colonized person, such as can occur when a person turns a patient, gives a patient bath, or performs other patient care activities that require direct personal contact (Ducel et al., 2002; Jalalpour et al., 2007; Kampf and Kramer, 2004; Raymond and Aujard, 2000; Sehulster and Raymond, 2003). Direct contact transmission can also occur between two patients, with one serving as the source of the infectious microorganisms and the other as a susceptible host. Indirect contact transmission involves contact of a susceptible host with a contaminated intermediate object, usually

*Corresponding author. E-mail: shilla.jalalpour@yahoo.com, jalalpour@iaush.ac.ir.

inanimate, such as contaminated instruments, needles, dressings or contaminated gloves that are not changed between patients.

Additionally, the improper use of saline flush syringes, vials, and bags have been implicated in disease transmission in the U.S., even when healthcare workers had access to gloves, disposable needles, intravenous devices and washed their hands frequently which is the single most important measure to reduce the risks of transmitting microorganisms from one person to another or from one site to another on the same patient (Ducel et al., 2002; Jalalpour et al., 2007; Kampf and Kramer, 2004; Raymond and Aujard, 2000 and Sehulster and Raymond, 2003). Total counts of bacteria on the hands of medical staff ranged from 3.9×10^4 to 4.6×10^6 . Their number increases with the duration of clinical activities on the average by 16 cells per min. Some clinical situations that are associated with a higher bacterial load on the hands of health care workers include: direct contact with patients, respiratory tract care, contact with body fluids, and after an interruption while caring for a patient (Kampf and Kramer, 2004). In general, however, it is difficult to clearly assign a specific risk of hand contamination to certain patient care activities. Nurses can contaminate their hands with 100 to 1,000 colony-forming units (CFU) of *Klebsiella* spp. during clean-up activities, while 10 to 600 CFU/ml can be found on nurses' hands after touching the groins of patients heavily contaminated with *Proteus mirabilis*. In intensive care units (ICU), a number of direct contacts were found between the hands of the health care workers and the patients (Kampf and Kramer, 2004). Between 4 and 16% of the hand surface is exposed by a single direct contact, and after 12 direct contacts, up to 40% of the hand surface may have been touched. The transmissibility of transient bacteria depends on the species, the number of bacteria on the hand, their survival on skin, and the dermal water content (Kampf and Kramer, 2004).

Environmental source is a means of transmission of infectious agents and the presence of the pathogen does not establish its causal role; its transmission from source to host could be through indirect means, e.g., via hand transfer. Although microbiologically contaminated surfaces can serve as reservoirs of potential pathogens, these surfaces generally are not directly associated with the transmission of infections to either staff or patients. The transfer of microorganisms from environmental surfaces to patients is largely via hand contact with the surface (Ducel et al., 2002; Jalalpour et al., 2007; Sehulster and Raymond, 2003).

Antibiotic resistance is the ability of a microorganism to withstand the effects of an antibiotic (Onyango et al., 2010; Olusesan et al., 2010). It is a specific type of drug resistance and evolves naturally via natural selection through random mutation, though it can also be engineered. Once such a gene is generated, bacteria can transfer the genetic information in a horizontal fashion

(between individuals) by plasmid exchange. If a bacterium carries several resistance genes, it is called multiresistant or informally, a superbug. Resistance costs money, livelihoods and lives, and threatens to undermine the effectiveness of health delivery programs (Kim, 2000; Jalalpour et al. 2007, 2009b). Antibiotic resistance is the result of evolution via natural selection or programmed evolution. The antibiotic action is an environmental pressure which allows those bacteria that have mutation to survive and live on to reproduce. They then pass this trait to their offspring, which will be a fully resistant generation (Kim, 2000; Jalalpour et al., 2007, 2009b).

The costs associated with nosocomial infections can be devastating to facility. Illness can spread quickly among patients and staff, causing huge expenses due to treating patients and employees' sick days. In fact, the average U.S. hospital spends over \$6 million per year for hospital acquired infections cost, and they are the leading cause of death among long term care residents. The key to preventing nosocomial infections is good hand hygiene, educating staff and providing them with hand hygiene products that are non-irritating; this can significantly reduce nosocomial infections and the associated costs (Stone et al., 2002). To implement a successful hand hygiene program, soaps, hand sanitizers, and moisturizing lotions are needed throughout the facility. Medline offers a complete array of convenient and easy to use hand hygiene products for staff, patients and visitors. By providing effective hand hygiene products and comprehensive training on proper usage techniques, the facility will be a safer and more profitable place. The Centers for Disease Control (CDC) recommend that healthcare workers (HCWs) wash their hands with soap and water if their hands are visibly soiled, dirty, or contaminated. Also, wash hands before eating, after using a restroom and if exposure to *Bacillus anthracis* is suspected or proven, but if visibly soiled, workers should use an alcohol-based hand cleaner for routinely decontaminating of their hands (Stone et al., 2002). For generations, hand washing with soap and water has been considered a measure of personal hygiene. The concept of cleansing hands with an antiseptic agent probably emerged in the early 19th century (Boyce and Pittet, 2002).

As early as 1822, a French pharmacist demonstrated that solutions containing chlorides of lime or soda could eradicate the foul odors associated with human corpses and that such solutions could be used as disinfectants and antiseptics. Transmission of health care associated pathogens from one patient to another via the hands of HCWs requires the following sequence of events: Organisms present on the patient's skin, or that have been shed onto inanimate objects in close proximity to the patient, when transferred to the hands of HCWs, must be capable of surviving for at least several minutes on the hands of personnel. Next, hand washing or hand antiseptics by the worker must be inadequate or omitted entirely, or the agent used for hand hygiene must be

Table 1 Frequency of bacterial strains on nursing staff.

Bacteria	<i>Staphylococcus</i> spp.		<i>Bacillus</i> spp.		Enterobacteriaceae							
	N	%	N	%	N	%						
Number / percent	28	35	48	60	4	5						
Species	<i>S. epidermidis</i>		<i>S. aureus</i>		<i>Bacillus</i> sp.		<i>B. cereu</i>		<i>E. coli</i>		<i>K. pneumoniae</i>	
	N	%	N	%	N	%	N	%	N	%	N	%
Number/percent	24	30	4	5	35	43.75	13	16.25	2	2.5	2	2.5

inappropriate. Finally, the contaminated hands of the caregiver must come in direct contact with another patient, or with an inanimate object that will come into direct contact with the patient. Health care associated pathogens can be recovered not only from infected or draining wounds, but also from frequently colonized areas of normal, intact patient skin (Boyce and Pittet, 2002).

The aim of this study is to evaluate the frequency of antibiotic resistant bacterial strains isolated, from Azzahra hospital nursing staff in Iran. This study was intended to answer the following questions:

1. What is the frequency of bacterial on Iranian nursing staff?
2. What is the frequency of antibiotic resistant bacteria on Iranian nursing staff?
3. What is the antibiogram pattern of bacterial isolated from Iranian nursing staff?

MATERIALS AND METHODS

Sampling

A total of 80 bacteria were collected from nursing staff and were isolated in Azzahra hospital in Esfahan, Iran during the period from 2007 to 2009. Samples were randomly collected from and cultured in Blood Agar and EMB Agar (Merck) via Fingerprint Technique (Merck) (Jalalpoor et al., 2009a, b; Sehulster and Raymond, 2003).

Bacterial strains

Bacterial identification was performed with microbiological methods e.g. Gram stains and biochemical tests with the BioMerieux database system and the use of differential culture medium. In the first step, specimen were grown on sheep blood and EMB agars then incubated at 37°C under aerobic conditions (Jalalpoor et al., 2009a; Washington et al., 2006).

Antibiotic susceptibility

Antibiotic susceptibility was performed with its standard disc diffusion agar (Jalalpoor et al., 2010a; Wikler et al., 2006). The susceptibility of the isolates was determined against 9 antibacterial agents by disc diffusion method. They included, Penicillin, Cephalexin, Clindamycin, Co-Trimoxazole, Ampicillin, Erythromycin, Tetracycline, Vancomycin and Gentamicin (Merck).

Statistical analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) version 14. Chi-square and Fisher test were used for the determination of significance of association. $P \leq 0.05$ was considered significant.

RESULTS

According to the results, *Staphylococcus* spp. 28 (53%), *Bacillus* spp. 48 (60%), *Enterobacteriaceae* 4 (5%) were constituents of the isolated bacteria from nursing staff (Table 1). According to the antibiogram pattern, 17.5, 59.5, 69.5, 41, 70.5, 50, 78, 97.5 and 64% of *Staphylococcus* spp., 28, 30.5, 59, 65, 65, 77.5, 98, 93.5 and 100% of *Bacillus* spp., and 25, 73, 6, 75, 65, 50, 64, 15 and 95% of *Enterobacteriaceae* were sensitive to Penicillin, Cephalexin, Clindamycin, Co-trimoxazole, Ampicillin, Erythromycin, Tetracycline, Vancomycin and Gentamicin respectively (Table 2).

DISCUSSION

In comparing the results obtained in this research with that obtained from previously published articles of similar research, it shows a high frequency of antibiotic resistant strains on nursing staff. According to the results, *Staphylococcus* spp. 53%, *Bacillus* spp. 60%, *Enterobacteriaceae* 5%, were constituents of the isolated bacteria from nursing staff. The result of a previous study (about bacterial epidemiology in hospitals) demonstrated that the frequency of *Staphylococcus* spp. and *Bacterial* spp. were 38.85% and they were the most bacterial strains that were isolated from nursing staff (Jalalpoor et al., 2009a, b, 2010b, c; Mansuri et al., 2007; Nasiry, 2000). The result of same previous study in other countries shows that the hands of HCWs that were colonized with *S. aureus* were between the range of 10.5 and 78.3%. Up to 24,000,000 cells were found per hand, and the colonization rate with *S. aureus* was higher among doctors (36%) than among nurses (18%), as was the bacterial density of *S. aureus* on the hands of doctors and nurses which was 21 and 5% respectively, with more than 1,000 CFU per hand (Kampf and Kramer, 2004).

Table 2 Antibiotic sensitivity pattern of bacteria isolated from nursing staff.

Bacteria	Antibiotic								
	Gentamicin (%)	Vancomycin (%)	Tetracycline (%)	Erythromycin (%)	Ampicillin (%)	Co-rimoxazole (%)	Clindamycin (%)	Cephotaxime (%)	Penicillin (%)
<i>Bacillus spp.</i>	100	100	96	89	80	73	68	61	56
<i>B. cereus</i>	100	87	100	66	50	57	50	0	0
<i>S. aureus</i>	50	100	66	50	51	25	66	50	0
<i>S. epidermidis</i>	78	95	90	50	90	57	73	69	33

The carrier rate may be up to 28% if the HCWs contact patients with an atopic dermatitis which is colonized by *S. aureus* and which can survive on hands for at least 150 min; Vancomycin resistant Enterococcus (VRE) survives on hands or gloves for up to 60 min (Kampf and Kramer, 2004). Colonization rates of gram-negative bacteria on the hands of HCWs have been described as ranging from 21 to 86.1%, with the highest rate being found in ICUs. The number of gram-negative bacteria per hand may be as large as 13,000,000 cells, the colonization may be long lasting, even in nursing homes, and a rate of 76% has been described for nurses' hands (Kampf and Kramer, 2004). Different species of gram-negative bacteria exhibit different colonization rates, for instance, the colonization rate is 3 to 15% for *Acinetobacter baumannii*, 1.3 to 25% for *Pseudomonas spp.*, and 15.4 to 24% for *Serratia marcescens*. *Klebsiella spp.* were found on the hands of 17% of the ICU staff samples, with up to 10,000 bacteria per hand with most of the gram-negative bacteria surviving on the hands for 1 h or more (Kampf and Kramer, 2004). During a third outbreak, caused by *Bacillus cereus* in a neonatal ICU, 11 (37%) of 30 fingerprints from HCWs were positive for *Bacillus spp.* (Kampf and Kramer, 2004).

Conclusion

Approximately one third of nosocomial infections are preventable. Cleaning is necessarily in the first step of any sterilization or disinfection process. Cleaning and removing organic matter, salts and visible soils interfere with microbial inactivation (Madani et al., 2009; Mielke, 2010; Rosenthal et al., 2010), so modern infection control is grounded in the work of Ignaz Semmelweis, who in the 1840s demonstrated the importance of hand hygiene for controlling the transmission of infection in hospitals. The importance of hands in the transmission of hospital infections has been well demonstrated and can be minimized with appropriate hand hygiene (Ducel et al.

2002; Jalalpour et al., 2009c; Kampf and Kramer, 2004; Raymond and Aujard, 2000 and Sehulster and Raymond, 2003).

ACKNOWLEDGMENTS

This article was written as a result of the thesis "The study of the production of β -lactamase, surface layer and nano structure in some isolated pathogen bacteria from clinical and hospital environmental samples". This thesis was introduced as the country's top microbiology thesis in 2009; it was among the defended theses during the years 2004 to 2008 in Islamic Republic of Iran. The authors would like to acknowledge Alzahra Hospital Management, Isfahan University, Management of Science research lab of Isfahan University, journals managers of Isfahan University of Medical Sciences, Azzahra Hospital Infection control committee, Dr Rooha Kasra Kermanshahi, Dr Ashraf Sadat Noohi, Dr Hamid Zarkesh Esfahani, Mr. Ardeshir Talebi, Mehrdad Memarzadeh, Kamyar Mostafavizadeh, Sinai Mobasherizadeh, Fariborz Kianpour, Mohsen Hosseini Balam, Ms. Kobra Maqhsudi, Mr Ali Mehrabi and all the people who helped us to achieve this research.

REFERENCES

- Boyce J M, Pittet D (2002). Guideline for Hand Hygiene in Health Care Settings. *Infect. Cont. Hosp. Epidemiol.*, 23(12): S3.
- Ducel G, Fabry j, Nicolle L, Girard R, Perraud M, Pruss A, etal (2002). Prevention of hospital- acquired infections, A practical guide, Department of Communicable Disease, Surveillance and Response. 2nd edition, Available at WHO/CDS/CSR/EPH/ 2002.12.
- Jalalpour S, Kasra Kermanshahi R, Noohi A, Zarkesh H (2007). Study of β -lactamase and S-layer Production in some of Isolated Pathogen Bacteria From Clinical and Environmental Hospital Samples. MSc thesis, Iran, Tehran, Islamic Azad University Science and Research Branch, Tehran, pp. 105-157.
- Jalalpour S, Kasra Kermanshahi R, Nouhi AS, Zarkesh Esfahani H (2009a). The comparative frequency of β -lactamase production and antibiotic susceptibility pattern of bacterial strains isolated from staff

- hands and hospital surfaces in Alzahra Hospital-Isfahan. *Iranian J. Med. Microbiol.*, 3(4): 37-45.
- Jalalpoor S, Kasra Kermanshahi R, Nouhi AS, Zarkesh Esfahani H (2009b). The Prevalence of Nano-structure Surface Layer in *Bacillus Cereus* Strains Isolated from Staff Hands and Hospital Surfaces. *J. Isfahan Med. School*, 27(100): 632-645.
- Jalalpoor S, Kasra Kermanshahi R, Nouhi AS, Zarkesh Esfahani H (2009c). Comparison of the Frequency β -lactamase Enzyme in Isolated Nosocomial Infectious Bacteria. *J. R. U. M. S.*, 8(3): 203-214.
- Jalalpoor S, Kasra Kermanshahi R, Nouhi AS, Zarkesh Esfahani H (2010a). The role of nanostructured surface layer and production of β -lactamase in penicillin resistant *Bacillus cereus* strains. *Iranian J. Med. Microbiol.*, 4(1): 18-26.
- Jalalpoor S, Kasra Kermanshahi R, Nouhi AS, Zarkesh Esfahani H (2010b). Survey Frequency of β -lactamase Enzyme and Antibiotic Sensitivity Pattern in Isolated Pathogen Bacteria from Low and High Hospital Contact Surfaces. *Pajuhandeh J.*, 15(2): 77-82.
- Jalalpoor S, Kasra Kermanshahi R, Nouhi AS, Zarkesh Esfahani, Mobasherizadeh S (2010c). Survey Prevalence and Resistance to some Beta lactame antibiotic in *B.cereus* st. Isolated of Azzahra Hospital. *Iranian J. Biol.*, 23 (4): 470-477.
- Kampf G, Kramer A (2004). Epidemiologic Background of Hand Hygiene and Evaluation of the Most Important Agents for Scrubs and Rubs. *Clin. Microbiol. Rev.*, 17(4): 863-893.
- Kim JM (2000). Multicentre surveillance study for nosocomial infections in major hospitals in Korea. *Am. J. Infect. Control*, 28: 454-458.
- Madani N, Rosenthal VD, Dendane T, Abidi K, Zeggwagh AA, Abouqal R (2009). Healthcare-associated infections rates, length of stay, and bacterial resistance in an intensive care unit of Morocco: Findings of the International Nosocomial Infection Control Consortium (INICC). *Int. Arch. Med.*, 2: 29.
- Mansuri F, Banitabay Hematyar S (2007). Overview of Bacterial Culture in Operations room in selective Hospital in Isfahan Medical Science university (1384-85). The National Student Congress on The Role of Health Service Staff in Prevention of Hospital Infections, Iran-Isfahan Medical Science University, p. 128.
- Mielke M (2010). Prevention and control of nosocomial infections and resistance to antibiotics in Europe - Primum non-nocere: elements of successful prevention and control of healthcare-associated infections. *Int. J. Med. Microbiol.*, 300(6): 346-350.
- Nasiry RB (2000). Overview of Bacterial in Operations room Environment in Hospitals Shahid Beheshty University. The 3th National Congress of Microbiology; Iran-Hamedan.
- Olusesan AG, Nonyelum O, Claire-Lorentz o E, Olorunmola EJ (2010). Plasmid DNA analysis and conjugative study of antibiotic resistant *Salmonella typhi* and *paratyphi* to ten selected antibiotics in Zaria, Nigeria. *Afr. J. Pharm. Pharmacol.*, 4(4): 158-164.
- Onyango DM, Kakai R, Nyandago WE, Ghebremedhin B, Konig W, Kong B (2010). Integron-plasmid mediated antibiotic resistance and virulence factors in clinical *Salmonella enterica* serovars in rural Western Kenya. *Afr. J. Pharm. Pharmacol.*, 4(7): 490-497.
- Raymond J, Aujard Y (2000). European Study Group. Nosocomial Infections in Pediatric Patients: A European, Multicenter Prospective Study. *Infect Control Hosp. Epidemiol.*, 21: 260-263.
- Rosenthal VD, Maki DG, Jamulitrat S (2010). International Nosocomial Infection Control Consortium (INICC) report, data summary for 2003-2008, issued June 2009. *Am. J. Infect. Control*, 38(2):95-104.
- Sehulster L, Raymond YW (2003). Guidelines for Environmental Infection Control in Health-Care Facilities. U.S. Department of Health and Human Services Centers for Disease Control and Prevention (CDC), Atlanta GA 30333.
- Stone P, Larson E, Kawa L (2002). A systematic audit of economic evidence linking nosocomial infections and infection control interventions: 1990-2000. *Am. J. Infect. Cont.*, 30: 145-152.
- Washington CW, Stephen DA, Janda WM, Koneman EW, Schreckenberger PC Procop GW, Woods GL (2006). *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*, Sixth edition .USA: Lippincott Williams & Wilkins., pp: 775-779.
- Wikler MA, Cockerill FR, Craig WA, Dudley MN, Eliopoulos GM, Hecht DW, Ferraro MJ, Swenson JM, Hindler JF, Patel JB, Powell M, Turnidge JD, weinstein MP, Zimmer BL (2006). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard. Ninth Edition, 26(1).