EFFECTIVE SOURCES OF NOSOCOMIAL INFECTION: STAFF HANDS AND **HOSPITAL SURFACES**

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ABSTRACT

Nosocomial infections (NIs), also called hospital-acquired infections are infections acquired during hospital care which are not present or incubating at admission. Nosocomial infections remain a major global concern in all countries. Microorganisms are present in great numbers in hospital environment. Both infected persons and persons at increased risk of infection congregate in the hospitals. The subject of this study was to survey effective sources of nosocomial infections in hospitalized patients. Papers related to the importance of staff hands and hospital surfaces in transmission of bacteria in hospital were extracted from pubmed, elsevier science and yahoo from 1995 to 2011. For this study key words which were search included hospital surfaces, staff hand, transmission bacteria and nosocomial infection. Staff hands and hospital surfaces have important role in spread and transmission of bacteria in hospital. There is a consensus that the control Bacterial population in these sources, lead to the control of spread and transfer bacteria and so nosocomial infections in hospital.

Hospital surfaces can serve as reservoirs of pathogenic bacteria such Staphylococcus aureus, Salmonella, Clostridium perfringens, Campylobacter, Listeria monocytogenes, Vibrio parahaemolyticus, Bacillus cereus and Escherichia coli and have important role in infection chain. Bacteria on hospital surfaces have low potential to spread. Staff hands have very contact with hospital surfaces and are more sources to transmission Bacteria in hospital. Increased Sanitation Staff hands and hospital surfaces has been considered to be the most important tool in control of transmission bacteria in hospital.

Key Words: Nosocomial Infection, Hospitalized Patients, Nosocomial Infections, Staff Hands, Hospital Surfaces

INTRODUCTION

Nosocomial infections occur worldwide and affect both developed and resource-poor countries. Infections acquired in health care settings are among the major causes of death and increased morbidity among hospitalized patients. They are a significant burden both for the patient and for public health. A prevalence survey conducted under the auspices of WHO in 55 hospitals of 14 countries representing 4 WHO Regions (Europe, Eastern Mediterranean, South-East Asia and Western Pacific) showed an average of 8.7% of hospital patients had nosocomial infections. At any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospital (Ducel et al., 2002; Johnson, 2006; Kampf and Kramer, 2004). The highest frequencies of nosocomial infections were reported from hospitals in the Eastern Mediterranean and South-East Asia Regions (11.8 and 10.0% respectively), with a prevalence of 7.7 and 9.0% respectively in the European and Western Pacific Regions (Ducel et al., 2002; Johnson, 2006; Kampf and Kramer, 2004; Raymond and Aujard, 2000; Stone et al., 2002).

In this review effective sources of Nosocomial infections have been investigated in the published literature. Related papers of importance of staff hands and hospital surfaces in transmission Bacteria in hospital were extracted from pubmed, elsevier science and yahoo from 1995 to 2011. For this study key words which were searched included hospital surfaces, staff hand, transmission Bacteria and nosocomial infection.

The health-care environment contains a diverse population of microorganisms. Microorganisms are present in great numbers in moist, organic environments, but some also can persist under dry conditions. Environmental source or means of transmission of infectious agents, 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 transferal (jalalpoor et al., 2009a,b; Boyce and Pittet, 2002; Ducel et al., 2002; Kampf and Kramer, 2004).

The surface of the hospital has been considered one of a number of potential reservoirs for the pathogen, but not the de facto source of exposure. An understanding of how infection occurs after exposure, is also important in evaluating the contribution of the environment to health-care-associated disease. All of the components of the chain must be operational for infection to occur:

1) Adequate number of pathogenic organisms (dose); 2) Pathogenic organisms of sufficient virulence; 3) A susceptible host; 4) An appropriate mode of transmission or transferal of the organism in sufficient number from source to host; 5) The correct portal of entry into the host (Sehulster and Raymond, 2003). Although microbiologically contaminated surfaces can serve as reservoirs of potential pathogens, these surfaces generally are not directly associated with transmission of infections to either staff or patients (Sehulster and Raymond, 2003). The transferral of microorganisms from environmental surfaces to patients is largely via staff hands contact with the surface (Jalalpoor *et al.*, 2009a,b; Sehulster and Raymond, 2003).

Normal human skin is colonized by various bacteria; different areas of the body have varied total aerobic bacterial counts (e.g., 1 x 10⁶ colony forming units (CFUs/cm² on the scalp, 5 x 10⁵ CFUs/cm² in the axilla, 4 x 10⁴ CFUs/cm² on the abdomen, and 1 x 10⁴ CFUs/cm² on the forearm). Total bacterial counts on the hands of a medical personnel have ranged from 3.9 x 10⁴ to 4.6 x 10⁶. In 1938, bacteria recovered from the hands were divided into two categories: transient and resident (Boyce and Pittet, 2002; Kampf and Kramer, 2004). Transient flora, which colonize the superficial layers of the skin, are more amenable to removal by outine handwashing (Boyce and Pittet, 2002; Kampf and Kramer, 2004). They are often acquired by HCWs during direct contact with patients or contact with contaminated environmental surfaces within close proximity of the patient. Transient flora are the organisms most frequently associated with health-care—associated infections. Resident flora, which are attached to deeper layers of the skin, are more resistant to removal. In addition, resident flora (e.g., coagulase-negative staphylococci and diphtheroids) are less likely to be associated with such infections. The hands of Health Care Workers (HCWs) may become persistently colonized with pathogenic flora (e.g., *S. aureus*), gramnegative bacilli, or yeast. Investigators have documented that, although the number of transient and resident flora varies considerably from person to person, it is often relatively constant for any specific person (Boyce and Pittet, 2002; Kampf and Kramer, 2004).

S. aureus, Escherichia coli and spore forming Bacteria are the common bacteria causing nosocomial infections in hospital and community.

S. aureus is the most common gram-positive bacterium causing nosocomial Infections (NIs) (Kampf and Kramer, 2004; Steinbrecher et al., 2000). Methicillin resistance is increasing in S. aureus (MRSA) worldwide, leading not only to NIs but recently also to community-acquired infection, colonization of health care workers' hands with S. aureus has been described to range between 10.5 and 78.3%. Up to 24,000,000 cells can be found per hand, 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 (21 and 5%, respectively, with more than 1,000 CFU per hand) (Kampf and Kramer, 2004). The carrier rate may be up to 28% if the health care worker contacts patients with an atopic dermatitis which is colonized by S. aureus and MRSA has been isolated from the hands of c. 16.9% of health care workers. VRE can be found on the hands of up to 41% of health care workers. Hand carriage of pathogens such as S. aureus, MRSA, or S. epidermidis has repeatedly been associated with different types of NI (Kampf and Kramer, 2004). The analysis of outbreaks revealed that dermatitis on the hands of health care workers was a risk factor for colonization or for inadequate hand hygiene, resulting in various types of NI. Transmissibility of VRE has also been demonstrated. The hands and gloves of 44 health care workers were sampled after care of VRE-positive patients. Gloves were VRE positive for 17 of 44 healthcare workers, and hands were positive for 5 of 44, even though they had worn gloves (Kampf and Kramer, 2004). One health care worker was even VRE positive on the hands although the culture from the glove was negative (Kampf and Kramer, 2004). S. aureus can survive on hands for at least 150 min; VRE survives on hands or gloves for up to 60 min. On inanimate surfaces, S. aureus and MRSA may survive for 7 months, with wild strains surviving longer than laboratory strains. VRE may survive on surfaces for 4 months. The long survival on surfaces, together with the relatively short survival on hands, suggests that contaminated surfaces may well be the source of transient colonization despite negative hand cultures (Kampf and Kramer, 2004).

Escherichia coli is the most common gram-negative bacterium, causing mainly NIs (Kim *et al.*, 2000). Overall, gram-negative bacteria are found in up to 64% of all NIs, colonization rates of gram-negative bacteria on the hands of health care workers 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 (Kampf and Kramer, 2004).

The colonization may be long-lasting, even in nursing homes, a rate of 76% has been described for nurses hands, colonization with gram-negative bacteria is influenced by various factors. For example, it is higher before patient contact than after the work shift, hands with artificial fingernails harbor gram-negative bacteria more often than those without (Kampf and Kramer, 2004; Hedderwick *et al.*, 2000). Higher colonization rates with gramnegative bacteria also occur during periods of higher ambient temperature and high air humidity, transient hand carriage of various gram-negative bacterial species has quite often been suspected to be responsible for cross-transmission during outbreaks resulting in various types of NI (Kampf and Kramer, 2004; Ganeswire *et al.*, 2003). Most gram-negative bacteria survive on the hands for 1 h or more. Survival on inanimate surfaces has been reported to be different for the different gram-negative species, with most of them surviving for many months . In general, gram-negative bacteria survive for longer on inanimate surfaces than on human skin (Kampf and Kramer, 2004). The main spore-forming bacterium causing NIs is *Clostridium difficile*. It is estimated that between 15 and 55% of all

cases of nosocomial antibiotic-associated diarrhea are caused by *C. difficile* (Barbut and Petit, 2001; Miller *et al.*, 2002). Patients with diarrhea caused by *C. difficile* have on average 3.6 additional hospital days attributable to the NI, The overall mortality is 15% (Morris *et al.*, 2002). Extraintestinal manifestations are very uncommon (1%). Patients can be contaminated from, for instance, the hands of hospital personnel and from inanimate surfaces (Barbut and Petit, 2001).

In one study, the hands of 59% of 35 health care workers were *C. difficile* positive after direct contact with culture-positive patients. Colonization was found mainly in the subungual area (43%), on the fingertips (37%), on the palm (37%), and under rings (20%) (Kampf and Kramer, 2004). In another study, 14% of 73 health care workers were culture positive for *C. difficile* on their hands. The presence of *C. difficile* on the hands correlated with the density of environmental contamination (Kampf and Kramer, 2004). During a third outbreak, caused by *Bacillus cereus* in a neonatal ICU, 11 (37%) of 30 fingerprints from health care workers were positive for *Bacillus* spp. (Van der Zwet *et al.*, 2000). Transmission of *C. difficile* in an endemic setting on a general medical ward has been shown to occur in 21% of patients, with 37% of them suffering from diarrhea, another spore-forming bacterium has been described as well: *B. cereus* was transmitted to the umbilicus in 49% of newborns on a maternity ward; the hands of 15% of the health care workers were found to be culture positive (Kampf and Kramer, 2004). Vegetative cells of *C. difficile* can survive for at least 24 h on inanimate surfaces, and spores survive for up to 5 months (Boyce and Pittet, 2002; Kampf and Kramer, 2004).

Prevention of nosocomial infections is the responsibility of all individuals and services providing health care. Everyone must work cooperatively to reduce the risk of infection for patients and staff. This includes personnel providing direct patient care, management, physical plant, provision of materials and products, and training of health workers. Infection control programmes are effective provided they are comprehensive and include surveillance and prevention activities, as well as staff training. There must also be effective support at the national and regional levels (Jalalpoor *et al.*, 2007; Madani *et al.*, 2009; Mielke, 2010; Rosenthal *et al.*, 2010a,b; Sehulster *et al.*, 2003). The major preventive effort should be focused in hospitals and other health care facilities. Risk prevention for patients and staff is a concern of everyone in the facility, and must be supported at the level of senior administration. A yearly work plan to assess and promote good health care, appropriate isolation, sterilization, and other practices, staff training, and epidemiological surveillance should be developed. Hospitals must provide sufficient resources to support this programme (Jalalpoor *et al.*, 2007; Madani *et al.*, 2009; Mielke, 2010; Rosenthal *et al.*, 2010a,b; Sehulster *et al.*, 2003).

Conclusion

Nosocomial infections remain a major global concern. Overall national prevalence rates have been described as ranging between 3.5 and 9.9%. They lead to additional days of treatment, increase the risk of death and increase treatment costs. Staff hands and hospital surfaces have important role in NIs (Astagneau *et al.*, 2001; Garcia *et al.*, 2001; Madani *et al.*, 2009; Mielke, 2010; Orsi *et al.*, 2002, Sehulster *et al.*, 2003).

Bacteria on hospital surfaces have low potential to spread. Staff hands have very contact with hospital surfaces and are more sources to transmission Bacteria into hospital. Increase Staff Hand and hospital surfaces hygiene has been considered the most important tool in control of transmission Bacteria (Jalalpoor *et al.*, 2007; Madani *et al.*, 2009; Mielke, 2010; Rosenthal *et al.*, 2010a,b; Sehulster *et al.*, 2003).

The studies have shown high frequency of resistance strain isolated from staff hands, Hospital surfaces and nosocomial infection (Jalalpoor *et al.*, 2009b,c,e; 2010b,c,e; 2011).

According previously result frequency of Surface layer in *B.cereus* st. isolated of staff hand were 84/6% and frequency of Surface layer in *B.cereus* st. isolated of hospital surfaces were 7/7%. According to antibiogram results, surface layer non producer strain, in comparative Surface layer producer strain, were more sensitive to antibiotics (Jalalpoor *et al.*, 2009a; 2009d; 2010a; 2010d).

The studies indicated that *Staphylococcus* spp. 28 (35%), *Bacillus* spp. 48 (60%) *Enterobacteriaceae* 4 (5%) consist of isolated bacteria from staff hands and according result of acidometric test in isolated bacteria from staff hands 61.9% of strains produce β –lactamase, respectively was in *Staphylococcus* spp., *Bacillus* spp. and *Enterobacteriaceae* 71%, 64.72% and 50% (Jalalpoor *et al.*, 2009b, 2010e, 2011). So Based on similar previously study result, *Staphylococcus* spp. 54.7%, *Bacillus* spp. 25%, *Enterobacteriaceae* 10.7%, other gram negative bacteria 4.15%, *Pseudomonas* spp. 3.6%, *Streptococcus* spp. 1.85% consist of isolated bacteria from hospital surfaces and according result of acidometric test 77.94% of this strains produce β –lactamase, respectively was in *Staphylococcus* spp., *Bacillus* spp. and *Enterobacteriaceae* 82.7%, 68.4% and 80.35% (Jalalpoor *et al.*, 2010c,e, 2011).

Approximately one third of nosocomial infections are preventable. Cleaning is the necessary first step of any sterilization or disinfection process. Cleaning is removing organic matter, salts, and visible soils, all of which

interfere with microbial inactivation (Jalalpoor et al., 2007; Madani et al., 2009; Mielke, 2010; Rosenthal et al., 2010a,b; Sehulster et al., 2003).

Hand washing frequently is called 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. Although hand hygiene is important to minimize the impact of this transfer, cleaning and disinfecting environmental surfaces as appropriate is fundamental in reducing their potential contribution to the incidence of healthcare-associated infections (Jalalpoor *et al.*, 2007, Madani *et al.*, 2009; Mielke, 2010; Rosenthal *et al.*, 2010a,b; Sehulster *et al.*, 2003).

The importance of hands in the transmission of hospital infections has been well demonstrated and can be minimized with appropriate hand hygiene .Compliance with hand washing, however, is frequently suboptimal. This is due to a variety of reasons, including: lack of appropriate accessible equipment, high staff-to-patient ratios, allergies to hand washing products, insufficient knowledge of staff about risks and procedures, too long a duration recommended for washing, and the time required (Jalalpoor *et al.*, 2007; Madani *et al.*, 2009; Mielke, 2010; Rosenthal *et al.*, 2010a,b; Sehulster *et al.*, 2003).

To minimize the transmission of microorganisms from equipment and the environment, adequate methods for cleaning, disinfecting and sterilizing must be in place. Written policies and procedures which are updated on a regular basis must be developed for each facility (Jalalpoor *et al.*, 2007; Madani *et al.*, 2009; Mielke, 2010; Rosenthal *et al.*, 2010a,b; Sehulster *et al.*, 2003).

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REFERENCES

- Astagneau, P., C. Rioux, F. Golliot and G. Brucker (2001). Morbidity and mortality associated with surgical site infections: results from the 1997–1999 INCISO surveillance. *J Hosp Infect*, 48: 267-274.
- Barbut, F. and J.C. Petit (2001). Epidemiology of *Clostridium difficile*-associated infections. *Clin. Microbiol Infect*, 7: 405-410
- Boyce, J.M. and D. Pittet (2002). Guideline for Hand Hygiene in Health-Care Settings. Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/ IDSA Hand Hygiene Task Force 51/RR-16.
- Ducel, G., J. Fabry, L. Nicolle, R. Girard, M. Perraud, A. Pruss and A. Savey (2002). *Prevention of hospital-acquired infections, A practical guide*. Department of Communicable Disease, Surveillance and Response, Editors;, 2nd edition, Available at WHO/CDS/CSR/EPH/ 2002.12.
- Ganeswire, R.K., L. Thong and S.D. Puthucheary (2003). Nosocomial outbreak of *Enterobacter gergoviae* bacteraemia in a neonatal intensive care unit. *J Hosp Infect*, 53: 292-6.
- Garcia, M., C. Lardelli, M. Jimenez, C. Bueno, J.D. Luna-del-Castillo and R. Galvez-Vargas (2001). Proportion of hospital deaths potentially attributable to nosocomial infections. *Infect Control Hosp Epidemiol.*, 2: 708–714.
- Hedderwick, S.A., S.A. McNeil, M.J. Lyons and C.A. Kauffman (2000). Pathogenic organisms associated with artificial fingernails worn by health care workers. *Infect Control Hosp Epidemiol.*, 21: 505-509.
- Hilton, M., J.M. Chen, C. Barry, M. Vearncombe and A. Simor (2002). Deoxyribonucleic acid fingerprinting in an outbreak of *Staphylococcus aureus* intracranial infection after neurotologic surgery. *Otol Neurotol*, 23: 550-554.
- Jalalpoor, S. H., R. Kasra Kermanshahi, A.S. Nouhi, and H. Zarkesh Esfahani (2007). Study Production of β-lactamase and Surface layer,Nano Structure in some of Isolated Pathogen Bacteria From Clinical and Environmental Hospital Samples. MSc, Thesis, Islamic Azad University Science and Research Branch.Iran, Tehran.46-84.

- Jalalpoor, S.H., R. Kasra Kermanshahi, A.S. Nouhi and H. Zarkesh Esfahani (2009a). The Prevalence of Nano-structure Surface Layer in *Bacillus Cereus* Strains Isolated from Staff Hands and Hospital Surfaces. *Journal of Isfahan Medical School*, 27(100): 632-645.
- Jalalpoor, S.H., R. Kasra Kermanshahi, A.S. Nouhi and H. Zarkesh Esfahani (2009b). 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 Journal of Medical Microbiology*, 3(4): 45-37.
- Jalalpoor, S.H., R. Kasra Kermanshahi, A.S. Nouhi and H. Zarkesh Isfahani (2009c). Survey effect of in-vivo and invitro condition on expression of surface layer genes in bacteria. *Journal of the Iranian Chemical Society*, 6 (suppl): S11.
- Jalalpoor, S.H. and H. Abousaidi (2009d). Survey Role and Important of Surfaces Structure and beta lactamase of Bacillus cereus in Drug Resistant. *Journal of Microbial World*, 2 (3): 169-176.
- Jalalpoor, S.H., R. Kasra Kermanshahi, AS Nouhi and H. Zarkesh Esfahani (2009e). Comparison of the Frequency β-lactamase Enzyme in Isolated Nosocomial Infectious Bacteria. *Journal of Rafsanjan University of Medical Science*, 8(3): 203-214.
- Jalalpoor, S.H., R. Kasra Kermanshahi, A.S. Nouhi and H. Zarkesh Esfahani (2010a). The Role of nanostructured surface layer and production of β-lactamase in penicillin resistant *Bacillus cereus* strains. *Iranian Journal of Medical Microbiology*, 4(1): 18-26.
- Jalalpoor, S.H., R. Kasra Kermanshahi, A.S. Nouhi and H. Zarkesh Esfahani (2010b). The specification of nano structure superficial layers in some of the pathogen bacteria. *Zahedan Journal of Research in Medical Science*, 12(4): 3-10.
- Jalalpoor, S.H., R. Kasra KermansA.hahi, A.S. Nouhi and H. Zarkesh Esfahani (2010c). Survey Frequence of β-lactamase Enzyme and Antibiotic Sensitivity Pattern in Isolated Pathogen Bacteria from Low and High Hospital Contact Surfaces. *Pajuhandeh Journal*, 15(2): 77-82.
- Jalalpoor, S.H., R. Kasra Kermanshahi, A.S. Nouhi and H. Zarkesh Esfahani (2010d). Prevalence of Nano Structure S-layer and β-lactamase in Bacillus cereus strains. *Journal Medical Science of Islamic Azad University*, 20(3): 157-163.
- Jalalpoor, S.H., R. Kasra Kermanshahi, A.S. Nouhi, H. Zarkesh Esfahani and S. Mobasherizadeh (2010e). Survey Prevalence and Resistance to some Beta lactame antibiotic in *B. cereus* st. Isolated of Azzahra Hospital. *Iranian Journal of Biology*, 23 (4): 470-477.
- Jalalpoor, S.H. (2011). Frequency of Beta Lactamase Enzyme in Isolated Pathogen Bacteria from Hospital In-Vivo and In-Vitro Condition. *Journal of Isfahan Medical School*, 29(131): 1-9.
- Johnson, L. (2006). Hand Hygiene Guideline From the Centers for Disease Control and Prevention. P.A.C.E. APPROVED.11/2006; Ver 5.10:6-7.
- Kampf, G. and A. Kramer (2004). Epidemiologic Background of Hand Hygiene and Evaluation of the Most Important Agents for Scrubs and Rubs. *Clin Microbiol Rev.*, 17(4): 863-93.
- Kim, J.M., E.S. Park, J.S. Jeong, K.M. Kim, J.M. Kim, H.S. Oh *et al.* (2000). Multicenter surveillance study for nosocomial infections in major hospitals in Korea. Nosocomial infection surveillance committee of the Korean Society for Nosocomial Infection. *Control Am J Infect Control*, 28: 454-458.
- Madani, N., V.D. Rosenthal, T. Dendane, K. Abidi, A.A. Zeggwagh and R. Abouqal (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. and S.H. Banitabay Hematyar (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. 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): 346-50.
- Miller, M.M., M. Hyland, M. Ofner-Agostini, Gourdeau and Ishak (2002). Morbidity, mortality, and healthcare burden of nosocomial *Clostridium difficile*-associated diarrhea in Canadian hospitals.Infect *Control Hosp Epidemiol.*, 23: 137-140.
- Morris, A.M., B.A. Jobe, M. Stoney, B.C. Sheppard, C.W. Deveney and K. E. Deveney (2002). *Clostridium difficile* colitis: an increasingly aggressive iatrogenic disease. *Arch Surg.*, 137: 1096-1100.
- Nasiry Razin B (2000). Overview of Bacterial in Operations room Environment in Hospitals Shahid Beheshty University. The 3th National Congress of Microbiology; Iran-Hamedan.
- Orsi, G.B., L. Di Stefano and N. Noah (2002). Hospital-acquired, laboratory- confirmed bloodstream infection: increased hospital stay and direct costs. *Infect Control Hosp Epidemiol.*, 23: 190–197.

- Raymond J, Aujard Y (2000). European Study Group. Nosocomial Infections in Pediatric Patients: A European, Multicenter Prospective Study. Infect Cont Hosp Epidemiol 21:260–63.
- Rosenthal, V.D., D.G. Maki and S. Jamulitrat (2010a). International Nosocomial Infection Control Consortium (INICC) report, data summary for 2003–2008, issued June 2009. *Am J Infect Control*, 38(2): 95–106.
- Rosenthal, V.D., D.G. Maki, C. Rodrigues, C. Alvarez-Moreno, H. Leblebicioglu, M. Sobreyra-Oropeza *et al.* (2010b). Impact of International Nosocomial Infection Control Consortium (INICC) strategy on central line-associated bloodstream infection rates in the intensive care units of 15 developing countries. *Infect Control Hosp Epidemiol.*, 31(12): 1264-72.
- Sehulster. L., and Y.M. Raymond (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.
- Steinbrecher, E., D. Sohr, A. Nassauer, F. Daschner, H. Ruden and P. Gastmeier (2000). Die haufigsten Erreger bel Intensivpatienten mit noskomialen Infektionen. *Chemother J.*, 9: 179-183.
- Stone, P., E. Larson and L. Kawar (2002). A systematic audit of economic evidence linking nosocomial infections and infection control interventions: 1990-2000. *Am. J Infect Cont.*, 30: 145-52.
- Tenorio, A. R., S.M. Badri, N.B. Sahgal, B. Hota, M. Matushek, M.K. Hayden *et al.* (2001). Effectiveness of gloves in the prevention of hand carriage of vancomycin-resistant *Enterococcus* species by health care workers after patient care. *Clin Infect Dis.*, 32: 826-829.
- Van der Zwet, W.C., C.A. Parlevliet, P.H. Savelkoul, J. Stoof, A.M. Kaiser, A.M. Van Furth *et al.* (2000). Outbreak of *Bacillus cereus* infections in a neonatal intensive care unit traced to balloons used in manual ventilation. *J Clin Microbiol.*, 38: 4131-4136.

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