Original article

Isolation of common aerobic bacterial pathogens from the environment of seven hospitals, Ahvaz, Iran

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Abstract

Introduction and objective: Hospital acquired infections are serious problems in patients care and adversely affect the mortality and morbidity despite antimicrobial therapy and advances in supportive care. The researchers aimed to determine the contamination of inanimate hospital environment to bacterial agents and their susceptibility to various antimicrobial agents. Seven different teaching hospitals were included in this study.

Materials and methods: From April 2006 to January 2007, 1208 samples (1156 wet swabs, eight water dialysis and 44 hand washing samples) were taken from surface and medical instruments in different hospitals' wards. Susceptibility test for bacterial isolates was done by disk diffusion assay.

Results: In the present study 57% of samples were positive and more than 10 species were isolated. Coagulase negative staphylococci (36.1%) and *Klebsiella pneumoniae* (8.9%) were the predominant isolates among Gram-positive and negative bacteria, respectively. Hands (79.5%), kitchen (71.4%), staffs' room (61.1%) and equipments (57.8%) were the most infected sites. Gram-negative enteric bacilli (50%) in food service personnel and Gram-positive cocci (46.6%) in medical personnel were predominant isolates from hand specimens. 60% of *Staphylococcs aureus* yielded methicillin resistant (MRSA).

Conclusion: Lack of a universal procedure for surveillance of nosocomial infection, presence of MRSA and some other pathogenic bacteria, poor hand hygiene and heavy contamination of some important surfaces are the most important problems in our hospitals.

Keywords: Hospital environment; Nosocomial infection; Bacterial pathogen; Bacterial contamination

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Introduction

In attempting to control and/or prevent nosocomial infections, an attack on the chain of infection at its weakest link is generally the most effective procedure [1]. The environment significantly influences multiple factors in the chain of infection. Although microbiologically contaminated surfaces can serve as reservoirs for pathogens, these surfaces generally are not directly associated with transmission of infections to either staff or patients [2].

The transmission of microorganisms from environmental surfaces to patients is largely via hand contact with the surface. Although hand hygiene is important to minimize the impact of this transfer, cleaning and disinfecting environmental surfaces appropriately is fundamental in reducing their potential contribution to the incidence of healthcare-associated infections [3]. Based on Centers for Disease Control and Prevention (CDC) classification, medical and surgical instruments are categorized to "critical," "semi critical," and "noncritical."

Environmental surfaces can be further divided into medical equipment surfaces (e.g., knobs or handles on hemodialysis machines, X-ray machines, instrument carts, and dental units) and housekeeping surfaces (e.g., floors, walls, and tabletops) [4]. Routine environmental-surface sampling (e.g., surveillance cultures) in health-care setting is neither cost-effective nor warranted. Surface sampling is used currently for research, as part of an epidemiologic investigation, or as part of a comprehensive approach for specific quality assurance purposes.

As a research tool, surface sampling has been used to determine a) potential environmental reservoirs of pathogens, b) survival of microorganisms on surface, and c) the source of environmental contamination [5,6]. We performed two studies about nosocomial infection in our province several years ago [7,8]. Because of this reason, we have made a decision to carry out a fundamental project to determine bacterial contamination of hospital environments as an important factor in nosocomial infection chain. The purpose of this study was to assess common aerobic pathogenic bacteria in the environments of the seven medical university hospitals in Ahvaz, Iran.

Materials and methods

During eight months (April 2006 to January 2007) 1208, samples were taken from different sites of seven hospitals (Emam Khomeini, Golestan, Razi, Sina, Shafa, Abuzar and Taleghani) that included almost 2000 beds (five general hospitals, one burn center and a center for cancerous patients). Sampling procedures were based on CDC guidelines for environmental infection control [9].

Environmental sampling was conducted in seven different wards, operating rooms, intensive care units, orthopedic/surgery, neonatal, kitchen and dialysis rooms. Furthermore, we evaluated bacterial contamination of personnel hands and some medical equipment too. All surface samples were taken after decontamination. To show the presence of antibiotic-resistant of bacteria in hospital environments, we did susceptibility test for all isolates. A questionnaire including eight questions about period of sampling, types and how to use disinfectants, members of infection control team, training infection control nurses and interpretation of sampling results was also prepared. This information has been taken from an infection control practitioner (ICP) in each hospital.

Sample/rinse method was used for sampling in the present study. Cotton tipped sterile swabs that were moistened in sterile brain-heart infusion broth (BHI) (Merck,

Germany) were used to take samples from different surfaces. In each sampling, approximately 25cm^2 was covered by moistened swab. The samples were categorized to clinical (patients area) and non-clinical surfaces (common area). The main target of sampling was hand contact surfaces. Although those were in an abundant group, we chose particularly important ones listed in table two [10].

The surfaces destined for food preparation are not analogous to all surfaces in a hospital. Two main factors, food staffs hands and food preparation surfaces were assessed [11]. To assess the quality of sterilizing equipments (autoclave and oven) we randomly evaluated some sterile packages from each hospital. A simple and innovative method of hand sampling was used to asses the presence of bacteria, particularly methicillin resistant *Staphylococcus aureus* (MRSA) that might be transmitted by personnel's hands.

Totally 35 people, including doctors, nurses, health care workers and food service personnel were included in this part of study. All samples were obtained from personnel during working hands. Samples of food staff were taken of their bare hands during food preparation. They washed their hands for 30 seconds in a sterile bag which contained 15ml of sterile Tryptic soy broth (Merck, Germany). The samples were transported to the laboratory in a cool-box and culture was carried out on the same day. A standard volume (100µl) was inoculated to the following culture media: MacConkey agar and blood agar (5.0% sheep blood) (Merck, Germany) and Mannitol salt agar (Merck, Germany) for selective isolation of *S. aureus* [12,13].

Out of seven hospitals that were included in this study, five possessed dialysis equipments. All hospitals used reverse osmosis (RO) water treatment systems through which water was collected in a strong tank as a reservoir. Water samples were taken from each water supply and then cultured on Tripticase soy agar (Merck, Germany) using pour plate method in the laboratory within 30 minutes after sampling. Colonies were counted after 48h of incubation at 35°C. All samples cultured on blood agar as an enriched media and MacConkey agar as selective media for Gram-negative bacteria. *S. aureus* was isolated using Manitol salt agar from other *Staphylococcus* species. Isolation and identification of microorganisms were done according to the standard procedure [13].

Disk diffusion method was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) with a 1µg oxacillin and Mueller-Hinton agar (Merck, Germany) to determine of MRSA. Furthermore, to evaluate the susceptibility pattern of other isolates, five antimicrobial agents including Ceftizoxim (30µg), Cephalotin (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Ceftazidime (30µg), Amikacin (30µg) and Trimethoperim/ Sulphamethoxazole (1.25/23.75 µg) were used. All discs were purchased from Padtanteb, Iran. All methodological variants were assessed using the same inoculums which were standardized to 0.5 McFarland turbidity. Two standard strains were processed in parallel as controls for the disk diffusion test: *Enterococcus faecalis* ATCC 29212 and *S. aureus* ATCC 25923 [14,15].

Results

Personnel's hands (medical and kitchen staff) were one of the highest contaminated sites in our study. Quantitative culture was performed to determine hands' microbial contamination. Results were categorized into two parts, personnel's hands in different medical wards and food service

personnel. Based on our results Gram-positive cocci *(S. aureus,* CoNS and *Enterococcus spp.*) in medical personnel and Gram-negative enteric bacilli *(Klebsiella pneumoniae* and *Enterobacter* spp.) in food service personnel were the most common isolates (Table 1). Four MRSA isolates were obtained of hand samples which were culture positive.

Sixty percent of all *S. aureus* isolates examined yielded MRSA. MRSA isolates were recovered from various sites such as patient's bed, staff rooms and particularly medical staff's hands. MRSA contamination rate in burn units was greater than in nonburn units. One of the most important points among our results concerned understanding the high contaminating site in the hospital environment. In comparison with the other sites, hands (79.5%), kitchen (71.4%), staffs' room (61.1%), equipments (57.8%) and nurses' station (51.3%) were common contaminated sites. Washed dishes (80.7%), food preparing surfaces (78.9%) in kitchen and patients' bed (69.5%) in equipments were the most infected sites.

Altogether 1208 samples were taken from different sites and 694 (57.4%) were culture positive to bacteria. Over 10 different bacterial species were isolated, of which the most common was Coagulase negative staphylococci (250/694; 36.1%), followed by *Bacillus* spp. (124/694; 17.9%), *S. aureus* (95/694; 13.7%), *K. pneumoniae* (62/694; 8.9%), *Enterococcus* spp. (51/694; 7.3%), *Pseudomonas aeruginosa* (42/694; 5.9%) and *Enterobacter* spp. (27/694; 3.9%) (Table1). However, we didn't find multidrug-resistant bacteria, but susceptibility pattern for some isolates was very considerable. However, *P. aeruginosa* was slightly more resistant than other bacteria. *P. aeruginosa* was resistant to Amikacin, Gentamicin, Cephalotin and Ciprofloxacin 83%, 79%, 78% and 53%, respectively (Table 2). The results showed *S. aureus* and *Enterococcus* spp. were the main isolates from staff's room. Two samples of eight water dialysis were contaminated with *P. aeruginosa.* To confirm, we took a couple of samples from each water supply in hemodialysis ward and these two contaminated samples belonged to one center. Culture technique was according to the Association for the Advancement of Medical Instrumentation (AAMI) standard procedure. The AAMI recommendations for microbial contamination are based on techniques using Tryptic soy agar as the medium and incubations at 37°C for 48h [16].

Among four sample sites in kitchen evaluated for bacterial contamination, food preparing surfaces were the most contaminated sites. CoNS were isolated in 23 (31%) samples and similar frequency was reported in the case *S. aureus* and *K. pneumoniae* (31.5%). The level of bacterial contamination on environmental surfaces of hospitals was variable. As shown in table one, we found the following sites with considerable percentage of bacterial contamination: Taps and showers 80%, patient's bed and cabinets 72.5%, drug trolleys 63.7%, neonatal incubators 59% and nurse's stations 51%.

Discussion

The prevalence of bacterial contamination among all hospitals that were included in this study has not been determined accurately yet and our study is the first in our province. Compared to other studies, we employed a fairly large number of samples and isolates so this is beneficial for interpretation [17,18]. All of dialysis units were equipped with water purification systems based on reverse osmosis (RO) which, along with sound pre-treatment processes, is almost capable of removing chemical contaminants completely.

Table 1: Frequency of number and type of isolates according to sample site

Sample site	Sample size	[c]Positive cultures (%)	*S. aureus*	*K. pneumoniae*	*E. coli*	*Enterobacter spp.*	*P. aeruginosa*	*Enterococcus spp.*	*Proteus spp.*	CoNS	*Acinetobacter spp.*	*Bacillus spp.*
Nurses' station	144	74 (51.3)	8	5	2	1	2	2	-	33	-	21
[a]Equipments	697	403(57.8)	59	34	11	14	27	25	-	143	11	79
Staffs' room	126	77(61.1)	11	6	1	2	1	12	-	31	-	13
Incubator & ventilator component	84	28(33.3)	-	-	-	2	3	2	-	13	3	5
[b]Kitchen	105	75(71.4)	10	10	7	3	5	8	3	23	2	4
Hands(kitchen & medical staff)	44	35(79.5)	7	7	1	5	2	2	1	7	-	3
Dialysis water	8	2(25)	-	-	-	-	2	-	-	-	-	-
Total (%)	1208	694(57.4)	95(13.7)	62(8.9)	22(3.1)	27(3.9)	42(6)	51(7.3)	4(0.5)	250(36.1)	16(2.3)	125(18)

a: Equipments: Patients' bed, Tap & shower, Patients' cabinet, Drug trolley, Blood pressure cuff, Laryngoscope, Stethoscope; b: Wash dishes, Food trolley, Food preparing surfaces, Meat grinder; c: Positive culture: grow overall cfu/cm^2

Table 2: Susceptibility pattern of isolates were taken from hands of personnel and hospital environment

Antibiotics	Disk content (µl)	*K. pneumoniae*	*Enterobacre spp*	*P. aeruginosa*	*Acinetobacter baumanni*
Ceftizoxime	30	52%	15%	45%	51%
Cephalotin	30	71%	69%	78%	79%
Ciprofloxacin	5	10%	10%	53%	25%
Gentamicin	10	52%	90%	79%	68%
Ceftazidime	30	76%	20%	25%	53%
Amikacin	30	23%	88%	83%	57%
Trimethoperim/ Sulphamethoxazole	1.25 / 23.75	53%	68%	ND	ND

ND: not done

In the present study two samples were contaminated with *P. aeruginosa*. There are many situations where certain types of Gram-negative bacteria can persist and actively multiply in aqueous environments associated with hemodialysis equipment which can directly or indirectly affect patients through septicemia or endotoxemia [19].

The most widely accepted standards of water purity are those recommended by AAMI and the European Pharmacopea, which respectively allow bacterial growth of <200 and <100cfu/ml, and an endotoxin concentration of <2 and <0.25IU/ml [16,20]. Andrulli *et al.* [21] reported that 17.8% of water dialysis samples had bacterial count more than AAMI standard (200cfu/ml). The results of multicentre studies indicate that the microbial quality of dialysis fluids is still a constantly neglected problem, particularly as there is evidence of a possible relationship between dialysis fluid contamination and a long-term morbidity [20].

All hemodialysis units which were included in this study used storage tank in their systems. These systems can greatly increase the volume of fluid but they are capable to serve as a niche to water bacteria. Now the ministry of health intends to substitute new online RO system machines for the old machines. Although we didn't report MRSA prevalence in each hospital separately, 60% yield of MRSA is a high prevalence. Boyce *et al.* [22] showed that MRSA contamination in inanimate environment of burn units (up to 64%) is greater than that in nonburn units (ranged from 1% to 18%) and in a similar study, French *et al*. [23] demonstrated that 72% of inanimate sites in hospital environment were positive for MRSA.

The fact that most Gram-positive bacteria, such as MRSA and Vancomycine Resistant *Enterococcus* (VRE) contaminate the inanimate environment has been well established. They can survive for months on an inanimate surface. The major reservoirs for MRSA include colonized or infected patients, personnel in the hospitals and the major mechanism is done via the unwashed hands of health care workers [24]. Presence of bacteria was different from ward to ward and hospital to hospital based on activities of each hospital. For example *S. aureus* was the predominant isolate in burn hospital; whereas *Enterococcus* was the main isolate in orthopedic / surgical hospital.

In our series, Gram-negative enteric bacilli (*K. pneumoniae,* and *Enterobacter* spp.) were the predominant isolates from food staff hands and *CoNS* and *S. aureus* were the third and the fourth agents. Aycicek *et al.* [11] demonstrated that *S. aureus* and CoNS were the most common isolates and *Escherichia coli* was reported as the fifth isolate from hand specimen of hospital food handlers. The presence of coliform or Gram-negative enteric bacilli on the hands is an indicator for fecal contamination and also poor hand hygiene.

In this study, the isolation of CoNS from food preparation surface was high (31%). However *S. aureus* and *E. coli* were presented to be the main agents for food poisoning, but there are some reports for production of enterotoxin by CoNS [25]. The presence of food poisoning bacteria on surfaces and food staffs hands are a serious alarm or warning for every hospital. Bacterial contamination on healthcare provider's hands was a lot different from food service personnel. In this group Gram-positive cocci (*S. aureus* 33%, CoNS 26% and *Enterococcus* spp. 13%) were the predominant isolates.

Various studies have reported the following pathogens with a considerable percentage on healthcare provider's hands: *Acinetobacter* spp. 15%, *Clostridium difficile* 14-59%, *Klebsiella* spp. 17%,

MRSA up to 16.9%, *Pseudomonas* spp. 1.3-25%, VRE to 41% and yeasts (including *Candida*) 23-81% [3]. Contaminated environmental surfaces are also an under-recognized source of hospital infections. Many surfaces in hospitals contain viable pathogens that may be variable in different wards or hospitals. Various studies have reported that nosocomial pathogens exist on many instruments that belong to healthcare providers such as stethoscopes, blood pressure cuff and laryngoscope [26].

The most important way to control the spread of nosocomial infections via these instruments is to disinfect or clean them regularly. Although, we reported one case of bacterial contamination in surgical instrument after sterilization, this is a crucial problem that can directly threat patient's life. Moreover, few studies have been conducted concerning sterilization of surgical instruments and medical devices such as endoscopes. Cleaning must also precede sterilization or high-level disinfection [27].

These findings have emphasized the followings: (1) High level of bacterial contamination on hospital environmental surfaces. (2) A standard procedure of steady sampling, interpretation and documentation is required. (3) The hygiene training level of healthcare worker and food service personnel is insufficient. (4) The number of nurses particularly infection control nurses is inadequate. (5) Types, quantity and usage of disinfectant reagent need to be reconsidered. (6) All personnel should be familiarized to rules of hand washing and using proper gloves too.

Conclusion

As a conclusion, hospital environment is a complicated ecosystem and many interventions are needed for optimal infection control. Lack of a universal procedure for surveillance of nosocomial infection, poor hand hygiene, and high level of bacterial contamination on hospital environmental surfaces and high prevalence of MRSA and *Pseudomonas* as common isolates are the most important problems in our hospitals.

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