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Methicillin- and Vancomycin-Resistant Staphylococcus Aureus on Computer Keyboards Located in Different Wards of a Third-Level Hospital in Tehran, Iran

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Abstract

Introduction: Nosocomial infections are one of the health problems in all societies. The aim of this study was to evaluate contamination of computer keyboards located in different wards of Rasoul-e-Akram hospital in Tehran with methicillin-resistant *Staphylococcus aureus*.

Material and Methods: This cross - sectional study performed to identify the microorganisms in the Rasoul-e- Akram Hospital, 90 samples collected from different wards of this hospital by sterile swab for culture on EMB agar and blood agar media and used for differentiation test to identify the types of microorganisms. Data analyzed by using SPSS24 Software.

Results: The results of this study showed that *Staphylococcus* bacteria are the most common type of microorganisms in infectious wards (59.41%) includes *Staphylococcus coagulase negative (42.02%) and S. aureus (17.39%)*. Also S. *aureus isolated* from PICU (50%), ICU(25%) and operating room (25%) were *MRSA. one* S. *aureus* was isolated with vancomycin MICs >2µg/ml.

Conclusion: Results showed the high incidence of microorganisms that causes nosocomial infections in different wards and there is need to use proper disinfectants to prevent the spread of these agents in a hospital environment.

Keywords: Nosocomial infections; Hospital; Hethicillin-resistant *Staphylococcus aureus*

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Introduction

Every year, millions of people across the world suffer from Healthcare-associated Infections (HAI). Infections may also occur at surgery sites, known as surgical site infections [1]. HAIs may be caused by infectious agents from endogenous (body sites) or exogenous sources (patient care personnel, visitors, patient care equipment, medical devices or the health care environment) [2]. These infections of ten have little or nothing to do with the primary reason for the hospital visit but are consequence of deprived hygiene in the healthcare setting [3]. Healthcare equipment is commonly shared between hospital workers, who may have various hygiene practices. Computers and telephones are now found in all healthcare locations and their disinfection is often ignored more than medical devices. Therefore, the opportunity for the transmission of contaminating bacteria is potentially great [4-8]. There has been much debate over the infection risk to patients from contaminated health care surfaces [9]. It has been reported that, devices and different items in hospital environments, such as telephones and computers, are highly associated with HAIs transmission [3-7].

It is now recognized that the health care environment may facilitate transmission of several important health care-associated pathogens, including vancomycin- resistant *enterococci* (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) [8,10]. These pathogens are frequently transmitted between patients and healthcare workers and consequently to the devices that are commonly used by other staff [10,11].

It is highly recommended that computer keyboards and mice used in patient care areas should

Table 1: Frequency of positive microbial culture according to type of microbial Contamination.

Bacterial isolates	No. of bacterialisolates from		
	Keyboards	mice	Total (%)
Staphylococcus coagulase negative	35	23	58(42.02)
S. aureus	18	6	24(17.39)
Bacillus spp	10	10	20(14.49)
Diphtheroidspp	10	7	17(12.31)
Gram negative bacteria (fermentative)	0	1	1(0.74)
Pseudomonas spp	0	1	1(0.74)
Fungal	17	0	17(12.31)
Total	90	48	138(100)

Table 2: The frequency of Staphylococcus species of isolated from variety of wards hospital.

Wards Hospital	Number of computers	Number of sample	*Staphylococcus species(n)*	
	In wards		CoNS	S.aureus
Emergency	4	8	8	0
PICU	3	12	5	6
ICU	2	8	4	4
Operating Room	2	8	4	4
Internal	3	6	5	0
Blood	3	6	5	0
Skin	3	6	5	0
Obstetrics and gynecology	3	6	5	0
Ophthalmology	2	8	5	2
SportClinic, heart	3	6	4	0
ClinicofNeurology	1	2	1	0
Psychiatry	2	8	4	4
lungandENT	3	6	1	4
Neurosurgery	2	4	1	0
Pediatric	2	4	1	0
Total€	38	98	58	24

be disinfected each day however it is neglected in almost all sectors of the public health system. In this study the contamination of computer keyboards located in different wards of Rasoul-e- Akram hos0pital with methicillin-resistant *Staphylococcus aureus* was investigated.

Materials and Methods

Sampling

A cross-sectional study conducted at Rasoul-e-Akram Hospital, Tehran, Iran, since April 1 t\o August 31/2015.Samples were taken from 98 keyboards and mice of multiple-user computers in 15 departments of the Hospital using pre-sterilized swabs (Table 1).The sterile swabs immersed in Trypticase Soy Broth (TSB) and used to obtain samples from the computer keyboards and mice by slowly, but firmly, rotating the swab on the different buttons and surfaces of the computers. The swabs were then re-immersed in the TSB broth to prevent from drying and were taken immediately to the microbiology laboratory for further processing (Table 2).

Isolation and Characterization

Swabs were cut in sterile condition and the head swabs were inoculated into TSB media in5 ml tubes. The tubes were incubated at 37ºC for 24 hours and aliquots were inoculated on blood agar, McConkey agar and Sabouraud dextrose agar plates. The preliminary characterization of the isolates performed based on colony morphology, Gram-staining, catalase and oxidase tests, bacterial isolates. Also, a slide coagulase test (Microgen Staph, UK) used to differentiate isolates of staphylococci into *S.aureus* and coagulase-negative *staphylococci.* A tube coagulase test used for confirmation (Figure 1).

Detection of Methicillin-Resistance

Methicillin-resistant *S. aureus* (MRSA) strains were confirmed on Mueller Hinton Agar (MHA)containing 4% NaCl (Oxoid) and supplemented with6 µg/ml Oxacillin (Oxoid). All *S. aureus*strains were tested for methicillin-resistance using the disk diffusion method (Cefoxitin 30 µg;, B D, Germany) in 33 to 35°C ambient air for 24 hours to identify MRSA (Table 3).

Antimicrobial susceptibility testing

Susceptibility of *S. aureus* isolates to Cefoxitin (Fox), Penicillin (P), Erythromycin (E), Clindamycin (Cd), sulfamethoxazole trimethoprim (sxt),Ciprofloxacin (Cip), Gentamicin (GM),Tetracycilin(Te) and Vancomycin(V) (MAST Group, United Kingdom) was determined by disc diffusion method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) [12]. All methicillin resistant

Table 3: Frequency of MRSA of isolated from variety of wards hospital.

Source sample	Noof *S.aureus*	No. of **MRSA (%)**	No. of **MSSA (%)**
ICU	4	1(25)	3(75)
PICU	6	3(50)	3(50)
Operating Room	4	1(25)	3(75)
Ophthalmology ward	2	0	2(100)
Psychiatry Ward	4	0	4(100)
Lung and ENT ward	4	0	4(100)
Total	24	5(20.8)	19(79.2)

strains were collected and MICs of vancomycin among MRSA isolates were determined by Etest (AB, Bio merieux, France) according to the manufacturer's instructions and were repeated by CLSI guidelines [12].

Detection of Coding Genes for Methicillin Resistance

DNA extraction

The genomic DNA was extracted from S. aureus isolate using QIA amp genomic DNA kit (Qiagen, Germany) as per manufacturer's protocol [13,14].

PCR-Amplification and Detection of *mecA* Genes

Detection of *mecA* gene by polymerase chain reaction (PCR) was considered the gold standard. A *mecA* negative strain of *S. aureus* (ATCC® 25923 (Cefoxitin zone 23–29 mm)) and A *mecA* positive strain of *S. aureus* (ATCC® 43300 (Cefoxitin zone ≤ 21 mm)) were used as negative and positive controls, respectively. The primers used for PCR-amplifications were forward 5'-TCGAGGTAAGGTTGGCC-3', Reverse 5'-AGTTCTGCAGTACCGGATTTGC-3' [15]. The PCR mixture was prepared in a final volume of 25 µL. The amplification mixture consisted of a 2.5 µL template DNA, 2 µL primers, 2 µL of a 10-fold concentrate PCR buffer, 2 µL dNTP, 0.5 mM $MgCl_2$, 15 µL D.W. and 1 U of Taq DNA (QiaGen, Germany). The PCR program for the detection of the *mec*A gene was: initial denaturation at 94°C: 3 min, followed by 30 cycles of amplification with 94 °C: 30 seconds, annealing at 55 °C: 30 seconds, and extension at 72 °C: 30 seconds, except for the final cycle, which had an extension step of 4 minutes. PCR products were then visualized on a 1% Agarose gel *MecA* positive isolates detected as 480 bp long amplicons observed on the agarose gel (Figuer 1).

Statistical Analysis

Data obtained in the study were descriptively and statistically analyzed using $SPSS_{24}$ software .The means were separated using one sample t-test (P<0.05) is significant and (P>0.05) is not significant.

Results

We investigated the bacterial contamination of two objects used daily: computer keyboards, computer mice. A total of 98 samples were collected from the two different objects. 87% percent of the total samples collected were contaminated with mixed bacterial growth. Coagulase-negative staphylococci dominated the isolates. The second most common bacterial growth in all specimens was Gram-positive bacilli. Potential pathogens isolated from all specimens were: *Staphylococcus aureus, Pseudomonas spp*. and *Gram negative bacilli.*

All computer keyboards, mice were cultured and analyzed for pathogenic organisms. 1 (1.0%) of the 98 samples were colonized with six different bacteria. 58samples (59.18%) grew gram-positive bacteria, and 2sample (2.0%) grew gram-negative bacteria. 17 sample (17.3 %) grew fungi. The fungal isolates are as follows Aspergillus sp., Mucor sp., Penicillum sp. The most commonly isolated species was Aspergillus.

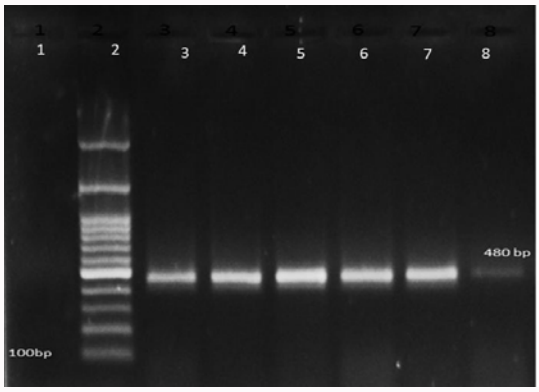

Figure 1: Polymerase chain reaction amplification of mecA gene. Lane1: Control negative, Lane 2: Ladder 100bp, Lane 3,4,5,6,7,8 : sample

In all coagulase-positive isolates, the mecA gene was detected by PCR.

In this study, *Staphylococcus aureus* was also isolated 24(17.39 %), of which 19(79.2%) were sensitive to methicillin and 5(20.8%) were methicillin resistant. S. *aureus* isolated from PICU (50%) ,ICU(25%) and operating room (25%) were *MRSA* .Vancomycin minimum inhibitory concentration (MIC) of resistant isolates was also determined using Etest. *one S. aurous* was isolated with vancomycin MICs >2µg/ml.

Discussion

The emergence of nosocomial infections, especially types of antibiotic resistance, is one of themaj or problems in the hospitals and environmental pollution has fundamental role in the development of nosocomial infections. Surfaces and different equipment in different hospital wards are suitable sites for colonization no f microorganisms. Different bacteria and fungi are able to survive on in animate surfaces for a long time. For instance *Staphylococci* can survive in dried blood for 3 months. Computer devices are widely used in hospital wards. Various studies in different parts of the world had assessed the microbial contamination on computer keyboards. In the present study, all the collected samples from mice and keyboards of computers were contaminated with human pathogenic bacteria. Gram positive bacteria were more frequently isolated from all surfaces compared to

Gram negative. This could be due to the fact that survival of Gram positive species on laminate surfaces is greater than that of Gram negative organisms [13].

Coagulase-Negative *Staphylococcus* is found on skin or in the nasal environment and only survives on dry skin. Its emergence on keyboards is due to the frequent use of these devices. Methylamine resistant *Staphylococcus aureus* (MRSA) which was isolated from the ICU key board, can cause infections in patients [14-16].Distributions of MRSA isolates were varied in different wards which partly reflected the fact that some patients, e.g., critically ill patients in ICUs, had a greater chance of becoming colonized or infected. *Enterococci* are part of the normal flora in human gutand are adapted to extreme conditions.

These results are somewhat similar with the findings of Alemu. study at the Vali –e- asre Hospital of Birjand, the bacterial contamination of computer keyboards were included *Enterobacteriaceae, bacilli*gram-positive spore-bearing, *CoNS*, *diphtheroid*and *S. aureus* [17]. Bacterial contamination of computer keyboards in hospitals of Isfahan province were also included *Bacillus* species, *CoNS*, *S. aureus*, *Enterococcus* species, *Micrococcus* species, gram negative bacilli, methicillin- resistant *S. aureus* and vancomycin- resistant *Enterococci* [18-21].

According to guidelines proposed by the Centers for Disease Control and Prevention (CDC) in the USA, difficult-to-clean hospital equipment (eg. computer keyboards) should be protected from potential contamination by means of special protective covers [22-26].

This study has demonstrated that microbial contamination of multiple-user computer keyboards may be a common mechanism of transfer of potentially pathogenic bacteria among users.

Conclusion

The isolation of the bacteria from computer keyboards and mice is a clear indication that the sterilization system of health care centers is not effective in reducing the level of the organism on these surfaces to an acceptable level. Microbes are everywhere, therefore, it is highly recommended that hand- washing hygiene should be adopted before and after using the computers to reduce the microbial transmission. Computer keyboards and mice should also be cleaned with alcohol or other disinfectants on a regular basis.

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