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Predictive Parameters of Heterotroph Plate Count Bacteria Amplification in Hospital Water Lines

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This research illustrates microbial instability of water from point of heterotrophic bacteria (HPC) amplification in accordance with residual chlorine, pH, temperature, dissolved oxygen (DO) and alkalinity (Alk) in hospital water. Hundred-fifty samples were collected from hot and cold tap water systems. Residual chlorine, pH and temperature were determined promptly in place; HPC was cultured on R2A culture media and spread plate method. DOand Alk was determined by Winkler and photometric methods, respectively. HPC was detected in 96% of samples therefor fifty-two percent had higher than the recommended standards and HPC density in samples been 947±998 CFU/ml. The highest contamination was detected in cooling systems, gynecological, NICU and sonography ward (1500-2000 CFU/ml) and the lowest was inendoscopy, laboratory and drug store (125-175 CFU/ml). Spearman correlation and multivariate linear regression revealed that HPC density in cold water has negative correlation with Cl_2, temperature and DO and positive correlation with pH and Alk. In warm water HPC density has negative correlation with temperature, pH, and DO and positive correlation with Cl_2 and Alk. Chi^2 test revealed that higher densities (>500 CFU/ml) in cold water was more frequently than warm water (OR: 2.3). HPC has unexpected distribution in water. Occurrence of high densities of HPC can be affect the presence of hazardous bacteria so, it is advised to implement the routine test of HPC monitoring with indicator bacteria and remark the effective efforts for hospitals' water disinfection for assurance of water safety.

Key words: Heterotrophic Bacteria, Water, Hospital.

Access to the safe and reliable drinking water from point of quality and quantity is one of the most important and difficult challenges in the health status of communities[1]. In recent years despite comprehensive progress towards the Millennium Development Goals, responsible organizations including United Nations reported that at least 11% of world's population has no access to the safe drinking water[2]. Water born disease leads to over than 50,000 people die daily and in developing countries the estimated mortality in children under five years is about 4 million[3]. Although, tremendous amount of researches have been investigated on water treatment, contributing to improved, treated water quality but, the quality of drinking water at the consumers' taps was neglected. Several researches reported that entered treated drinking water to the distribution system has appropriate status from point of physicochemical and microbial aspects, but the relevance complicated physicochemical and biological processes lead to its deterioration at the

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consumers' taps[4]. Therefore, despite observance of the relevant current regulations which are set by the World Health Organization in the developed and developing countries, proliferation and multiplication of pathogens and opportunistic pathogenic bacteria possesses several risks for human health and the treated drinking water still causes waterborne disease. This phenomenon may relevant to bacterial regrowth and biofilm accumulation in drinking water distribution systems which was reported by many of investigators[5]. Despite many of reports have demonstrated that regrowth or amplification of native bacteria in drinking water is not a threat to human health however, many bacteria occurrence in drinking water may act as an opportunistic pathogen and pose a health risks, especially for vulnerable population with an impaired immune systems[6]. HPC is one of the most important components of biofilm systems and native bacteria in water distribution systems[7]. Although HPC as an ancillary indicator of bacterial quality of water cannot detect pathogenic bacteria but, these bacteria represent a wide range of bacteria types, including *Escherichia, Klebsiella, Enterobacter, Citrobacter, Serratia, Helicobacter*, etc, which are now known to pose a public health risk to consumers[8, 9]. Despite, no epidemiological evidences have been linked to directly HPC bacteria but, several studies implied that these organisms may lead to health concerns in the immune compromised populations[9, 10]. Nosocomial infectious is one of the most legitimated health challenges which tend to occur more commonly in immune compromised patients[11] so, it can be told that its morbidity may influenced by the hospital water HPC which represent a wide range of pathogens. Due to this hazardous potential of HPC, several countries were set obvious guideline for these organisms. The United States directed less than 500 CFU/ml of HPC as an accepted level for drinking water. In Australia, the guideline is 100CFU/ml and 500 CFU/ml for disinfected and none-disinfected waters, respectively[6]. Due to, safety of drinking water especially in hospital is still a concern, auditing and inspection of water distribution system is regarded as important to the supply of safe drinking water[12]. Since, the hospital water is one of the most important and controllable source of nosocomial pathogens, survey of microbial water quality from point of HPC amplification and their relevant parameters for providing of the effective efforts for HPC elimination is important. The aim of this research was investigation of treated drinking water quality from point of HPC multiplication and its relevant parameters in hospital water.

MATERIALS AND METHODS

Sampling analysis

A total of hundred-fifty aliquots of 2 L water specimens were taken with sterilized bottles from hospitals water; consisting of seventy-nine samples of cold tap water (temperature range: 9-29ºC) and seventy-one samples of warm tap water (temperature range: 30-60ºC) for determining of HPC and the selective chemical parameters.The identified physicochemical qualities of samples are in accordance with the Iranian regulations for drinking. Grab sampling procedure was used for the sampling from several wards of the hospital. Briefly, 2 L of the warm and cold water was collected after letting the water runs for approximately 3 min, corresponding to the hottest and coldest water in the hospital water lines. Residual chlorine removal was performed via 3 % sodium thiosulfate. Samples were taken to the laboratory at 4°C and processed for bacterial tests within maximum of 8 h after collections(13).HPC was cultured on R2A agar culture mediabased on spread plate count method and incubation on 35±0.5°C for 48±2 h and enumeration was done via HPC counter(13). The results were expressed as colony-forming units per liter (CFU × L^{-1}), on the basis of plate counts of bacteria colonies. Since high densities of HPC were expected; HPC culture was performed with appropriate dilution which considered in HPC density calculations. To obtain the reproducibility of the collected data, the mean of two plates (duplicate readings) was reported.

Physical and chemical analyses

Temperature, pH and residual chlorine was measured on site promptly[13]. Residual chlorine concentration was determined using N, N-diethyl-p-phenylenediamine sulfate colorimetric method. The value of temperature and pH was determined using the portable digital thermometer (T-Bar ST-9296) and pH meter (METTLER TOLEDO). Alkalinity and DO was determined in laboratory by Winkler and photometric methods, respectively.

Statistical analysis

Normal distribution of data was determined by Kolmogorov-Smirnov test. Descriptive statistic was used for description of data and the chi-square tests($Ç^2$), Spearman's rank correlations, Mann-Whitney U test and Multivariate linear regression, were conducted to analysis the correlation between parameters using statistical package software(SPSS 15) in which statistical results were interrupted at the level of significance $p<0.05$. All statistical analysis was performed with the HPC density and selective chemical parameters including pH, temperature, DO and Alk which stated in the manuscript.

RESULTS

HPC was detected in 144 of samples (96%) in which 52% of samples had higher than the recommended standards (500CFU/ml) and HPC density in samples been 947±998 CFU/ml. The highest densities of HPC was observed in cooling systems, gynecological, NICU and sonography ward (1500-2000 CFU/ml) and the lowest was in endoscopy, laboratory and drug store (125-175 CFU/ml). Chi^2 test revealed that the HPC density (500<CFU/ml< 500) in cold and warm water has significant difference($p<0.001$) therefore, the higher densities (>500 CFU/ml) in cold water was more frequently than warm water (OR: 2.3). We next evaluated the HPC density in hospital wards (Table 1).Since, occurrence of the high densities of HPC as an opportunistic bacteria in some of the hospital wards are crucial which can pose health risks on vulnerable groups, HPC contamination evaluated in selected wards individually in cold and warm water (Figure 2and 3).

Distribution of HPC in cold and warm water revealed that both cold and warm water has high densities of HPC. Therefore, HPC density is higher than the recommended guidelines by WHO and CDC (500 CFU/ml).

Comparison of the mean values of residual

Table 1. Distribution of samples and HPC density in hospital wards

Hospital ward	Cold water		Warm water	
	Samples N.	HPC (CFU/ml)	Samples N.	HPC (CFU/ml)
Operating room	6	175	6	558
CCU	3	598	3	625
ICU)	3	1449	6	1035
Internal ward	8	1771	8	418
Laboratory	2	140	3	125
Radiology	2	45	7	308
Pharmacy	1	250	1	0
Endoscopy	1	75	1	270
Gynecological	3	1558	4	1778
Dentistry	12	668	2	2309
NICU	2	1962	2	1390
Infants	2	1420	2	1200
Emergency	2	1443	3	1352
CTS&Sonography	1	1340	2	1693
Surgery	4	857	5	11
Nuclear Medicine	0	0	2	390
Orthopedic	3	1164	3	30
Urology	1	650	1	115
Neuro-phycology	1	800	1	30
Hemodialysis	1	735	1	0
Clinic	1	710	1	1750
Cooling Systems	14	1995	0	0
Kitchen	1	625	3	57
Etc. (Installation)	1	1260	4	1305
Water inlet	4	850	0	0

chlorine, temperature, pH, DO and alkalinity in accordance with HPC density (500<HPCd” 500 CFU/ml) was performed via Mann-Whitney U test(Table 2). As shown in Tab. 2 in cold water the mean of residual chlorine and temperature has significant difference in 500<HPCd” 500 CFU/ml status. Additionally in warm water the mean of temperature, pH and alkalinity has significant difference.

Statistical analysis via Spearman correlation and Multivariate linear regression tests revealed that in cold water residual chlorine has negative and significant difference but in warm water total alkalinity has positive and significant difference in 500<HPC ≤ 500 CFU/mlstatus. Although, other parameters have positive or negative correlation but theirinfluences are not significant (C.I= 95%).

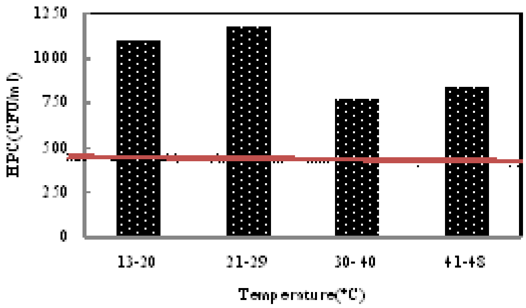

Fig. 1. HPC distribution versus water temperatureand recommended cut-off point

Table 2. Comparison the mean values of measured parameters and HPC density (Mann-Whitney U test)

Water Characteristic	Unit	Water System	HPC >500 CFU/ml (n=78) Mean	HPC ≤ 500 CFU/ml (n=78) Mean	*p* value	Med(IQR)
Res. Chlorine	mg/l	C.	0.50	0.75	0.006*	0.6(0.5-0.6)
		W.	0.29	0.39	0.28	0.1(0-0.7)
Temp.	˚C	C.	18.96	22.48	0.001*	20(17-22)
		W.	40	43.57	0.04*	43(43-48)
pH		C.	7.87	7.82	0.64	7.90(7.80-8.0)
		W.	7.68	7.78	0.03*	7.70(7.50-8.0)
DO	mg/l	C.	5.68	6.09	0.41	6(5-7)
		W.	4.86	5.37	0.14	5(5-6)
T. Alkalinity	mg/l $CaCO_3$	C.	161.96	136.30	0.13	130(120-150)
		W.	137.50	122.45	0.004*	120(120-145)

C: Cold water, W: warm water

Table 3. Correlation of measured parameters ver. HPC density

Water Characteristic	Unit	Water System Type	Linear correlation r_s	Linear correlation *p*	Multivariate linear regression β	Multivariate linear regression *p*
Res. Chlorine	mg/l	C.	-0.42	<0.001*	-0.33	0.003*
		W.	0.04	0.73	0.02	0.9
Temp.	˚C	C.	-0.20	0.07	-0.09	0.39
		W.	-0.27	0.02*	0.009	0.94
pH	——-	C.	0.08	0.50	0.01	0.93
		W.	-0.26	0.02*	-0.12	0.51
DO	mg/l	C.	-0.22	0.6	-0.2	0.19
		W.	-0.26	0.04*	-0.08	0.46
T. Alkalinity	mg/l $CaCO_3$	C.	0.14	0.20	0.10	0.51
		W.	0.54	<0.001*	0.43	0.002*

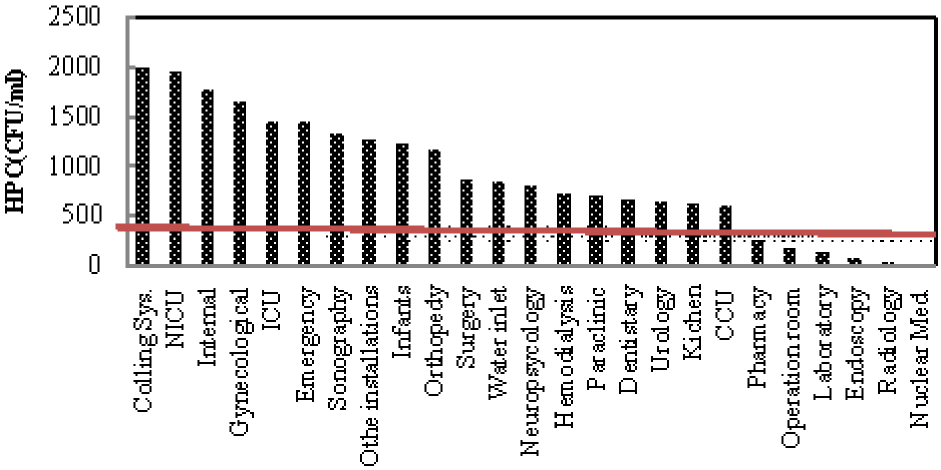

Fig. 2. Cold water HPC density in hospital wards and recommended cut-off point

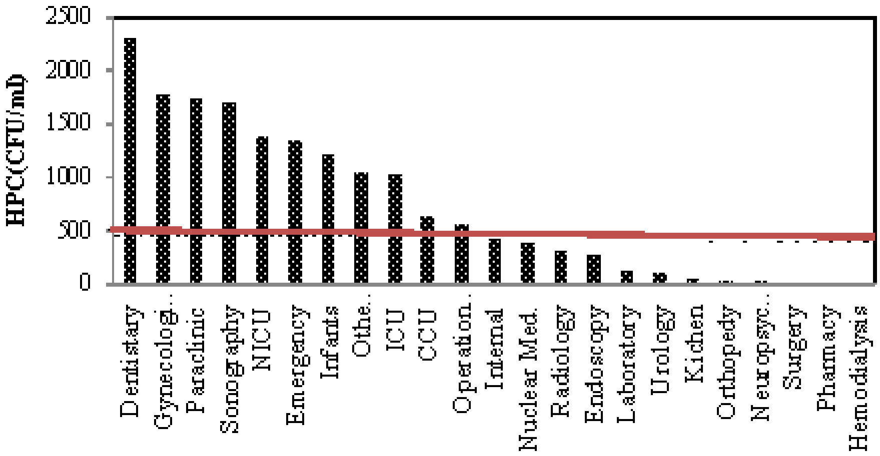

Fig. 3. Warm water HPC density in hospital wards and recommended cut-off point

DISCUSSION

The results of this field research in selected military hospital revealed that 96% of samples were positive for HPC in which 52% of samples had higher than the recommended standards (500 CFU/ml) and HPC density in samples been 947±998 CFU/ml. The mean of HPC density in water inlet to hospital was 850 CFU/ml which higher than the WHO and Iranian recommended guidelines. Previous studies in Iran (Tabriz; North-west area, Ardebil; North-west area, Esfahan; central zone area, and Semnan; central zone area) demonstrate that higher HPC density than the recommended guidelines is one of the water microbial quality problem in Iran(14). Therefore, in this study which done in Tehran (Iran capital) the high density of HPC was detected in water inlet to hospital. This phenomenon may relevant to water distribution system age and residual chlorine concentration. Hence, the result of this study shows that chlorine concentration in some of samples was zero (data not shown). Since HPC represent a wide range of bacteria types (*Acinetobacter, Aeromonas, Flavobacterium, Klebsiella, Legionella, Moraxella, Mycobacterium, Serratia,Pseudomonas and Xanthomonas*) which are now known to pose a public health risk to consumers, higher densities of these organisms in hospital water line, especially in several critical wards including NICU, ICU, CCU, surgery, ect, may possess several health risks including nosocomial infections in immune compromised patients. So, the risks of infection by drinking water HPC estimated at levels as low as 7.3 per billion for low exposures to *Aeromonas*, to 98 per 100 for patients on antibiotic treatment exposed to high levels of *Pseudomonas*[5, 16]. Also risk assessment of HPC shows the importance of the strains acquired resistance against antibiotics and revealed the high load of bottled drinking water heterotrophic bacteria with multiple drug resistance poses crucial health hazards to the consumers, especially to immunocompromised individuals[17]. In addition, previous research reported the significant correlations between the rates of highly credible gastrointestinal illness and HPC which demonstrate the HPC importance in hospital water lines[18]. So, occurrence of the high densities of HPC in hospital drinking water can be used as a predictive tool for hazard prediction and highlighting the effective techniques for the eradication of these opportunistic bacteria from hospital water sources and installations as a potential hazard sources.

The results of this study implied both cold and warm water have high densities of HPC. This phenomenon may revealed to ecology of these organisms and it can be claimed that HPC can have unexpected cold and warm water distribution(9)so, our findings suggest that the cold and warm water supply of healthcare facilities may be heavily contaminated with HPC; therefore, emphasizing the importance of assessing the cold and warm water supply of healthcare facilities for HPC and other opportunistic bacteria monitoring. The results revealed negative and significant effects of the residual chlorine on HPC density in cold water and non-significant effects in warm water. This phenomenon may demonstrate the complicated and effective adaptation systems in these organisms which induce HPC resistance in various statuses.Other findings of this study implied that alkanity has significant influences in both cold and warm water HPC density. Since calcium is the main component of alkalinity and hardness, the effects of this parameter can be attributed to calcium role on bacteria ecology and HPC multiplication and colonization in hospital water[19].

CONCLUSION

Although the selected hospital is taken the treated water in accordance with Iranian regulations for drinking but they have high densities of HPC which were not comply with WHO, CDC and Iranian guidelines. This phenomenon implies that conventional residual chlorine is not effective for HPC control in hospital water line or theseorganisms have complicated resistance systems which led to their resistance, proliferation and multiplication.It can be concluded that prevention of HPC and other relevant opportunistic bacteria proliferation and amplification with individual and effective disinfection procedures is necessary.Since several hospital wards have high densities of HPC, this status may lead to occurrence of other opportunistic bacteria including *legionella spp*. which poses the legitimated public health concerns worldwide. Occurrence of the high densities of HPC in cold and warm water revealed that these organisms have unexpected water distribution. So, policy making from point of water quality assurance and its potential effects in nosocomial infections in hospitals needs to be better understood.

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