SOURCES OF ENVIRONMENTAL NOSOCOMIAL INFECTIONS AND THE BACTERICIDAL EFFICIENCY OF THE DISINFECTANTS USED IN WARDS AT MAHARAT NAKHON RATCHASIMA HOSPITAL

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Abstract

This study investigated sources of environmental nosocomial infections (NI) in wards at Maharat Nakhon Ratchasima Hospital. The 415 samples were collected from various environmental sources within the common wards. It was found that the important microorganisms were *Pseudomonas aeruginosa (Ps. aeruginosa), Stenotrophomonas maltophilia (S. maltophilia), Acinetobacter baumannii (A. baumannii), Klebsiella pneumoniae (K. pneumoniae), Escherichia coli (E. coli)* and *Enterobacter cloacae (E. cloacae).* These microorganisms could be associated with human diseases. They were found in most of the environmental sources within the wards except in plaster on patients' skin, 70% alcohol, and providine® antiseptic solutions. Therefore, a study about the bactericidal efficiency of the disinfectants which were commonly used in the wards (savlon® 1:100, pose-cresol®, and sodium hypochlorite 1:20) were obtained from the hospital's Pharmacy Department and unused disinfectants from the wards' stocks. From tests, Ps. aeruginosa ATCC 15442 was killed by sodium hypochlorite (1:20) but not by savlon® (1:100) and pose-cresol®. Then, repeated tests for the 2 disinfectants with low efficiency were undertaken from stocks freshly prepared by the Pharmacy Department. It was found that savlon® (1:100) could kill Ps. aeruginosa ATCC 15442, but only within 48 hours even though it was stated to expire 7 days after dilution. Pose-cresol® could not kill Ps. aeruginosa ATCC 15442 within 24 hours even though it was stated to expire 30 days after dilution. So, the savlon® (1:100) can be used as a disinfectant solution within 48 hours, but after that it can be used to reduce smell and clean the sexual organs only. At present, pose-cresol® is not used for soaking clinical thermometers anymore because it has no bactericidal activities.

Keywords: Environmental nosocomial sources, *Pseudomonas* spp., bactericidal efficiency, disinfectant, wards, Maharat Nakhon Ratchasima Hospital

Introduction

Nosocomial infections (NI) are major health problems in both developed and developing countries. They are associated with a high mortality rate and the cost of treatment is enormous. NI were significant problems throughout the world and increase every year

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(Alvarado *et al*., 2000).

In Thailand, NI surveillance reported that there was an 11.7% incidence rate and 7% mortality rate or 14000 cases in 1988 which cost about 1000 million baht per year (Danchaivijitr and Choklolkaew, 1989). Urinary tract infections (UTIs) are the most common type of nosocomial infections since they are 40% of all NI in hospitals per year (Burke and Zavasky, 2009). In addition, several studies have reported that about 80% of nosocomial UTIs occur after using instrumentation, especially catheterization (Asher *et al*., 1988). Because nearly 10% of all hospitalized patients are catheterized, preventing UTIs during catheterization was a major factor in decreasing NI. Most nosocomial UTIs were caused by gram-negative coliform bacteria, particularly *Escherichia coli (E. coli)*, *Pseudomonas* spp., and organisms from the Enterobacter group (Haley *et al*., 1985). Within the hospital, *Pseudomonas aeruginosa (Ps. aeruginosa)* was a major cause of UTI and was commonly found in the environment e.g. soil, water, and other moist locations (EHA Consulting Group, 2012). The treatment for *Ps. aeruginosa* infection is difficult, in part because of this organism's antibiotic resistance. Therefore it is common and is becoming more widespread (Wistreich and Lechtman, 1988).

In 2010, Maharat Nakhon Ratchasima Hospital reported that there were 128 cases of NI from 1,441 chart reviews. There were 27 cases of nosocomial UTIs which were the most prevalent of the infections (12 cases were on Foley's catheterization) (Maharat Nakhon Ratchasima Hospital, 2010).

In Thailand, the environmental NI sources in hospitals and the efficiency of disinfectants have not been reported. Therefore, this study investigated the important bacteria from the environments which could be the sources of NI and investigated the bactericidal efficiency of the diluted chemical disinfectants that were commonly used in wards. The results from this study will be information to make medical staff aware of the sources and to develop preventive measures to reduce the incidence of NI in Maharat Nakhon Ratchasima Hospital and other hospitals.

Materials and Methods

The Environmental Nosocomial Infection Sources

Specimens from the environmental sources were taken from 31 wards of 6 departments at Maharat Nakhon Ratchasima Hospital. The specimens were cultured for *Pseudomonas* spp., *Stenotrophomonas maltophilia (S. maltophilia), Acinetobacter baumannii (A. baumannii), Klebsiella pneumoniae (K. pneumonia), E. coli,* and *Enterobacter cloacae (E. cloacae)*. The total number of investigated samples was 415.

The environmental sampling sources were

1. Plaster on patients'skin
2. Hand-wash basins (nursing areas)
   - 2.1 Sinks for hand washing (nursing areas)
   - 2.2 Faucet aerators for hand washing (nursing areas)
   - 2.3 Bar soap for hand washing or faucets for hand washing solutions (nursing areas)
3. Equipment-wash basins
   - 3.1 Sinks for equipment washing
   - 3.2 Faucet aerators for equipment washing
4. Potable water for patients (provided by wards)
5. Antiseptic solutions (during use)
   - 5.1 70% alcohol
   - 5.2 Providine® antiseptic solution
6. 0.9% normal saline solution (during use)
7. Patients' mattresses
8. Patients' coats (before use)
9. Patients' coats (after use)
10. Air conditioners (air grilles)
11. Flush toilets

The specimens from the environments were taken by cotton swabs moistened with sterile 0.9% normal saline solution, placed in a sterilised medium, and transferred to blood agar plates and MacConkey agar plates. All plates were incubated at 35-37°C for 24 h. Suspected colonies were grown in trypticase

soy broth (TSB) at 35-37°C for 5 h. and then identified by biochemical tests (Blazevic and Ederer, 1975).

The Bactericidal Efficiency of the Chemical Disinfectants

The Preparations of the Chemical Disinfectants

Nine samples (3 diluted chemical disinfectants × 3 samples) of unused diluted chemical disinfectants from the wards' stocks which were supplied by the Pharmacy Department at Maharat Nakhon Ratchasima Hospital were tested for the efficiency of the disinfectants against Ps. aeruginosa ATCC 15442. The diluted chemical disinfectants which were commonly used were savlon®, pose-cresol®, and sodium hypochlorite.

Two chemical disinfectants were prepared by the Pharmacy Department as follows:

Savlon® (1.5% chlorhexidine gluconate: 15% cetrimide) was diluted with sterilised water in a ratio 1:100.

5 gm pose-cresol® was diluted with 1000 ml. sterilised water.

Another chemical disinfectant was prepared by the wards by diluting sodium hypochlorite with water from faucet aerators in a ratio 1:20.

TheEfficiency of Disinfectants Tested by the AOAC Use-Dilution Test (Association of Official Analytical Chemists, 1990)

Test of Organisms' Preparation

The bacteria used for testing were of *Ps. aeruginosa* ATCC 15442. They were cultured for 48 h. at 37°C in 10 mlTSB. Bacteria cultures were then collected and put into a beaker size 100 ml. The amount used for testing was about 20 ml.

Carriers' Preparation

Cleaned carriers (10 carriers per 1 bacterium) were put into 1N sodium hydroxide overnight before washing them with distilled water many times. The carriers were dried and then put into 0.1% asparagin solution and sterilized at 121°C for 15 min.

Use-Dilution Method

1. All 10 carriers were transferred into a beaker containing the bacteria culture (from 2.2.1). After 10 min, sterilised forceps were used to remove each carrier and put it onto sterilised filter paper that was in the bottom of a sterilised petri dish in a vertical position with the same distance between each of them; the petri dish was closed and then incubated at 37°C for 60 min.

2. Each sample of the diluted chemical disinfectants was prepared and used for testing. Ten tubes were used per 1 sample and each tube contained 5 ml of sample.

3. The prepared carriers were put into the tube dilution (1 carrier per 1 tube). The timer was started when the first carrier was put into the first tube; the following carriers were put into the following tubes at 1 minute intervals. After 10 minutes, each carrier, which was in a diluted disinfectant tube, was transferred into subculture media by a sterilised technique using holdfast hooked the carrierThis does not make sense. The first carrier was transferred first, followed by the rest at 1 minute intervals until finished.

4. The carriers were cultured in the subculture tubes for 48 h at 37°C and the turbidity in the subculture tubes was observed and reported as apositive result.

5. The efficiency tests were performed by the AOAC use-dilution test from the first day of preparation until expiration at 2-day intervals (1, 3, 5,…).

Evaluation

The evaluations for the efficiency of the disinfectants should reveal no bacterial growth in any of the 10 subculture tubes with the diluted disinfectants. If the growth of Ps. aeruginosa ATCC 15442 was found in even 1 tube, it would indicate that the dilution in the group of samples was not safe enough to be used for killing the bacteria.

The Low Bactericidal Efficiency of the Disinfectants Before the Expired Date

The efficiency test of the disinfectants with low efficiency was repeated at 2-day intervals after preparation to prove whether they were efficient or not before the expiry date. The low efficiency disinfectants were freshly prepared by the Pharmacy Departmentat

at Maharat Nakhon Ratchasima Hospital. The efficiency tests were performed using the AOAC use-dilution test from the first day of preparation until the expiration at 2-day intervals (1, 3, 5,…).

Results

Pseudomonas spp., A. baumannii, K. pneumoniae, E. coli, and *E. cloacae* in the environmental sources of NI in the wards at Maharat Nakhon Ratchasima Hospital were studied. The results were shown in Table 1. Bacteria were found in most of the environmental sampling sources within the wards except in the 70% alcohol and providine® antiseptic solutions. *Burkholderia cepacia, Burkholderia mallei,* and *Burkholderia pseudomallei* were not found in most of the environmental sampling sources. The microorganisms found in the environments were *A. baumannii* which was found in 13.01% (54 specimens); *Ps. aeruginosa* which was found in 7.22% (30 specimens); and *K. pneumoniae* which was found in 4.57% (19 specimens). The environmental sampling sources which were found to contain the highest number of species of screened microorganisms were the sinks for equipment washing (28 specimens), the sinks for hand washing (nursing areas) (26 specimens), and the flush toilets (20 specimens).

Table 2 showed that sodium hypochlorite (1:20) was the most effective disinfectant against *Ps. aeruginosa* ATCC 15442 since no bacterial growth occurred in any of the 10 subculture tubes with the diluted disinfectants. Savlon® (1:100) and pose-cresol® did not pass the criteria because *Ps. aeruginosa* ATCC 15442 was found in 3-8 tubes within 48 hours, indicating that both of the diluted chemical disinfectants could not kill the *Ps. aeruginosa* ATCC 15442 even before the expiry date. To confirm the results, the chemical disinfectants freshly prepared by the Pharmacy Department were used to repeat the checks for the real efficiency from the first day of preparation until the expiry date at 2-day intervals (1, 3, 5,…). The results were shown in Table 3. The efficiency tests showed that savlon® (1:100) could kill *Ps. aeruginosa* ATCC 15442 within 2 days only, but pose-cresol® could not possibly kill *Ps. aeruginosa* ATCC 15442 even within the first day of preparation. This suggested that both savlon® (1:100) and pose-cresol® had very poor efficiency and did not work as efficiently as stated in the manufacturers' details.

Discussion

The important microorganisms were found in most of the samples collected from environmental sources, except in plaster on patients' skin, 70% alcohol, and providine® antiseptic solutions in common wards at Maharat Nakhon Ratchasima Hospital. The species of Pseudomonas found in the environmental sources were *Ps. aeruginosa* and *S. maltophilia*. These 2 Pseudomonas spp. cause human diseases.

In general, the medical equipment such as the central lines of the ventilators and catheters which are made from plastics are sterilised by gas sterilisation; other equipment such as scalpels and speculums are sterilised with autoclaves. But there is some medical equipment, such as thermometers, that cannot be sterilised with autoclaves or hot air ovens, thus pose-cresol® solution is used for soaking to reduce the pathogens. Normally, antiseptic solutions (70% alcohol, providine®) and isotonic solution (0.9% normal saline) are used for dressing wounds on the skin. For the urogenital tract, which has sensitive soft tissue, 70% alcohol or providine as antiseptic solutions cannot be used because they will irritate the soft tissue. In the past, for an external genital wash before per vaginal examination or for washing when the genital organ had an infection, every hospital used savlon® (1:100), but later it was found that savlon was not up to standard and used savlon® or not were not differentThis does not make sense. After that, some hospitals changed to the use of 0.9% normal saline solution to clean the urogenital tract,and many hospitals changed the solutions used for cleaning the organisms or wounds of patients. The 0.9%

Table 1. Type of the microorganisms which were isolated from the environmental sources in wards at Maharat Nakhon Ratchasima Hospital (415 samples)

Micoorganisms	*Ps. aeruginosa*	*S. maltophilia*	*A. baumannii*	*K. pneumoniae*	*E.coli*	*E. cloacae*	Total + ve
The environmental sources							
1. Sinks for hand washing (nursing areas)	9	3	8	2	2	2	26
2. Faucet aerators for hand washing (nursing areas)	3	3	10	1	1	0	18
3. Bar soap for hand washing or faucets for hand washing solution (nursing areas)	3	0	1	2	0	3	9
4. Sinks for equipment washing	4	1	12	8	2	1	28
5. Faucet aerators for equipment	4	1	9	2	0	0	16
6. Potable water for patients (provide by wards)	0	0	2	0	0	0	2
7. 0.9% normal saline solution (during use)	1	0	1	0	0	0	2
8. Patients' mattresses	0	0	3	1	0	0	4
9. Patients' coats (before use)	0	0	1	0	0	0	1
10. Patients' coats (after use)	1	0	0	0	2	0	3
11. Air conditioners (air grilles)	1	0	0	0	0	0	1
12. Flush toilets	4	0	7	3	5	1	20
Total	30	8	54	19	12	7	130
Percentage from 415 samples	7.23	1.93	13.01	4.58	2.90	1.69	31.34

Notes Ps. aeruginosa = Pseudomonas aeruginosa, S. maltophilia = Stenotrophomonas maltophilia, A. aumannii = Acinetobacter, baumannii, K. pneumoniae = Klebsiella pneumonia, E. coli = Escherichia coli, E. cloacae = Enterobacter cloacae

normal saline solution or savlon® 1:100 are used to flush the vulva before inserting urinary catheters, during catheterizations, and before cervical examinations. For patients with cervical cancer, the savlon® 1:100 was used only to reduce smell.

Some wards at Maharat Nakhon Ratchasima Hospital regularly used savlon®

Table 2. The bactericidal efficiency of diluted chemical disinfectants used in wards at Maharat Nakhon Ratchasima Hospital

Diluted chemical disinfectants	Lab results			Date	
	Positive samples / Total samples			Diluted	Expiry
	day1*	day 3*	day 5*	date	date**
Savlon® 1:100					
Sample 1	3/10	4/10	10/10	25/9/2008	2/10/2008
Sample 2	6/10	6/10	10/10	28/9/2008	5/10/2008
Sample 3	8/10	8/10	10/10	25/9/2008	2/10/2008
Pose-cresol®					
Sample 1	8/10	8/10	10/10	18/9/2008	18/10/2008
Sample 2	0/10	6/10	10/10	22/9/2008	22/10/2008
Sample 3	0/10	6/10	10/10	22/9/2008	22/10/2008
Sodium hypochlorite 1:20					
Sample 1	0/10	0/10	0/10	1/10/2008	29/10/2008
Sample 2	0/10	0/10	0/10	1/10/2008	29/10/2008
Sample 3	0/10	0/10	0/10	1/10/2008	29/10/2008

Notes * *samples collected on 1/ 10/ 2008 and at 2-day intervals*
** *Expiry date that was stated in the manufacturer's details*

Table 3. The repeated bactericidal efficiency of diluted chemical disinfectants with low efficiency from freshly prepared by Pharmacy Department at Maharat Nakhon Ratchasima Hospital

Diluted chemical disinfectants	Lab results		
	positive samples/ total samples		
	day1*	day 3*	day 5*
Savlon® 1:100			
Sample 1	0/10	7/10	10/10
Sample 2	0/10	6/10	10/10
Sample 3	0/10	6/10	10/ 10
pose-creso®l			
Sample 1	10/10	-	-
Sample 2	10/10	-	-
Sample 3	10/10	-	-

Notes **samples collected on 30/10/2008 and at 2-day intervals*

1:100 to clean the sexual organ (flushing the vulva before inserting urinary catheters, during catheterizations, before cervical examinations, and to reduce smell for the patients with cervical cancer) which was different from Siriraj Hospital and Songklanagarind Hospital that used 0.9% normal saline solution to clean the sexual organ, while using savlon® 1:100 only to reduce the smell from cervical cancer patients.

Normally, the disinfectant solutions used in hospitals must not contain bacteria. Surprisingly, in this study, bacteria (*Ps. aeruginosa* and *A. baumannii*) were found in 0.9% normal saline solution (during use). This solution is usually used for wound dressing and blocking blood vessels to get medicine through the vessels. By observing and interviewing the nurses, it was found that sometimes they used 0.9% normal saline solution more than 24 h after opening a bottle without specifying the time the bottle was opened. Moreover, the researchers discovered that the 0.9% normal saline solution bottles were punctured with syringes and there was solution still remaining on the rubber cork which might have been contaminated by the bacteria that passed into the bottles. When the contaminated solution was used on patients, it would cause the wounds and the blood vessels to be infected in another way (Pattarachai, 2006).

From the bactericidal efficiency test of disinfectants used in wards at Maharat Nakhon Ratchasima Hospital, the only chemical disinfectant that passed the criterion and was able to kill the *Ps. aeruginosa* ATCC 15442 was sodium hypochlorite (1:20) while savlon® (1:100) and pose-cresol® did not pass the criteria. Therefore, the researchers were interested in savlon® (1:100) and pose-cresol® because they were ineffective before the expiry date. The repeated tests for these 2 disinfectants with low efficiency were done with freshly diluted solutions from the Pharmacy Department at Maharat Nakhon Ratchasima Hospital; they were tested for bactericidal efficiency from the first day when they were diluted until the expiry date (they were tested every second day). The efficiency test results showed that savlon® (1:100) was able to pass the criteria (no bacterial growth in any of the 10 subculture tubes) for upto 48 h after dilution. However, its bactericidal effect on *Ps. aeruginosa* ATCC 15442 did not pass the criteria after 48 h, indicating that this diluted disinfectant did not work as efficiently as stated in the manufacturer's claim that it was effective for up to 7 days. Furthermore, Maharat Nakhon Ratchasima Hospital had used pose-cresol® for soaking clinical thermometers, but the bactericidal efficiency test for pose-cresol® produced a very poor result indeed. It was not able to kill Ps. aeruginosa ATCC 15442 within 24 h even though it is claimed to be effective for 30 days. Now pose-cresol® is not used for soaking the clinical thermometers anymore because it has no bactericidal activity.

The conclusion from the results of these tests is that both savlon® (1:100) and pose-cresol® had very poor efficiency and they did not work as efficiently as stated in the manufacturers' claims. If they were continued to be used at the same concentrations in the hospital, there would be the risk of spreading the bacteria from one patient to another.

The causes of the failure in such standard dilutions of the chemical disinfectants that were used in the hospital could be possibly due to their low quality, or low constancy or efficiency in their dilution to save costs. From the standard disinfectant solutions which were produced by the companies, an important part in the quality of the chemical disinfectants may be how they were diluted and used in the hospitals. Other factors such as the mixing containers and the environments around the mixing areas were also important. By using low quality chemical disinfectants, there was a high risk rate because it was not only harmful to the patients directly but it also caused nosocomial infections that could spread outside the hospital later on. The effectiveness of the chemical disinfectants used in the hospital has been a concern for many years. One hundred and two samples of the chemical disinfectants from 32 hospitals throughout the country were tested for their disinfectants

qualities. The results showed that 43.1% samples were not up to standard (Department of Medical Sciences Center of Thailand, 1993 Not mentioned in References). Thus, chemical disinfectants which were not up to standard were one of the reasons for nosocomial infections. Therefore, it is important to determinate the expiry date of savlon® which is diluted with sterilised water in a ratio 1:100 based on the stability factors (physical stability and chemical stability) of the production in each hospital. Moreover it depends on the preparation of the sterilised water for dilution, the packaging size, and the method for cleaning the package before pouring into the chemical disinfectants (Theanthong, 2010).

The information about the sources of the microorganisms found in the wards was reported to the head of Infection Control at Maharat Nakhon Ratchasima Hospital for control and prevention management, and also reported to the head of the Pharmacy Department at Maharat Nakhon Ratchasima Hospital regarding the efficiency of the diluted chemical disinfectants which were not up to standard. It is very important to control the quality of the chemical disinfectants which are diluted and used in the hospital.

Today, Maharat Nakhon Ratchasima Hospital and many hospitals do not use pose-cresol® anymore because there is no difference between using it or not as was confirmed by the very poor bactericidal efficiency results.

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