Key concept : Hospital infection control, Contamination of saline used in surgical procedures

The Contamination Levels and Exchange of Saline Used in Surgical Procedures*

Hae S. Yoon** · Hae H. Song***

INTRODUCTION

The number of hospitalized patients has increased 10-20% each year in Korea since 1989, and 46%-69% of all hospitalized patients have inpatient surgery (Annual Report of Catholic Medical Center, 1993). Patients undergoing surgery are exposed to the risk of nosocomial infections, especially surgical site infections. Surgical site infection rates were reported to be 3.1% to 11.6% (Choi et al., 1998; Horan et al., 1993; Jeong et al., 1996; Park & Kim, 1995; Rostein et al., 1992). These infections result in significant patient morbidity and prolonged hospitalization with concomitant additional costs and psychological trauma (Shulkin et al., 1993). Most postoperative wound infections occur as a result of contamination of the surgical wound, which originates from the bacteria that enter into the operative tissue during surgery. The causative pathogens are derived of microorganisms shed by patients, the operating room environments and operating teams (Eickhoff, 1994; Pittet & Ducel, 1994; Wiley & Ha'Eri, 1979). The postoperative wound infections can also break out due to inadequate aseptic surgical techniques and procedures involving the surgical instruments and materials, patients, operating room personnel and possibly the air in the operating room (Schwan, Bengtsson, Hambraeus & Raurell, 1977; Shaw & Douglas, 1973).

Whether airborne microbes are a potential source of operative wound infection has been the subject of debate since 1950. Some studies have suggested that airborne bacteria have played an important role in surgical site infections (Lidwell, 1981; Lidwell & Phil, 1986; Lidwell et al., 1987), while other studies have suggested little effect (Ayliffe, 1991; Whyte, Hambraeus, Laurell, & Hoborn, 1991). Several studies concluded that the contamination of

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** Department of Nursing, College of Gachon Gil

*** Department of Biostatistics, College of Medicine, The Catholic University of Korea

saline used in operation was the very cause of postoperative wound infection; postoperative eye infection occurred when unsterilized saline was used in eye operation (Ayliffe et al., 1965; Ayliffe et al., 1966), and wound infection following breast plastic surgery was associated with the use of saline contaminated by Serratia marcescens surgical wound infection (Pegues et al., 1991).

In Korea saline is usually prepared in a tray of 500-1000 mL, with other surgical materials and instruments at the start of a surgery and is used to the end without being exchanged. Saline put in a bowl or a tray can be used in many ways, such as to soften the surgical suture, to irrigate an antiseptic solution of suture materials, to prevent suture materials and gauze from drying, and to clean the blood and tissue fluid from surgical instruments. Thus, saline is contaminated as the surgery proceeds and the use of contaminated saline in an operation will inevitably contaminate an operative site. Possible sources of contamination of saline are operative tissue and organs, surgical instruments and materials, operating room air, and the hands of the operating room personnel. Authors consider that one critical factor related to surgical wound infection is the possible use of contaminated saline in surgical procedures. There are many studies on air contamination in operating rooms, but there are few studies on saline which is easily contaminated during operation.

This study was undertaken to estimate the contamination levels of saline used in surgical procedures, to identify a risk factor for contamination of saline used to operation and to examine the contamination levels at different operative stages so that an appropriate timing and method of saline exchange could be suggested. The specific objective of this study is to compare the contamination levels in saline exposed to the air and saline used in operation, to compare the contamination levels at different operative stages, to compare the contamination levels in saline samples from different types of operations and to examine contamination levels in saline changed right after excision of the organ.

LITERATURE REVIEW

There has been many debates since 1950 whether an airborne microbe is an important route of transmission that contaminates the operative wound. Some studies suggested that airborne bacteria had an important role to surgical site infections (Lidwell, 1981; Lidwell & Phil, 1986; Lidwell et al., 1987), but other studies suggested little effect (Ayliffe, 1991; Whyte, Hambraeus, Laurell, & Hoborn, 1991). Most postoperative wound infections occur as a result of contamination of the surgical wound, which originates from the bacteria that enter into the operative tissue during surgery. The causative pathogens are derived of microorganisms shed by patients, the operating room environments and operating teams (Eickhoff, 1994; Pittet & Ducel, 1994; Wiley & Ha'Eri, 1979). The postoperative wound infections can also break out due to inadequate aseptic surgical techniques and procedures involving the surgical instruments and materials used, patients, operating room personnel and possibly the air in the operating room (Schwan, Bengtsson, Hambraeus & Raurell, 1977; Shaw & Douglas, 1973).

A significant effect of airborne contamination on surgical wound infection has been mentioned in a substantial number of studies (Burke, 1963; Lidwell, 1981; Lidwell & Phil, 1986; Lidwell et al., 1987), while many other researchers have expressed skepticism about such a source of surgical wound infection in the

operating room(Ayliffe, 1991; Eftekhar, 1973). Especially Burke(1963) identified the same Staphylococci from operating room air and operative tissue among 68% of surgical patients. Kundsin quoted by Eickoff(1994) concluded that airborne spread in the operating room accounted for 20% to 24% of all postoperative wound infections.

On the other, there have been a few reports on the differential role of airborne contamination according to the type of surgical procedures. Schwan and colleagues (1977) and McQuarrie and coworkers(1990) found that airborne bacteria had little effect on clean contaminated or dirty operations. On the other hand, many researchers have expressed a skeptical view on airborne contamination causing surgical wound infection. Eftekhar(1973) quoted Charnley's comments that despite of air cleanliness being achieved 25 times better by an unconventional isolation type of enclosure the infection rate was reduced only by 50%. Ayliffe(1991) found that although the number of airborne bacteria decreased 50% after installation of ventilation system, postoperative wound infection rate increased from 8.8% to 12.6%. Though airborne bacteria in the operating room decreased by 13 times, wound contamination was reduced only by 50%(Whyte et al., 1992). After an extensive review, Eickhoff(1994) concluded that airborne transmission accounted for only 10% of all nosocomial infection.

There are studies that differentiate the role of airborne contamination in the development of surgical wound infection depending on the types of operations. Airborne bacteria had little effect on clean contaminated or dirty operation (Schwan et al., 1977; McQuarrie et al., 1990). If there was no infection due to bile or skin pathogens, airborne contamination would be important in bringing about surgical wound infection(Whyte et al., 1992).

Several studies concluded that the contamination of saline used in operation was the very cause of postoperative wound infection; postoperative eye infection occurred when unsterilized saline was used in eye operation (Ayliffe et al., 1965; Ayliffe et al., 1966), and wound infection following breast plastic surgery was associated with the use of saline contaminated by Serratia marcescens(Pegues et al., 1991). Richter, Lang, Zur & Nissenkorn (1991) reported that wound infection was found in 19 (23.5%) out of 81 patients when the patients urine was infected, but only in 6 (8.7%) out of 69patients when the patients urine was sterile. It was also reported that Enterobacter, Klebsiella and Pseudomonas species isolated from saline used in operation were the pathogens in surgical wound infections (Raymond & Aujard, 2000). These results suggest that contamination of saline used in operation could lead to surgical wound infection.

MATERIALS AND METHODS

Design

The contamination level of saline was defined as the number of bacterial colonies isolated from a saline sample of 50 ml. A saline sample of 50 ml was passed through a membrane filter, which was then put on a rabbit's blood agar of 15 ml in a petri dish and cultured at a 37 ℃ in an incubator for 24-48 hours. The diameter of cocci necessary to cause hospital infection is 0.2(m with a major and minor diameter of 0.5 (m and 0.5-1(m respectively. So a membrane filter with a diameter 47 mm and a pore size of 0.2(m was used to filter microorganisms. Viable micro-organisms retained on the filter membrane were cultured and identified with gram cocci identification card and Vitek system.

Sampling of saline at three different operative

stages was undertaken. The first sampling was taken just before the incision of the skin and the second sampling was undertaken when the specimen was completely excised. The third sampling occurred when the skin was sutured. In case of culture failure, two samples of 50mL were collected at each stage.

After the packages of operative drapes and instruments were placed on an instrument table, two trays(width, 25cm; depth, 30cm; height, 5cm) were placed on an instrument table, and each tray was filled with saline of 1000 mL. One tray of saline was used in the operation and the other was exposed to air alone. Saline samples from the first tray were called saline used in operation and those from the second tray were called saline exposed to air

For each operation one of two procedures was randomly chosen, either saline was used in the operation without being exchanged throughout the operation or saline was exchanged when the specimen was completely excised. For saline exchanged, the tray was emptied and filled with fresh saline of 1000 mL, but the tray itself was not exchanged. Thus, the contamination levels of saline at the third operative stage were possibly affected under the saline exchange condition by contamination of the tray.

Subjects

This study included 37 surgical procedures performed by a single surgeon at a 1500-bed hospital between October, 1997 and April, 1998. 22 cases of cholecystectomy, 10 cases of mastectomy and 5 cases gastrectomy were observed to determine and minimize any variation in operative techniques and asepsis procedures that might affect contamination levels of saline. An operative theatre in which saline samples were collected had the air shower system at the entrance, but no laminar air flow system in the operating room.

Statistical methods

The contamination levels of saline exposed in the air, saline used in operations and exchanged saline were analyzed using a nonparametric Wilcoxon rank sum test and the contamination levels from different types of surgical procedures were compared using a nonparametric Kruskal-Wallis test and t-test. The contamination levels at the three operative stages were compared with a repeated-measures analysis of variance (ANOVA). Mean values were mean colony counts per saline sample of 50 mL. All p values were two-sided.

RESULTS

1. The contamination levels of saline exposed to the air and saline used in surgery

   The contamination levels of saline exposed to the air and saline used in surgery are shown in Table 1. The mean colony counts per saline sample of 50 mL was 4.7 for saline exposed to air, but the mean colony counts of saline used in surgery, 21.2, was significantly larger($p = 0.005$).

2. The contamination levels of saline sampled at different stages of operation

   The contamination levels of saline sampled at different stages of operation are shown in Table 2. The contamination levels of the saline exposed to the air increased significantly as the surgery proceeded($p=0.001$), but did not reach a level considered to be clinically important and the statistical significance is due to a small sampling variation in the colony counts data. For the saline used in surgery, however, the mean contamination levels of the sample rose dramatically across three stages($p=0.041$). At the last operative stage, skin suture, the

<Table 1> Comparison of the contamination levels in saline exposed to air and saline used in operation

Saline	No. of samples	Colonies / 50 ml			p*
		Mean	Median	SD	
Exposed to Air	96	4.7	3.5	5.3	10.005
Used in Operation	57	21.2	7.0	53.3	

* Wilcoxon rank sum test
SD is standard deviation.

<Table 2> Comparison of the contamination levels in saline sampled at different stages of operation

Saline	Stages of operation	No. of samples	Colonies / 50 ml			p*
			Mean	Median	SD	
Exposed to Air	Pre-skin Incision	32	2.0	1.5	1.9	
	Post-Specimen Excision	32	4.9	4.0	4.1	0.001
	Skin Suture	32	7.3	5.0	7.1	
Used in Operation	Pre-skin Incision	19	1.6	1.0	1.5	
	Post-Specimen Excision	19	15.9	8.0	25.9	0.041
	Skin Suture	19	45.9	18.0	84.1	

* Repeated-measures ANOVA
SD is standard deviation.

<Table 3> Comparison of the contamination levels in saline sampled from different types of operations

Saline	Types of operation	No. of samples	Colonies / 50 ml			p*
			Mean	Median	SD	
Exposed to Air	Cholecystectomy	54	4.0	3.5	4.8	
	Mastectomy	30	4.8	4.0	3.6	
	Gastrectomy	12	7.6	3.0	9.2	0.212
Used in Operation	Cholecystectomy	66	17.2	3.5	47.4	
	Mastectomy	27	9.2	8.5	8.6	
	Gastrectomy	15	40.5	6.5	70.6	0.409

* Kruskal-Wallis test
SD is standard deviation.

<Table 4> Comparison of the contamination levels in saline sampled from different types of operations

Types of operation	Saline	No. of samples	Colonies / 50 ml			p*
			Mean	SD	t	
Cholecystectomy	Exposed to Air	54	4.0	3.5	2.25	0.028
	Used in Operation	66	17.2	47.4		
Mastectomy	Exposed to Air	27	4.8	4.0	2.56	0.014
	Used in Operation	30	9.2	8.5		
Gastrectomy	Exposed to Air	12	7.6	9.2	1.61	0.135
	Used in Operation	15	40.5	70.3		

* t-test
SD is standard deviation..

<Table 5> Summary of the contamination levels when saline was changed right after the excision of the organ

Stages of Operation	No. of samples	Colonies / 50 ml			p*
		Mean	Median	SD	
Pre-skin Incision	17	1.7	1.0	2.4	
Post-Specimen Excision	17	22.6	6.5	40.2	
Skin Suture	17	22.0	4.0	48.2	0.074

* Repeated-measures ANOVA
SD is standard deviation.

large variation of the colony counts are particularly striking for the samples of saline used in surgery, which implies that the contamination levels varied widely. The large sampling variation thus resulted in a statistical non-significance as shown in Table 3 and 5, even though the contamination levels of the saline used in surgery were observed to rise greatly at the later stage.

3. The contamination levels of saline samples from different types of surgery

   The contamination levels of saline samples collected from different types of surgery are shown in Table 3 and Table 4. The contamination levels of saline samples exposed to air demonstrated no gross indication of differences under different types of surgery(p=0.212), but there was a clear difference for those of saline used in surgery but did not reach statistical significance due to a large sampling variation(p=0.409). Mastectomy had the lowest mean colony counts, cholecystectomy had the second highest and gastrectomy had the highest mean colony counts.

   The mean colony counts of saline used in cholecystectomy(p=0.028)and mastectomy(p=0.014) were significantly larger than the mean colony counts of saline exposed to the air(Table 4). But there was no difference for those of saline used in gastrectomy(p=0.135).

4. The effect of exchanged saline on contamination levels

   The mean colony counts of saline used in the operations without exchange at skin suture was 45.9 and at post - specimen excision was 15.9(Table 2). In Table 5 the effect of exchanged saline on contamination levels are shown. Interestingly the mean contamination level at skin suture stage did not drop to the lowest level of pre-skin incision stage and the contamination levels at three different stages of the operations showed a marginal significant difference(p=0.074). The most frequently isolated microorganisms from saline exposed to air were coagulase negative Staphylococcus(74.5%) and Micrococcus(13.6%), while the most frequently isolated micro-organisms from saline used in operation were coagulase negative Staphylococcus (72.5%), Enterococcus(9.5%) and Enterobacter species (4.6%). Sampling demonstrated that saline used in operation contained Enterococcus (9.5%), Enterobacter species(4.6%), E. col I (2.8%), Alcaligenes species(1.2%), Klebsiella species(0.9%) and Pasteurella multocida (0.8%), but the saline exposed to air did not contain them.

DISCUSSION

The relative contribution of airborne bacteria in operating rooms among many other infection sources to postoperative wound infection remains to be controversial. A significant effect of airborne contamination on surgical wound infection was mentioned in a substantial number

of studies. Environmental contamination of surgical equipment through contaminated saline has been reported as the major source of operative wound infection. Our findings revealed that the contamination levels of the saline used in surgery differed depending on the types of surgery, but contamination levels of the saline exposed to the air didn't differ depending on the types of surgery But the contamination levels of saline used in surgery were significantly higher than those of saline only exposed to air, and that the colony counts of saline used in surgeries increased abruptly across the three stages of sampling, whereas those of saline exposed to air gradually increased across stages of surgery. One explanation of these findings is that there are sources of saline contamination other than airborne contaminants, notably tissues and organs, from the patient's skin and the hands of the surgical team.

Furthermore, the contamination levels of saline used in surgeries were accelerated as the operation was prolonged, but it was not likewise for those of saline only exposed to the air. These results of saline only exposed to the air are in accordance with those of Ferraz et als (1992); they demonstrated that the duration of surgery was not significant in clean operations during which surgical wounds had few chances to be contaminated by any other sources except the operating room air. From our findings we may conclude that the airborne bacteria have relatively insignificant effect on the contamination of saline, yet the mere demonstration of the contamination level of saline only exposed to the air does not fully establish insignificant airborne transmission to surgical infection. The contamination level of saline used in surgeries was higher for gastrectomy, the contaminated operation, than for mastectomy, the clean operation in our study, which indicates that not only the operating room air but also the infected organs contaminated saline used in surgeries.

Our results in regard to saline contamination levels associated with mastectomy and gastrectomy accord with those of Horan et al(1993) and Garcia et al(1997) who reported that the postoperative infection rate of gastrectomy and cholecystectomy were higher than herniorrhaphy and mastectomy respectively (Beck-Sague, Chong, Roy, Anderson & Jarvis, 1992). On the other hand, for a clean operation of mastectomy the colony counts of the saline used in surgery were 9.2 colonies, but those of the saline exposed to the air were 4.8 colonies in our study. This could explain that there are other sources than the operating room air which contaminate the saline used in surgery, and notably patient's skin and surgical team's hands are possible sources of contamination. The colony counts of saline used in surgery had much larger variations than that of saline exposed to the air in our study and this is in accordance with the findings that bacterial counts ranged from 0 to 2,000 in saline used for rinsing surgical instruments(Wise, Sweenly, Haupt & Waddell, 1959).

Our findings revealed that the P. multocida (0.8%) were isolated microorganisms from saline exposed to air whereas saline exposed to air did not contain them. P. multocida is part of the normal flora of many animals, but P. multocida is found in human patients with underlying pulmonary disease and acute epiglottitis (Weber, Wolfson, Swartz & Hopper, 1984; MayoSmith, Hirsch, Wodzinski & Schffman, 1986). The study by Weber et al(1984) and MayoSmith et al(1986) thus led us to the following interpretation that P. multocida found in our saline sample used in the surgical procedures was transmitted by air. Lee et al(1994) reported earlier that the most common microorganisms in the bile and in the blood of patients with biliary tract infection were E. coli,

Klebsiella species, Enterobacter species and enterococcus, which authors isolated from saline used in operation. All things considered, we conclude that the contaminants of saline used in surgical procedures were the operative tissue rather than the air in operating room.

Among the many necessary procedures for surgery is the necessity of ensuring the adequate quality of the air in the operating room and the quality of saline used in surgery. Saline used in surgery can be contaminated because of poor aseptic techniques and through a failure to exchange saline with fresh units at appropriate phases of surgery. It is very difficult to block the transmission route of airborne contamination, but it is relatively easy to block the transmission through saline contamination. In our study saline used in surgery was discarded after excision of specimen and a 1000 ml of clean saline was poured into the tray that was in place at the start of the operation. Even with this method of saline exchange, however, the colony counts at the post-specimen excision stage were not as low as to the level at pre-skin incision stage. This implies that the time and/or manner of exchange might have been ineffective for reducing the contamination level. We suggest as for a better exchange plan that saline and its tray are altogether exchanged after anastomosis of contaminated specimen.

At the time of preparing saline at Pre-Skin Incision stage, the colony counts of saline were 1.6 colonies. But at the time of Post Specimen Excision and Skin Suture, the colony counts of saline were 15.9 and 45.9 colonies, respectively. This indicates that saline begins to be contaminated after a bottle of sterile saline is poured into a sterile tray. The contamination level of saline used in operation becomes higher as the operation proceeds, and the speed of the contamination level is much accelerated for the prolonged operation.

Possible contaminant sources of saline used in operation could be operating room air, operative tissue of patient, surgical instruments and materials that are easily contaminated by the operative procedures, and the hands of a surgical team. In conclusion, a relatively important risk factor for contamination of saline used to operation is not the airborne bacteria of operating room but the operative tissues and the contamination level of saline used in operation becomes higher as the operation proceeds. Therefore, we recommend exchange of saline and its container and also exchange of gloves before the specimen is excised for a dirty or prolonged operation.

Accordingly, we suggest that saline and its tray be replaced after the anastomosis of contaminated specimen and as often as possible thereafter because the rate of the saline contamination accelerates for operations extending over a prolonged period. Yet, a mere demonstration of contamination levels of saline in our study does not fully establish significant transmission of saline contamination to surgical wound infection, and further studies into this possible means of transmission are recommended.

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- 국문 초록 -

주요개념 : 병원감염관리, 수술에 사용하는 생리식염수의 오염

수술에 사용하는 생리식염수의 오염수준 및 교환방법*

윤 혜 상**・송 혜 향***

본 연구는 수술에 사용하는 생리식염수가 수술의 종류와 수술진행단계에 따른 오염수준을 파악하여 생리식염수의 적절한 교환시점과 교환방법을 제시하기 위하여 시도되었다. 1500 병상 규모의 대학병원에서 1명의 일반외과 의사가 집도한 37건의 수술을 대상으로 하였다.

37개의 수술 각각에서 피부 절개전, 장기절제 후, 그리고 피부 봉합시의 3 시점에서 수술에 사용된 생리식염수와 공기에 노출시킨 생리식염수에서 각각 50 mL의 생리식염수를 채취하여 얻은 균주의 수를 비교하였다.

* 1997년 학술진흥재단 연구조성비 지원에 의해 이루어진 연구임
** 가천길대학 간호과
*** 가톨릭대학교 의과대학 통계학교실

공기에 노출시킨 생리식염수에 비해 수술에 사용된 생리식염수에서 균주가 보다 많이 검출된 것으로 나타났다. 특히 수술의 종류에 관계없이 수술 마지막 단계 즉 피부 봉합 단계에서 수술에 사용된 생리식염수의 오염수준이 급격히 증가한 반면 공기에 노출시킨 생리식염수의 오염수준 변화는 미미했다. 수술에 사용한 생리식염수에서는 Enterococcus(9.5%), Enterobacter species(4.6%), E. col i(2.8%), Alcaligenes species(1.2%), Klebsiella species(0.9%) and Pasteurella multocida(0.8%) 등의 균주가 검출되었으나 공기에 노출시킨 생리식염수에서는 이러한 균종이 검출되지 않았다.

수술실의 공기가 수술에 사용하는 생리식염수의 오염요인으로 작용하기보다는 수술조직이 생리식염수의 오염요인으로 작용하는 것으로 사료된다. 특히 수술에 사용하는 생리식염수의 오염가능성을 최소화시키기 위해 수술소요 시간이 길어지거나 또는 오염 수술의 경우 절제부위가 봉합된 후에 수술에 사용하는 생리식염수, 생리식염수를 담는 용기 및 봉합에 이용되는 봉합감자 등을 새로이 준비하여 피부 봉합에 이용해야 할 것으로 사료된다.