Comparative Identification of Bacterial Quality in liquid Soap between Nasser and European Gaza Hospitals, Khanyounis Governorate

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ABSTRACT— Background: Liquid soap used in hospitals dispensers can become contaminated bacteria. Many factors have an effect on the contamination of these dispensers. This study aimed to investigate the bacterial contamination in liquid soaps at hospital environment in Khanyounis Governorate. Methods: A total of 82 liquid soap samples (50.0% them from Nasser hospital and 50.0% from European Gaza Hospital) were collected in sterile cups from the same departments of both hospitals. After that, the cups were transported within one hour of collection to the microbiology laboratory. Bacteriological identification was performed. Collected data, and bacteriological identification results were analyzed by SPSS version 18. Results: In Nasser hospital, only one sample were found colonized by staph aureus, and two samples were found colonized by other staph, While, In European Gaza Hospital, 12 samples were colonized by staph aureus, and 13 samples were colonized by other staph. Open dispenser systems and washing of dispensers with water were directly proportional with bacterial growth in liquid soap among study hospitals environments (p-value = 0.033 & 0.000 respectively). In European Gaza Hospital, the departments with the largest number of contaminated soap samples were the pediatric department (n=6), surgery men department (n=4), surgery women department (n=4), Intimal women department (n=4). Conclusions: The contamination of liquid soap in European Gaza Hospital is attributed to high number of open dispenser systems (27 dispensers) that was observed in its departments, in addition to using of water instead of chlorine bleach for washing the dispensers before putting the new liquid soap.

Keywords--- Hand washing, Soap, Contamination, Gram positive bacteria, Gaza Strip.

1. INTRODUCTION

Bacteria are widely distributed, line the skin, mucous tissue and cover the intestinal tract of men and animals [1]. Many bacteria are harmless and some are beneficial to their host and provide nutrients or protection against pathogens and diseases, limiting the colonization abilities of harmful bacteria. In the hospital environment, however, bacteria have a different profile, mainly due to the indiscriminate use of antimicrobial agents, as these increase the selective pressure and also the opportunity to acquiring resistance mechanisms, causing the dissemination of antibiotics-resistant bacteria, entailing severe risks for health [1].

The responsibility for infection prevention and control means strict attention to the several aspects associated with biological risks and unhealthy environmental conditions, which need to be targeted and controlled. These include lack of cleaning material, inappropriate use of individual protection equipment, inadequate cleaning and disinfection routine of the units and collective-use equipment, including soap dispensers and flasks, improper destination of contaminated clothes and materials, among others. Hence, hospitals is an infection source for antibiotics-resistant bacteria.

Hand washing by health professional before having contact with patients is considered a fundamental hospital infection control measures [2]. Hand carriage of bacteria is an important route of transmission of infection between patients and health care workers. Hand hygiene has been considered to be the most important tool in nosocomial infection (NI) control [3]. Opened soap dispensers are exposed to infection from microbial skin flora and other bacteria that present in hospital environment. Infection of soaps lead to failure to perform appropriate hand hygiene among health care stuff which is supposed to be the leading cause of NI and the spread of multi-resistant microorganisms in the hospital environment.

In Brazil; a cross-sectional study was performed a bacteriological analysis of the liquid soap in dispensers that health professionals use for hand washing. Fifty-nine liquid soap samples were analyzed, of which 33 contained the following microorganisms: *Burkholderia cepacia, Pseudomonas putidas, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter clocae, and Pseudomonas luteola.* The units with the largest number of contaminated samples were the surgical (n=7) and the dermatological clinics (n=4). Contamination was also found in an original flask of the same lot of liquid soap used to fill up the dispensers [1].

In Iraq, A comparative cross-sectional research was designed to determine the colonization of the in-use hand washing soaps in hospital settings. Swabs from surfaces of bar soaps and from liquid soaps via their applicator tips; at the sinks of toilets of hospital staff and working rooms of the wards were taken. Conventional microbiologic methods were used for culture of the swabs and identification of the isolates. The results reported that colonization was detected 60% and 15.9% in bars and liquid forms respectively. A total of 44 microorganisms were isolated. *Pseudomonas aeruginosa* (41%) was the most frequent isolated bacteria followed by *Escherichia coli* (13.6%) and *Acinetobacter baumanii* (11.4%). And this lead to the conclusion that bar soaps could be colonized with microorganisms excessively [4].

Another study by Carrie et al. (2009) to evaluate opportunistic pathogens from contaminated bulk soap on the hands of students and staff in an elementary school. This study show hands had significantly less gram-negative bacteria after washing with sealed soap compared to after washing with contaminated bulk soap, 135 vs. 2047 (P < 0.0001) [5].

In Turkey, another study was conducted to determine the bacterial colonization of bar soaps and liquid soaps in hospital environments. This study showed that bacterial colonization was detected in 36 bars (72%) out of 50 bar soaps. Totally 44 microorganisms were yielded including mainly *Pseudomonas aeruginosa, Escherichia coli* and *Acinetobacter baumanii*. Seven species (15.9%) were isolated from liquid soaps, majority of which was *Pseudomonas sp*. Nosocomial infection rate dropped from 4.2% in 2003 to 2.2% [6].

In Institut für Medizinische Hygiene; a study shows the hygienic examinations were performed relating to microbial contaminations of different kinds of fluid soaps in relation to various systems of dispensers, This study reported that totally 492 specimens of fluid soap, sampled in 14 different hospitals including 4 different systems of dispensers. Out of 14 trade name products--containing antimicrobial additives--8 proved to be sterile. The rest--declared as free from antimicrobial additives--showed bacterial contaminations of different grade. One of them (without antimicrobial additives) proved to be excessively contaminated with *P. cepacia* in 108 specimens out of 281, i.e. 38% in the range from 10^3 - 10^6 CFU/ml [7].

To the best of our knowledge, no study has been reported to determine bacterial contamination in liquid soap dispenser in main hospitals of Khanyounis Governorate, Palestine. Therefore, this study aims to determination of bacterial contamination in liquid soaps that used by health professionals for hand washing at hospital environment (Nasser and European Gaza Hospitals as a study subject) in Khanyounis Governorate to shed the light in the contamination of liquid soap in use at all departments, units & rooms of these hospitals, trying to produce an important information for decision makers in health field about effectiveness of infection control programs applied in hospitals and clinics, to formulate standards and guidelines for soaps used in clinics & hospitals.

2. MSTERIAL & METHODS

2.1 Study design and Sample Size

The study was analytical cross sectional, 82 liquid soap samples (half of them from Medical Nasser Complex and the other half was from European Gaza Hospital) were taken from the same departments and sectors/unit (includes care units, stations, stuff rooms, working rooms, toilets and kitchens) which were contain liquid soap dispensers in both mentioned hospitals.

2.2 Liquid soap sampling, processing and bacterial identification

2.2.1 Liquid soap sampling and processing

Liquid soap samples were collected in a 100 ml sterile cups. After that, the cups were transported within one hour of collection to the microbiology laboratory in Medical Sciences Department in University Collage of Sciences and Technology. The pH of stock soap samples was determined using strip by dipstick method. After that, The soap samples was diluted to 1/10 in tubes containing 9 ml sterile normal saline (0.9%). Tubes were shacked and ten microliter of sample was cultured on both Blood agar and MacConkey agar and then was incubated for 24 hours at 37°C.

On the other hand, only the soap samples which were exhibited a bacterial growth in the blood agar after incubation and had a catalase +ve test, a ten microliter of original diluted liquid soap of these samples were taking and cultivated on MSA (a differential media for the *Staphylococcus*) and then was incubated for 24 hours at 37°C. After 24 hours, the total number of *staph aureus* and other *staph species* colonies per ml (CFU/mL) that was grown on MSA was counted. In the final step of the method, Coagulase test (tube method) was used for conformation the results of MSA.

2.2.2 Bacterial identification:

1. Growth on Blood Agar without growth on MacConkey agar means the bacteria is a gram positive.

- Positive catalase test means the bacteria is a one type of staph species. Procedure of this test includes the following:
 i. Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a sterile loop.
 - ii. Place 2-3a drop of H2O2 on to the slide and mix.
 - iii. A positive result is the rapid evolution of oxygen by bubbling whereas, the negative result is no bubbles.
- 4. Growth on MSA means the bacteria is a gram positive Staphylococcus (*Staph aureus* produce yellow colonies with yellow zones, whereas other *Staphylococci* produce small pink or red colonies with no color change to the medium). Moreover, the *Staph aureus* species which was produced yellow colonies with yellow zones in MSA were confirmed by coagulase test (tube method) where by which it is positive.
- 5. Coagulase test (tube method) procedure includes the following:

i. Several isolated colonies of test organism were emulsified in 1ml of fresh plasma until give a milky suspension. ii. Incubate tube at 37oC for 4 hours was done.

iii. Clot formation was checked, when it was negative at 4 hours, incubate at room temperature overnight and check again for clot formation was done.

2.2 Data analysis

Data obtained were analyzed using Statistical Package of Social Sciences (SPSS) system (Version 18.0). Descriptive statistics (Frequencies and cross tabulation) and Chi-Square test were applied. A significant result means that the P-value for the hypothesis tests is less than 0.05. The confidence intervals (CI) was reported as 95%.

3. RESULTS

The findings demonstrate that among 41 samples that were collected from liquid soap dispensers which were found in Nasser hospital, Only one sample (2.4%) were found colonized by *staph aureus*, and two (4.8%) samples were found colonized by other *staph*, whereas 38 (92.8%) samples were not colonized by any type of bacteria (neither gram positive bacteria nor gram negative bacteria) (table 4.1). On the other hand, in European Gaza Hospital, among 41 samples which were collected from their liquid soap dispensers, 12 (29.3%) of them were colonized by *staph aureus*, and 13 (31.7%) were colonized by *other staph*, in addition to *staph aureus* + other *staph* (mixed) were reported in 2(4.8%) samples, whereas 14 (34.2%) samples were not colonized by any type of bacteria (neither gram positive bacteria nor gram negative bacteria) (table 1).

Bacterial Growth on MSA	Nasser Hospital		European Gaza Hospital	
bacteriai Growth on MSA	Frequency (No.=41)	(%)	Frequency (No.=41)	(%)
Staph aureus	1	2.4%	12	29.3%
Other Staph	2	4.8%	13	31.7%
Staph aureus + Other Staph (Mixed)	0	0.0%	2	4.8%
No growth	38	92.8%	14	34.2%
Total (%)	41	100.0%	41	100.0%

Table 1: Percent comparison of *Staph* species which was isolated from both hospitals

It is necessary to mention that, liquid soap samples that were collected from all dispensers of both hospitals and cultured on MacCAP were not exhibit any bacterial growth on that media. On other statement, the study was not registered any gram negative bacteria in liquid soap dispensers in both hospitals. Moreover, the findings also showed that the mean of pH for all liquid soap samples was 6 in both mentioned hospitals. However, there was no statistically significant relation between pH of liquid soap and bacterial growth in both hospitals (p-value > 0.05).

Furthermore, in Nasser hospital, nearly, all liquid soap dispensers (37 dispensers) were found sealed, whereas in European Gaza hospital, nearly, one third of liquid dispensers (14 dispensers) were found sealed. However, there was a statistically significant association between sealing of liquid soap dispenser and bacterial growth in both hospital (p-value = 0.033).

On the other side, by the observation and notation of research team in addition to inquiry of the hospital cleaning

workers and some relevant health workers, cleaning workers of Nasser hospital were found used chlorine bleach for washing the dispensers (containers) before putting the new liquid soap, while cleaning workers of European Gaza hospital were accustomed to wash it with water only. However, there was a highly statistically significant association between using of chlorine bleach for washing of liquid soap dispenser and bacterial growth between both hospital (p-value = 0.000).

Bacterial Growth on MSA	Nasser Hospital		European Gaza Hospitals	
	Frequency (No.=41)	(%)	Frequency (No.=41)	(%)
Pediatric Department	(100 12)		(1100 12)	
Staph aureus	0	0.0%	3	43.0%
Other Staph	1	14.0%	3	43.0%
Staph aureus + Other Staph (Mixed)	0	0.0%	0	0.0%
No growth	6	86.0%	1	14.0%
Emergency Department				
Staph aureus	0	0.0%	2	67.0%
Other Staph	0	0.0%	1	33.0%
Staph aureus + Other Staph (Mixed)	0	0.0%	0	0.0%
No growth	3	100.0%	0	0.0%
Intensive Car Department				
Staph aureus	0	0.0%	0	0.0%
Other Staph	0	00.0%	0	0.0%
Staph aureus + Other Staph (Mixed)	0	0.0%	0	0.0%
No growth	3	100.0%	3	100.0%
Surgery Men Department	- -			
Staph aureus	0	0.0%	2	40.0%
Other Staph	0	0.0%	1	20.0%
Staph aureus + Other Staph (Mixed)	0	0.0%	1	20.0%
No growth	5	100.0%	1	20.0%
Surgery Women Department				-
Staph aureus	0	0.0%	0	0.0%
Other Staph	0	0.0%	3	50.0%
Staph aureus + Other Staph (Mixed)	0	0.0%	1	17.0%
No growth	6	100.0%	2	33.0%
Intimal Men Department			-	
Staph aureus	0	0.0%	0	0.0%
Other Staph	0	0.0%	1	20.0%
Staph aureus + Other Staph (Mixed)	0	0.0%	0	0.0%
No growth	5	100.0%	4	80.0%
Intimal Women Department	0	0.00/	2	75.004
Staph aureus	0	0.0%	3	75.0%
Other Staph	0	0.0%	1	25.0%
Staph aureus + Other Staph (Mixed)	0	0.0%	0	0.0%
No growth	4	100.0%	0	0.0%
Cardiac Department	0	0.0%	1	25.00/
Staph aureus	0		1 2	25.0%
Other Staph Staph aureus + Other Staph (Mixed)	0	0.0%	0	50.0% 0.0%
No growth	4	100.0%	1	25.0%
Hospital Kitchen	4	100.070		23.070
Staph aureus	1	33.3%	1	33.3%
Other Staph	1	33.3%	1	33.3%
No growth	2	66.7%	2	33.4%
Total (%)	4	100.0%	4	100.0%

Table 2: Percent comparison of Staph species which was isolated from departments of both hospitals of the study

Table 2 reveals the percent comparison of *staph species* which were grown on MSA with coagulase test confirmation after taken from liquid soap dispenser between departments of both hospitals of the study. In Nasser hospital, the only

detected *staph aureus* was isolated from liquid soap dispenser (30 CFU/ml) which found in the food preparing sector in hospital kitchen, while other *staph* species were isolated from two liquid soap dispensers; the first one was the dispenser which found in the bath room of the Hospital Kitchen (40 CFU/ml), the second one was the dispenser of the patients bathroom which found in pediatric department (20 CFU/ml) (table 2).

Table 3: Distribution of *Staph* species according departments and sectors (units) from which they isolated in both hospitals of the Study

Stamb Smaater	Isolation Department and Sector	Count	
Staph Species	_	(CFU/ml)	
	Nasser Hospital		
Staph aureus	Hospital kitchen, food preparing sector	30	
Other Staph	Hospital kitchen, bathroom	40	
	Pediatric department, patients bathroom	20	
	European Gaza Hospital	•	
Staph aureus	Pediatric department, bathroom outside patients room	5	
	Pediatric department, bathroom inside patients room	100	
	Pediatric department, treatment preparing room	200	
	Emergency department, reception sector	15	
	Emergency department, nursing room	20	
	Surgery men department, bathroom outside patients room	15	
	Surgery men department, patients room	30	
	Intimal women department, patients room No. 1	200	
	Intimal women department, patients room No. 2	400	
	Intimal women department, nursing room	50	
	Cardiac department, center cardiac operations room	100	
	Hospital kitchen, kitchen workers room	120	
Other Staph	Pediatric department, patients room No. 1	200	
	Pediatric department, patients room No. 2	120	
	Pediatric department, babies nursing room	30	
	Emergency department, patients room	15	
	Surgery men department, patients room	20	
	Surgery women department, surgery women room	30	
	Surgery women department, bathroom outside patients room	10	
	Surgery women department, doctors rom	40	
	Intimal men department, nursing room	60	
	Intimal women department, patients room	250	
	Cardiac department, catheter operation room	20	
	Cardiac department, patients room	30	
	Hospital kitchen, housewares sector	10	
Mixed	Surgery men department, treatment preparation room	150	
	Surgery women department, patients room	35	

On the other side, In European Gaza Hospital, *staph aureus* was isolated from six departments and twelve sectors (units) as mentioned in the following; Pediatric department (bathroom outside patients room, bathroom inside patients room and treatment preparing room), Emergency department (reception sector and nursing room), Surgery men department (bathroom outside patients room and patients room), Intimal women department (patients room No. 1, patients room No. 2 and nursing room), Cardiac department (center cardiac operations room) & Hospital kitchen (kitchen workers room). Whereas, other *staph* species were isolated from eight departments and thirteen sectors as mentioned in the following: Pediatric department (patients room No. 1, patients room No. 2 & babies nursing room), Emergency department (patients room), Surgery men department (patients room), Surgery women department (surgery women room, bathroom outside patients room), Surgery men department (patients room), Surgery women department (surgery women room, bathroom outside patients room), Intimal men department (nursing room & patients room), Cardiac department (catheter operation room & patients room) and Hospital kitchen (table 3).

4. **DISCUSSION**

The main bacteria isolated in the analyzed soap samples were gram +ve *staph aureus* and *other staph*. On the other hand, there was no evidence on isolation of gram –ve bacterial species. The result of the present study was not in agreement with findings of other studies conducted by Güneş et al (2003) in Turkey, Zeiny et al., 2009 in Iraq, and Joselany et al.,2011 in Brazil where these studies reported that Gram –ve bacteria species are the most common pathogens isolated from hand washing soap in hospital setting [1,4,6]. The incompatibility in the findings between the current study and mentioned previous studies may be due to the limited number of liquid soap samples collected from both hospitals (82 liquid soap samples and only 30 of them were found colonized by bacteria) in the current study as compared to mentioned previous studies, in addition to the fact that the liquid soap is a salty environment and may be a non-favorable medium for growth of gram –ve bacterial species. Nevertheless, this argument cannot be proven.

As is illustrated in the current study; departments of European Gaza Hospital were the sites with the largest number of contaminated soap samples. In this study, one limitation is the lack of identification of the main contamination cause in infected departments due to high number of factors that contributing to be in-related and have an effect. This factors may include: type of dispensing systems (opened or sealed), location of contaminated liquid soap dispenser (e.g. office, unit, department, etc..), how far the liquid soap dispenser away from patients access, number of patients and stuff who used liquid soap in the unit, handling of liquid soap dispensers inside the hospital environment, in addition to many other factors. On the other hand, it is important to not forget alert to contaminations in the industrial phase.

Therefore, this study reported two factors and suggested them to be the main causes of high contamination rate of liquid soap in European Gaza Hospital as compared to Nasser Hospital. The first one is sealing of dispenser, where in Nasser hospital, nearly, all liquid soap dispensers (48 dispensers) were found sealed, whereas in European Gaza hospital, nearly, one third of liquid dispensers (14 dispensers) were found sealed. However, there was a statistically significant relation between sealing of liquid soap dispenser and bacterial growth in both hospitals (p = 0.033). The second is handling of liquid soap dispensers inside the hospital environment, where the cleaning workers of Nasser hospital were found used chlorine bleach for washing the dispensers (containers) before putting the new liquid soap, while cleaning workers of European Gaza hospital were accustomed to wash it with water only. However, there was a highly statistically significant association between using of chlorine bleach for washing of liquid soap dispenser and bacterial growth between both hospital (p-value = 0.000).

The suggestions of the present study are in agreement with finding of other study conducted by Joselany et al. (2011) who was perform a bacteriological analysis for the liquid soap in dispensers that health professionals use for hand washing. Where he reported that open dispenser systems, as practiced at the institution under analysis, and the handling of liquid soaps inside the hospital environment are known risk factors for microbial contamination of these products [1].

However, the current study findings confirm this risk and also alert to contaminations in the industrial phase. Honestly, in European Gaza Hospital, this study observed that there were no heuristic protocol for the methods of cleaning, disinfection, exchanging of liquid dispensers in order to implemented by cleaning workers. Whereas, In Nasser hospitals this protocol is somewhat clear for all relevant health workers and is monitored by the hospital administration.

Furthermore, with regard to way of cleaning that followed by cleaning workers in European Gaza Hospital; soap dispensers are cleaned with water whenever the solution inside them finishes. Although the protocol mentions cleaning once per week [2], this routine often is not followed regretfully, and dispensers are refilled not when they are totally empty, but when they are somewhat empty, which can favor the growth of microorganisms.

As for the department with the largest number of contaminated soap samples in European Gaza Hospital, these were the pediatric department (n=6), surgery men department (n=4), surgery women department (n=4). Intimal women department (n=4). Thus, in addition to the two suggestions above, it can be inferred that the great contamination of these soap dispensers may be due to overcrowding of patients inside these departments in addition to present of waste products of surgical wounds inside it, thus, make these soap dispensers more vulnerable to bacterial contamination. However, this assertion cannot be proved.

5. CONCLUSION

Only gram +ve *staph aureus* and *other staph* were isolated from dispensers of liquid soap that were found in departments of both hospitals. On the other hand, there was no evidence on isolation of gram –ve bacterial species. On the other hand, The contamination of liquid soap sample in European Gaza Hospital is attributed to high number of open dispenser systems (27 dispensers) that was observed in the departments, in addition to using of water instead of chlorine bleach for washing the dispensers before putting the new liquid soap. Thus, the bacterial biofilm in the bottom of

dispenser is not affected. In departments of European Gaza Hospital, the bacterial contamination may be due to overcrowding of patients inside these departments in addition to present of waste products of surgical wounds inside it.

6. **RECOMMENDATION**

Administration of European Gaza Hospital is recommended to establish heuristic protocol for the methods of cleaning, disinfection, exchanging of liquid dispensers and monitor its implementation by cleaning workers. In addition, All open system dispensers should be replaced with closed system or discardable dispensers in both study hospitals. Furthermore, Using of chlorine bleach for washing of dispensers before putting the new liquid soap, short changing times, proper handling, provide patients with individual soap in addition to increase disinfection with gel alcohol are perceptible control measures cannot be excluded. Further studies is highly recommended to investigate all factors that contributing to be in-related and have an effect on the contamination of liquid soap dispensers, to authenticate the findings from this study.

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