

Role of Hand Washing Antiseptics in Open Containers as Source of Pathogens in Nosocomial Infections

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ABSTRACT

Contaminated hand washing antiseptics act as source of multi-drug and disinfectant resistant isolates in nosocomial infections through hands of Health care workers(HCW). Limited data available necessitated the present study. To determine rate of contaminated hand-washing antiseptic solutions in open containers(cHWAOC) by In-use test. To analyze role of cHWAOC in nosocomial infections as source of pathogens. Cross sectional prospective observational study of two months was conducted. 46 of 65 HWAOC from wards, OPDs, Casualty and ICUs were studied for microbial contamination by In-use test. Isolation, identification, antimicrobial susceptibility testing and antibiogram typing of isolates was done by standard laboratory procedures. Questionnaire survey was used to assess hand washing practices in HWAOC. Correlation of isolates from cHWAOC with nosocomial infections was done by antibiogram typing and temporospatial association. Rate of c HWAOC was 28.26% (13/46). Distribution of cHWAOC was 21.8%, 50%, 66.67% and 15.38% respectively in wards, ICUs, Casualty and OPDs(P >0.05 NS). With an overall association in 11 nosocomial infections. *Pseudomonas aeruginosa* (5 antibiogram types) was predominant isolate (29.41%), followed by *A. baumannii* [3 types] (17.64%) with association in 5 and 4 nosocomial infections respectively. Questionnaire survey revealed use of savlon in 80.43% of HWAOC with variable concentrations (2.5% to 17% v/v). Changing antiseptics in HWAOC was highly variable. Majority of the containers were washed by soap and water (73.9%). Higher rate of cHWAOC with *Pseudomonas aeruginosa* and *Acinetobacter baumannii* as most common isolates was observed. Possible role of cHWAOC as source of nosocomial pathogens could not be ruled out by antibiogram typing of isolates. Use of HWAOC in health care settings should be banned.

Key words:

INTRODUCTION

Seminal studies of Ignaz Semmelweis [1846] and Oliver Wendell Holmes [1843] have proved the role of hand washing with soap and water in between examination of two patients and disproved the role of just rinsing hands in antiseptics in preventing transmission of nosocomial infections in health care setting.^[1,2] However, entry of hand washing antiseptics in open containers [HWAOC] for hand

washing in health care setting, not recommended by Ignaz Semmelweis, Oliver Wendell Holmes, World Health Organization (WHO) or Centre for Disease Control (CDC), remains largely unknown.

Outbreaks of nosocomial infections have most frequently incriminated the environmental sources like, ventilators circuits, suction apparatus, nebulizers and other patient care equipments especially contaminated antiseptic solutions,

which form a suitable environmental niche for *P. aeruginosa* and other gram negative pathogens which have tendency to remain viable on both animate and inanimate objects around the patient, including antiseptic solutions.^{3,4} Contaminated antiseptics acting as a source of pathogens in nosocomial infections has been proved by several studies^{5,6}

Stringent hand washing with soap and water is followed by HCWs at our hospital in between two patients during examination, with the exception of some areas where antiseptic solutions in open containers are used. It was hypothesized that contaminated HWAOC may act as source of nosocomial pathogens, spreading through hands of HCWs after washing/rinsing their hands in such solutions.

Although, commonly used for hand washing both in resource poor government hospital and corporate hospitals, role of HWAOC in nosocomial infections has not been investigated by systematic studies. Hence the present study was conducted to determine rate of contaminated Hand Washing Antiseptics In Open Containers (cHWAOC) by In-use test and their role as source of pathogens in nosocomial infections.

MATERIALS AND METHODS

Study design

A hospital based cross sectional observational study of two months duration was conducted in a tertiary care hospital with prior approval from Institutional Ethical Committee. A total of 65 HWAOC from Intensive care units (ICUs), Out patient departments (OPDs), Emergency ward, General wards and labor room were included in the present study.

Specimen collection

One ml of In-use antiseptic solution from open container was drawn into sterile 2ml disposable syringe and immediately added to 9 ml of nutrient broth in a sterile universal container with aseptic precautions.

In-use test⁷

0.02 ml of diluted antiseptic in nutrient

broth from sterile universal container was spot inoculated onto ten different areas on two well dried nutrient agar plates. One plate was incubated at 37°C for three days and the other for 7 days at room temperature.

Antiseptic solution from an open container was considered as contaminated if there was growth in more than five spot inoculations on either plate.

Bacterial strains and susceptibility testing

Bacterial isolates from cHWAOC on nutrient agar plates were sub-cultured on Mac-Conkey agar and Blood agar (Hi media, Mumbai, India) and identified by standard laboratory procedures.⁸ Antimicrobial susceptibility testing was performed on Muller Hinton agar (Hi media, Mumbai, India) by Kirby Bauer's disc diffusion method as per guidelines of Clinical Laboratory Standards Institute (CLSI)⁹

Typing of bacterial isolates

Isolates were typed by ANTIBIOGRAM TYPING. Association of isolates from cHWAOC with nosocomial infections was done by circumstantial evidence (Temporospacial association) and bacterial isolates with identical antibiogram type from cHWAOC and cases. Results were quantitated by analyzing microbiology culture reports during past six months.



Fig. 1 :

Exclusion Criteria

1. Samples from freshly prepared HWAOC were not collected.
2. Samples from HWAOC not being used for hand washing were not collected.

Questionnaire survey

Hand washing practices in HWAOC was studied to determine:

- a. Concentration of antiseptic used in HWAOC
- b. Frequency of changing antiseptics in HWAOC
- c. Method of washing containers of antiseptics
- d. Number of health care workers washing hands in HWAOC/day

Statistical Analysis

Distribution of cHWAOC was analyzed by Chi-square test.

RESULTS

HWAOC were used in 26/54 wards, 4/7 ICUs, all three areas of casualty and all OPDs for hand washing in between examination of two patients. A total of 46 HWAOC, after excluding 14 were included in the present study.

The present study revealed a high rate of contamination, 28.26% (13/46), with Savlon as the only antiseptic used in all HWAOC. Rate of contamination was highest in casualty 66.67%(2/3) and lowest in OPDs 15.38(2/13). Distribution of cHWAOC in different areas of hospital was

statistically not significant. ($P > 0.05$)

Pseudomonas aeruginosa was the most common bacterial isolate from cHWAOC, 29.41%(5/17) followed by *Acinetobacter baumannii* 17.64%(3/17). *Rhizopus* spp, *Aspergillus* spp, and many *Mycelia sterilia* were also isolated. Aerobic spore bearers isolated were ignored. (TABLE 2)

Multiple antibiogram types of *Pseudomonas aeruginosa*[PA-1 to PA-5] and *Acinetobacter baumannii*[AB-1 to AB-3], *Klebsiella pneumoniae*[KP-1 and KP-2] and *Escherichia coli* [EC-1 and EC-2] and several other bacterial isolates which could not be typed, were observed. Multi drug resistant strains PA-3 and AB-1 were isolated from Surgical Intensive care unit and Intensive Cardiac care units respectively. PA-4, PA-5 and KP-2 were isolated from orthopedics OPDs.

Bacterial isolates from cHWAOC could be associated with 11 clinical cases[5 in ICUs] by criteria used in the study. (TABLE 3 to TABLE 6)

Questionnaire survey revealed variable concentration of Savlon used; 1:6 to 1:250 [2.5% to 17% v/v] with tap water, not prepared by designated health care worker, variable number of health care workers washing their hands in HWAOC[0-25/day] with most common being 0-5[19%] and 5-10[20%] excluding medical and nursing students. Different methods of washing containers: water alone 3(6.5%)/ water and soap 41(89.13 %)/ water and Hypochlorite 1(2.174%), Variable frequency of changing disinfectants: Once daily, 80.435 [37/46] and twice daily, 17.391%[8/46].

Table 1: Distribution of Contaminated Hand Washing Antiseptics in open Containers in Different Areas Of The Hospital

Areas of the Hospital	Number of Samples Tested	Number of Contaminated Samples	Percentage of Contaminated Samples
Wards	26	7	26.92
Icus	4	2	50
Casualty	3	2	66.67
Out Patient Departments	13	2	15.38
Total	46	13	28.26

Note: Icu= Intensive Care Units

DISCUSSION

The present study, first of its kind to the best of our knowledge conducted to determine role of cHWAOC in nosocomial infections revealed important and significant findings in a health care setting with a changing scenario in hand washing practices in terms of strict adherence to hand washing in sinks with alcohol based hand washing solutions/soap and water as per standard guidelines.

Diluted Savlon in HWAOC for hand washing, the only antiseptic, being used without any guidelines from the local Hospital Infection Control Committee in some areas of our hospital necessitated the present study to determine their role as source of nosocomial pathogens.

The present cross sectional study revealed a high rate of contamination, 28.26% (13/46) of HWAOC by In-use test, a simple, cheap yet clinically significant test suitable to assess the microbial contamination of in-use disinfectants. Similar studies on HWAOC are not available for comparison after thorough review of literature. However, a large multicentric longitudinal study conducted in Malaysian hospitals by Keah *et al.* reported a lower rate of contamination; 16.4% (1529/9265) and 5.3% (1/9) for various in-use disinfectants and Cetrimide [one of the ingredients of Savlon] respectively, but not from HWAOC.^[10] Gajadhare *et al.* have reported a contamination of 15% (9/60) for Savlon, both pre-use

and in-use, as an highly contaminated antiseptic among the three antiseptics used in the hospital¹¹

Rate of contamination was highest in Casualty and ICUs than in General wards with diverse and often multi-drug resistant bacterial isolates. Distribution of contaminated HWAOC in different areas of the hospital was not statistically significant. Higher incidence of nosocomial infections in areas of the hospital with cHWAOC or vice versa i.e. lower incidence in areas without cHWAOC was not observed, indicating several other sources of nosocomial pathogens.

The most common bacterial isolates from cHWAOC in the present study were *P. aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* often multi-drug resistant with multiple antibiogram types. *P. aeruginosa* was the most common bacterial isolate from cHWAOC since Savlon, a mixture of Chlorhexidine gluconate (0.3% v/v IP) and Strong Cetrimonium bromide (0.6% w/v BP) was the only antiseptic used in all HWAOC with Cetrimonium bromide acting as selective agent for *P. aeruginosa*. This probably highlights that other isolates from cHWAOC may also be disinfectant resistant rather than representing recent contamination. Gajdhare *et al.* have reported that *Pseudomonas spp.* as the only bacterial species isolated from all the contaminated disinfectants. However, Keah *et al.* have reported diverse, Gram negative nonfermenters, predominantly *Pseudomonas aeruginosa* from different disinfectants⁵

Table 2: Bacterial Isolates From Contaminated Hand Washing Antiseptics In Open Containers

Name Of The Bacterial Isolate	Number of Bacterial Isolates	Percentage
<i>Pseudomonas Aeruginosa</i>	5	29.41
<i>Pseudomonas Stutzeri</i>	1	5.88
<i>Acinetobacterbaumannii</i>	3	17.64
<i>Acinetobacterlwoffii</i>	1	5.88
<i>Klebsiellapneumoniae</i>	2	11.76
<i>Escherichia Coli</i>	2	11.76
<i>Staphylococcus Aureus</i>	1	5.88
<i>Coagulase Negative Staphylococci</i>	1	5.88
<i>Nocardia Species</i>	1	5.88
Total	17	100

In the present study, role of cHWAOC as source of pathogens by temporospatial association and identical antibiogram type of the isolate from cHWAOC and clinical cases could be established only in 11 nosocomial infections. However, Keahet *al.* and Gajdhare *al.* have not determined the role of contaminated disinfectants/antiseptics in nosocomial infections by correlating isolates with clinical cases.^{[10],[11]} Large outbreak of *P. maltophilia* was reported by Wishart MM from contaminated Savlon from Australia.^[5] Nosocomial infections due to contaminated disinfectants/antiseptics will have grave consequences^{5,6}

However, clinical correlation of isolates from cHWAOC with only 11 nosocomial infections appears largely, an underestimation of the role of cHWAOC as source of nosocomial pathogens. This fact is further strengthened by frequent isolation of bacteria with identical antibiogram types as that from cHWAOC from hand of Health Care workers as transient colonizers or carriers [apart from other antibiogram types] in our hospital. For association of bacterial isolates from cHWAOC with cases, molecular methods of typing like PCR, though costly would be more useful. Antibiogram types, PA-3, PA-5, AB-3, KP-1, KP-2 and EC-2 could not be associated

with nosocomial infections with the criteria used in the present study. However, this does not rule out that they have not caused any infection in the past or will not do so if cHWAOC are totally not banned. Contaminated disinfectants exhibit decreased efficacy and effectiveness. Large number of bacterial isolates from disinfectants have also exhibited resistance to commonly used antimicrobial agents thus posing a therapeutic challenge.^{[12],[13],[14]}

Role of cHWAOC as source of nosocomial pathogens in different areas of the hospital was probably not equally significant. Role of cHWAOC in CCU and MICU, 2 of the 4 ICUs with HWAOC, was clinically significant with *P. aeruginosa* (PA-4) associated with 2 infections and *Acinetobacter baumannii* (AB-1) with 3 infections respectively. Both strains were also isolated from hands of HCWs acting as carriers in respective ICUs. Role of HWAOC as constant source but with different pathogens at different point of time is a strong possibility in our hospital. The finding is further strengthened by the fact that *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* are most common pathogens at our ICUs, often multi-drug resistant with a complex and highly dynamic transmission and several environmental sources of pathogens¹⁵

Table 3: Antibiogram Types of *Pseudomonas aeruginosa* Isolates

Strains	Antibiogram Type	Area of the hospital	Number of Infections Caused
PA-1	R to: Cpt, Pit, Spx, I, Tob, Pc, Net, Gat, Le, Ak, Nx, Ctx S to: Others	Female Surgery Ward	2
PA-2	R to: Nil S to: All	Male Surgery Ward	1
PA-3	R to: Cz, Ax, Azm S to: Others	SICU	-
PA-4	R to: Azm, As, Ax,, Cis, Cpt, Spx, Le, Ctx, Amc, Nx, Tob S to: Gat, Net, Ctr, Pit, Ic, Pc, Ak	Orthopedics OPD [CCU]	2
PA-5	R to: Ctr, Azm, As, Ax, Cis, Cpt, Spx, Le, Amc, Nx, Tob S to: Pit, Ak, Ic, Net, Ctx, Gat	Orthopedics OPD	-

Note: SICU=Surgical Intensive Care Unit, CCU=Critical Care Unit, OPD= Out Patient Departments, PA-1 to PA-5= *Pseudomonas aeruginosa* strains [1-5], R=Resistant, S=Sensitive,

Although hand washing in sinks with soap and water or alcohol based hand washing solutions is ideal the same is practically impossible in hospital areas like ICUs, with sinks located in remote corners and heavy work load in terms of number of serious patients admitted in ICUs. This has already lead to replacement of HWAOC with alcohol based hand rubs in between two patients with terminal hand washing in sinks in majority of the ICUs.

Two of the three cHWAOC[66.05%] from casualty were contaminated with *P.stutzeri* and *P. aeruginosa* and *E. coli*[EC-2], from Green area and emergency triage room. This was a worrisome finding since new patients entering into the hospital might get colonized by disinfectant resistant and often multi-drug resistant bacteria. Due to lack of clinical correlation with cases, significance of cHWAOC from casualty could not be determined/proved from the present cross sectional study.

High rate of cHWAOC was observed, 26.92%[7/26] with multi-drug resistant bacterial isolates in wards where strict hand hygiene is being practiced in between two patients, but unfortunately in HWAOC with hand washing in sinks only at the

beginning and end of the duty shifts. Six of the seven cHWAOC were observed in surgical wards, the cause of which could not be identified. HWAOC were found to be persistently contaminated in surgical wards but with constantly changing bacterial flora, which was confirmed by sampling three times at different intervals. This confirms the role of cHWAOC as source of diverse bacteria which cannot be identified by a cross sectional study. Diverse, often multi drug resistant bacterial isolates from cHWAOC is not a complete list of nosocomial pathogens in our hospital since a cross sectional study like ours, probably underestimates the role of cHWAOC as potential source of nosocomial pathogens.

Rate of cHWAOC in OPDs was 15.38%(2/13) apparently not a significant risk factor considering the type of health care activities done in OPDs. Both (two) the cHWAOC were observed in OPDs of surgical specialty, the cause and the role of which in nosocomial infections could not be determined with certainty. However, certain procedures done in OPDs like, suture removal, wound dressing and cleaning of external fixators in Orthopedics OPD pose significant risk of acquiring nosocomial infections in patients.

Table 4: Antibigram types of *Acinetobacter baumannii* isolates

Strains	Antibiogram Type	Area of The Hospital	Number of Infections Caused
AB-1	R To : Cz, Ax, Ak, Net, Pi, Tob, Nx, S To: Others	Micu	3
AB-2	R To : Cz, Ax, Azms To : Others	Cardiology Ward	1
AB-3	R To : Cz, Ax, Amc, Azm, Cpt, S To : Others	Male Surgery Ward [220]	-

Note: Micu= Medical Intensive Care Unit, Ab-1 To 3(*Acinetobacterbaumannii*), R=Resistant ,

Table 5: Antibigram types of *Klebsiella pneumoniae* isolates

Strains	Antibiogram Type	Area of The Hospital	Number of Infections Caused
KP-1	R to: Ax, Cpt, Net, Cz, AzmS to : Others	Female Surgery Ward [215]	-
KP-2	R to : Ak, Cz, Tob, Net, Ax, S to : Others	Orthopedics OPD	-

Note: OPD= Out Patient Departments, R=Resistant , S=Sensitive

The In-use test used in the present study will not determine whether the contamination was due to an inadequate concentration of disinfectant or whether organisms were surviving or growing at or above the recommended concentration and also inadequate concentrations of disinfectant in the absence of bacterial contamination will not be detected¹⁶ Majority of the cHWAOC showed confluent growth in all ten spot inoculations indicating a high microbial burden probably indicating disinfectant resistant bacteria rather than heavy contamination¹⁰ Relatively high microbial burden in cHWAOC is indicative of probability of attaining an infective dose and of establishing a nosocomial infection through hands of HCWs. The risk is further magnified when cHWAOC were found in Surgical wards, Surgical OPDs and ICUs as observed in the present study.

In 43.48% (20/46) of HWAOC growth was observed in 1 to 4 spot inoculations, not declared as contaminated by the In-use test used in the present study. However, changing bacterial flora, often with high degree of microbial burden and variable number of health care workers washing their hands in HWAOC, even these HWAOC can be considered as potential source of nosocomial pathogens which probably could be proved by a longitudinal study.

To overcome limitations of In-use test and to complement methodology used in the present study to determine factors influencing the contamination of HWAOC, a questionnaire method of survey was done. Second author, Phase III MBBS student collected tactfully all the information by inquiry, since the same done by the first author, an Infection Control Officer would have led to bias in terms of false information [Theoretical] and over consciousness and over glorification of hand washing practices in HWAOC. This revealed several interesting findings, mainly a changing scenario from

HWAOC to Alcoholic hand rubs or conventional hand washing in sinks in majority of the areas of the hospital including ICUs.

Although findings of the questionnaire were analyzed objectively, the inferences drawn appear more or less subjective and to some extent arbitrary. Several studies have reported that unhygienic practices during preparation and distribution of disinfectants/antiseptics contribute significantly to their contamination in a hospital environment. Inappropriate source of water as diluent, failing to maintain adequate cleanliness of the container were found to be important source of contamination by other studies^{5,6,10}

Although, findings of questionnaire survey analyzed and interpreted, clearly indicate factors responsible for contamination of HWAOC, this does not necessitate teaching, training or establishing guidelines for hand washing practices in HWAOC since HWAOC should be totally banned in health care setting and should be replaced by conventional hand washing in sinks and limited use of hand washing with alcohol based hand rubs in between two patients in busy hospitals.

Limitations of the present study

1. Molecular methods of typing bacterial isolates from cHWAOC were not used.

Implications of the present study

1. HWAOC were immediately banned and replaced by conventional hand washing in sinks with soap and water and in ICUs by alcohol based hand rubs in between examination of two patients.
2. Clear guidelines to ban hand washing in HWAOC were incorporated in the Infection control Manual of our Hospital Infection Control Committee.

Table 6: Antibigram Types Of *Escherichia Coli* Isolates

Strains	Antibiogram Type	Area Of The Hospital	Number of Infections Caused
EC-1	R to : Ax, Amc, Cz, AzM S to : Others	Female Surgery Ward	2
EC-2	S to :	All Emergency Triage Room	-

Note: EC= *Escherichia coli*, R=Resistant, S=Sensitive

- Further In-vitro studies on disinfectant resistance will be conducted on preserved bacterial isolates from cHWAOC.

Conclusions of the present study

- Higher rate of contamination of HWAOC with *Pseudomonas aeruginosa* and *Acinetobacter baumannii* as most common isolates was observed.
- Role of cHWAOC as source of nosocomial pathogens though not proved conclusively, could not be ruled out by antibiogram typing and temporospatial association of isolates, necessitating further Phenotypic and Genotypic methods of typing of isolates.
- Variable concentrations of antiseptics and variable number of HCWs using HWAOC and improper washing of containers were found to be risk factors for contamination.
- Clear guidelines to be incorporated in WHO, CDC and other authorities regarding banning HWAOC in health care settings.

HCW= Health care worker
 WHO= World Health Organization
 CDC= Centre for Disease Control
 Cpt= Cefepime+Tazobactam
 Pit= Piperacillin+Tazobactam
 Spx=Sparfloxacin
 I=Imipenem
 Tob=Tobramycin
 Pc=Piperacillin
 Net=Netilmycin
 Gat=Gatifloxacin
 Le=Levofloxacin
 Ak=Amikacin
 Nx=Norfloxacin
 Ctx=Cefotaxime
 Amc= Amoxicillin+Clavulanic acid
 Ctr= Ceftriaxone
 Azm= Azithromycin
 As=Ampicillin+Sulbactam
 Ic= Imipenem+Cilastatin
 Cz= Cefozoline
 Ax= Ampicilli+Cloxacillin

Abbreviations

HWAOC= Hand washing antiseptics in open containers
 cHWAOC= Contaminated Hand washing antiseptics in open containers

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