Assessing The Effect of Selected Cleaning Agents (Bleach, Ethanol and Liquid soap) On Selected Nosocomial Organisms (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*) in Uganda. A Case Study of Kisubi Hospital.

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Abstract

Background

The major cause of disease and even human death are bacteria. Disinfectant is widely used in various ways as an effective agent to kill or eliminate bacteria, especially in the microbial laboratory. The most commonly used disinfectants in the laboratory are ethanol, bleach, and hand soap. This study assessed the effect of the common cleaning agents against bacteria.

Methodology

It was an experimental study on disinfectant efficacy of ethanol, bleach, and antibacterial hand washing soap on surface disinfection. Sampling was carried out between April and June of 2017. The samples were collected from our lady of Consolata kisubi hospital which is located on Nkima Road- Kisubi, Wakiso, Uganda. The samples collected were obtained from the hospital surfaces like door handles, beds, random floors, toilets, and work surfaces before and after cleaning.

Results

64 samples were collected from 32 sites. The selected nosocomial organism in the study included *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa.* Among the selected disinfectants, ethanol was the most effective against the available selected nosocomial organisms.

Conclusion

Bacteria is present on so many surfaces of the hospital, and the selection of appropriate cleaning agents is vital to achieve maximum cleaning. Bleach and liquid soap should not be used in combination if cleaning different surfaces in the hospital due to the effects of the inorganic compounds that might be present in the liquid soap that inactivates active ingredients used present in the Bleach.

Recommendation[a]

The hospital should come up with a minimum inhibitory concentration of the disinfectants that is effective to be used. The support staff in charge of cleaning should be trained on the right way of cleaning and disinfecting.

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1 Background

The major cause of disease and even human death are bacteria (Drexler *et al.,* 2010). Disinfectant is widely used in various ways as an effective agent to kill or eliminate bacteria, especially in the microbial laboratory. Disinfectants can be mainly divided into five agents: alkylating, sulfhydryl combining, oxidizing, dehydrating, and permeable(Mutuku, 2018). The most commonly used disinfectants in the lab are ethanol, bleach, and hand soap. Bleach, with the main constituent of sodium hypochlorite, affects by oxidizing the cell of microorganisms and attacking essential cell components including lipid, protein, and DNA (Gerald *et al.,* 1999). Ethanol, as a dehydrating agent, causes cell membrane damage, rapid denaturalization of proteins with subsequent metabolism interference and cell lyses (Fung, K. W, 2015). Hand soap, as a daily used disinfectant, normally works by stripping away the outer layer of oil on the skin and prevents bacteria present in the body from coming to the surface of the hand. Ethanol and bleach disinfectants are believed to have an immediate effect against most microorganisms according to many of the studies that have been done comparing disinfectant efficiency (S.Kumar, *et al,* 2010). For bacterial strains, *Escherichia coli* (*E.coli)*, *Staphylococcus aureus, Pseudomonas aeruginosa* have been used widely in the disinfectant test as a pathogen indicator.

Requirements for aseptic processing areas include readily cleanable floors, walls, and ceilings that have smooth, non-porous surfaces; particulate, temperature, and humidity controls; and cleaning and disinfecting procedures to produce and maintain aseptic conditions. These conditions combined with a careful and thorough evaluation of the chemical agents used for the cleaning and disinfection program should lead to achieving the specified cleanliness standards and control of microbial contamination of products.

According to the World Health Organization report (2002) on the prevention of hospital-acquired infections, it is believed that the major cause of nosocomial infections is majorly due to inefficient use of disinfectants to kill bacteria in hospitals.

The research intends to use such information to identify the common bacteria, and therefore suggest relevant disinfectants that seem to kill such bacteria.

2 Methodology:

Study design

It was an experimental study on disinfectant efficacy of ethanol, bleach, and antibacterial hand washing soap on surface disinfection. Sampling was carried out between April and June of 2017.

Study site

The samples were collected from our lady of Consolata kisubi hospital which was located on Nkima Road- Kisubi, Wakiso, Uganda and were immediately taken to the microbiology laboratory for analysis.

Data and Sample collection

The samples collected were obtained from the hospital surfaces like door handles, beds, random floors, toilets, and work surfaces before and after cleaning. The swabs collected from different areas and wards were well labelled and put in transport media which was peptone water. They were then stored in a cool box and transported to the microbiology laboratory where they were analysed.

MATERIALS:

MacConkey agar, Blood agar, Chocolate agar, Distilled water, Incubator, Heat source, Inoculating plate, Swabs, Disks, Inoculating loops, Mueller Hinton agar, the pH indicator dye, and bromothymol blue.

Laboratory Methods

Sample processing

The swabs were immersed in peptone water immediately after collection to enable the multiplication of microorganisms present on the swab.

Inoculation on blood agar and MacConkey agar

From peptone water, the swabs were inoculated on blood agar and MacConkey agar and streaked to obtain single colonies. The plates were then incubated at 37◦C for 24 hours.

Identification of bacteria strains on blood agar and MacConkey agar

After 24 hours, the plates were inspected for bacterial growth. The bacteria were identified according to colon size, shape, and hemolysis on blood agar. On MacConkey agar, lactose fermenters or non-lactose fermenters were obtained. The suspected colonies were further subcultured to obtain pure colonies that were confirmed using Gram staining and biochemical tests.

Confirmatory tests for the bacteria strains

Several biochemical tests were used to confirm the presence of the different bacteria strains from the individual subcultured colonies. These were;

Triple sugar iron test (TSI)

Principle

TSI agar contained three sugars, glucose, sucrose, and lactose, and also ferrous iron (iii) sulphate. Also, it had an indicator phenol red that changed color according to the pH change. For the lactose non-fermenters, they broke down the glucose-producing acid that lowered the pH turning the indicator to yellow in the butt. After breaking down the glucose the organisms broke down the amino acids that were present in the medium raising the pH that turned the indicator red in the slant. For the lactose fermenters, they broke down all the three sugars producing enough acid that turned the color of the indicator in the butt and the slant to yellow. The hydrogen sulphide produced by some organisms reacted with iron (iii) sulphate that turned the media black.

Procedure

TSI tubes were inoculated with a long straight wire. The well-isolated test colony recovered from a gar, plate was touched with the end of the inoculating needle which was then stabbed into the deep of the tube. When the inoculating wire was removed from the deep of the tube, the slant surface was streaked with a back and forth motion. Inoculated tubes were placed into the incubator at 350C for 18 to 24 hours.

Citrate Utilization test

Principle

The techniques were used to assist in the identification of enterobacteria. The test was based on the ability of an organism to use citrate as the only source of carbon and ammonia as its only source of nitrogen. In the presence of citrate utilizing microbe, sodium citrate that was contained in the medium was broken down and that resulted in a change in pH that caused the color of the indicator to change to blue.

Procedure

The agar was inoculated with a single colony from the subculture using a long straight wire. The well-isolated test colony recovered from a gar plate was touched with the end of the inoculating needle which was then stabbed into the deep of the tube. When the inoculating wire was removed from the deep of the tube, the slant surface was streaked with a back and forth motion. Inoculated tubes were placed into the incubator at 35◦C for 18 to 24 hours

SIM test (Sulphur indole motility)

SIM medium was a semisolid a gar that was used to determine hydrogen sulphide production, indole formation, and motility.

SIM medium was used to differentiate members of the family Enterobacteriaceae.

Haziness that spreads from the stab line indicated a positive test for motility.

A red color development after the addition of Kovacs reagents indicated indole production. A black precipitate indicated hydrogen sulphide production.

Urease test

The Urease test identified those organisms that were capable of hydrolyzing urea to produce ammonia and carbon dioxide. It was primarily used to distinguish urease-positive protease from other Enterobacteriaceae.

Principle

Many organisms especially those that infested the urinary tract had a urease enzyme that was able to split urea in the presence of water to release ammonia and carbon dioxide. The ammonium carbonate which turned the medium alkaline to form the indicator phenol red from its original orange-yellow color to bright pink. No color change was observed in the absence of urease-producing bacteria.

Procedure for urease test

Using a sterile wire loop, a single colony of the bacteria was streaked on the surface of the urea agar slant. The tube was left loosely capped to allow entry of oxygen. The tube was then incubated for 48 hours to 7 days at 35◦C. The color of the medium changed from light orange to magenta if the organism produced the enzyme urease.

3 Results

KEY

0 – no growth 1 - growth

X – no sample collected F- Floor

TDK – toilet door knob GW – general ward

OPD – out patient department US – ultra sound

ICU – intensive care unit AW – adult ward

BP – blood pressure MW – maternity ward

PWR – pediatric ward reception POW – post operative ward

Table 1. shows the results obtained after from using 70%ethanol, bleach and liquid soap againstE.coli, Staphylococcus aureus and Pseudomonas aureginosa

Sample collection site	Disinfectant used	E coli		Staphylococcus		Pseudomonas	
		be-fore	af-ter	Be-fore	After	be-fore	After
TDK(US and x-ray department)		0	0	1	1	0	0
AW (bed without patient)		0	0	0	0	0	0
AW (bed with patient)	Bleach and liquid	0	0	0	0	0	0
MW (POW) floor	soap	1	1	0	0	0	0
MW (POW) table		0	0	1	0	0	0
MW bed 1		0	0	0	0	0	0
MW nursery	70% ethanol	1	0	0	0	0	0
GW bed	Bleach and liquid	0	0	1	1	0	0
OPD bed	soap	x	0	X	1	x	0
Laboratory sample collection table	70% ethanol	0	0	1	0	0	0
PW ICU oxygen line	Bleach and liquid	1	1	0	0	0	0
PW ICU bed	soap	0	0	0	0	0	0
PW floor	Bleach and liquid	0	0	1	1	0	0
PW patients table	soap	0	0	0	0	0	0
Canteen table	liquid soap	0	0	1	1	0	0
Canteen chair		0	0	0	0	0	0
OPD(BP) machine		0	X	1	X	0	X
PW(ICU) door knob		0	0	0	0	0	0
PW bed rails		X	0	X	1	X	0
PWR nurses table		0	0	0	0	0	0
F-6		0	0	0	0	0	0
F-8		0	0	0	0	0	0
F-5	Bleach and liquid	0	0	1	1	0	0
F-7	soap	0	0	0	0	0	0
F-10		0	0	0	0	0	0
F-1		1	1	0	0	0	0
F-9		0	0	0	0	0	0
F-11		0	0	0	0	0	0
F-2		X	1	X	1	X	0
F-3		0	0	0	0	0	0
F-4		0	0	1	1	0	0

4 Discussions:

64 samples were collected from 32 sites. Samples were collected before and after cleaning for each site. The selected nosocomial organism in the study included *E.coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa.* Among the selected disinfectants, ethanol was the most effective against the available selected nosocomial organisms. This finding was in agreement with a study by (Gaonkar, *et al.,* 2006), and the used liquid soap and bleach had the least effectivity on the organisms.

Effect of Bleach and liquid soap when used as a disinfectant to *E. Coli*

The samples collected from the post-operative ward showed growth for *E.coli.* This included its floor, and the intensive care unit oxygen line. The samples collected after the sites were cleaned still showed growth of bacteria meaning that even after cleaning with bleach and liquid soap, the bacteria was still present. The results is not in agreement with what has been established by (WHO, 2014) that Bleach is a strong and effective disinfectant. Its active ingredient sodium hypochlorite is effective in killing bacteria, fungi and viruses, including influenza virus. Diluted household bleach disinfects within 10–60 minutes contact time. The inability of the combination of Bleach and liquid soap

to kill bacteria in this study is explained by (WHO, 2014) that the active ingredients present in bleach is easily inactivated by organic material, which are present in the liquid soap.

Effect of 70% Ethanol when used as a disinfectant to *E.coli*

The samples collected from the maternal ward nursery had *E. coli* before cleaning with 70% Ethanol, and after cleaning, the samples collected from the same area had no growth meaning that 70% Ethanol as a disinfectant was effect against the bacteria. Findings of this study agree with a study by (Salvage *et al.,* 2014) that reported 70% to be effective, and more at lower concentrations than the 70% standard.

Effect of Bleach and liquid soap to *Staphylococcus aureus (S.aureus)* :

Resulted from this study show that all samples from the toilet door knobs of the Ultra Sound scan, and the X-ray department had growth for *S. aureus* a result that is in agreement with a study by (Alonge *et al.*, 2019) that reported *Staphylococcus aureus* being present on door knobs at a frequency of 42.9%. The maternal ward, post-operative ward table, the general ward bed, Outpatient department bed, Laboratory sample collection table, pediatric ward floor, Canteen table, Outpatient department Blood pressure machine, pediatric ward bed rails, random hospital floors 5, 2, and 4 had a growth of *Staphylococcus aureus.* The use of bleach and liquid soap only cleaned Maternal ward post-operative ward table, and the outpatient department blood pressure machine but not any other site from which samples were collected after cleaning.

Effect of 70% ethanol as a cleaning agent to *Staphylococcus aureus (S.aureus)*

Ethanol (70%) was used as a cleaning agent when cleaning the laboratory sample collection table and the Maternal ward nursery at the hospital. Results of this study showed that bacteria was only present before cleaning, and after cleaning, the samples showed no growth for the bacteria on the laboratory sample collection table. This is in agreement with a study by (Salvage *et al.,* 2014) that reported 70% to be effective. The study reported no bacteria growth from samples collected from maternal ward nursery.

Effect of Bleach, liquid soap, and 70% ethanol used as a cleaning agent to *Pseudomonas Aureus (P.aureus).*

Pseudomonas aureus was not found to be present in this study from the 64 samples that were collected from 32 sites both before and cleaning at the hospital. This means that the effect of the cleaning agents against these bacteria was not fully studied.

5 Conclusions:

Bacteria is present on so many surfaces of the hospital, and the selection of appropriate cleaning agents is vital to achieve maximum cleaning. Bleach and liquid soap should not be used in combination if cleaning different surfaces in the hospital due to the effects of the inorganic compounds that might be present in the liquid soap that inactivates active ingredients used present in the Bleach.

Recommendation

The hospital should come up with a minimum inhibitory concentration of the disinfectants that is effective to be used. The support staff in charge of cleaning should be trained on the right way of cleaning and disinfecting.

Abbreviations

A References:

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Table 2. Abbreviations

CHO	Chocolate Agar
Mac	MacConkey Agar
E.coli	Escherichia Coli
ATCC	American Type Culture Collection
SIM	Sulphur Indole Motility
TSI	Triple Sugar Iron
SOP	Standard Operating Procedures
Mls	Milli liters
Mm	Millimeters
RPM	Revolution per minute
Hrs.	Hours
E.g.	Example
Etc.	Excreta
I.e.	That is to say
EDTA	Ettylenediamine tetra acetic acid
App	Appendix
CAMP	Christine ,Atkins, munch Peterson

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