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Original Article

Examining the Activity of Four Disinfectants together with Lactic Acid versus Bacterial Growths on High-touch Surfaces/ objects in a Referral Hospital of Jhelum. A Clinical Dermatology Study with Hospital Lab

Uzma Naeem[1*], Qamar ud Din Khan[2], Rafia Irfan[3]

[1]Department of Pathology, Combined Military Hospital Lahore.
[2]Department of Dermatology, Combined Military Hospital, Lahore.
[2]Department of Microbiology, Combined Military Hospital, Tarbela.

Abstract

Background The contamination by microorganisms on the worktops of hospitals is a potential reservoir for hospital-acquired infections. Disinfectants based on lactic acid have emerged as the safer choice.

Objective The objective of the study was to compare the in vitro activity of a lactic acid +2% sodium lauryl sulfate (4%), distilled water-based disinfectant and other commonly used disinfectants on isolated bacteria.

Methods A descriptive, cross-sectional survey was done in a tertiary-level hospital from April to September 2020. Samples of micro flora of environmental surfaces were collected. The disinfectants were tested by adding 50 µl of test solutions on culture plates, determining the diameter of zones of inhibition. The WHO formula for single-proportion trials calculated power and sample size. At 95% confidence coefficient and accuracy of ±5%, the following sample size was calculated as 363.

Results Out of 363 samples, bacterial contamination was detected on 244 (67.2%) and not detected/ not noted on 119 (32.8%). The prevalence of Gram-positive bacteria (56.1%) was higher than that of Gram-negative bacteria (43.9%). The most prevalent isolates were Staphylococcus aureus (S. aureus) and Pseudomonas aeruginosa (P. aeruginosa) and were highly sensitive to alcohol, phenol and sodium hypochlorite (97%) and equally sensitive to lactic acid (94%). The pattern of susceptibility was also found to be common for all disinfectants to multiple organisms.

Conclusion Lactic acid was found as an equally effective disinfectant as other disinfectants. Lactic acid is safe and can be useful in cleaning and infection control.

Keywords Bacterial contamination; Lactic acid; Disinfectant efficacy.

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Introduction

Biofilms, which are made up of tiny living things, are well known for being hard to get rid of on hospital surfaces like medical equipment and patient areas.[1] Cleaning and disinfecting surfaces well and often is important to lower the number of healthcare-associated infections (HAIs) in both patients and staff. This is important to stop and treat infections.[2] Biofilms can protect some pathogens for days or even months. The degree of infection is influenced by the surface's age, porosity, and moisture content.[3]

Address for correspondence
Dr. Uzma Naeem, Consultant Chemical Pathologist,
Department of Pathology,
Combined Military Hospital Lahore,
Email: uzmacpath61@gmail.com

People use a lot of different disinfectants, such as glutaraldehyde, sodium hypochlorite, chlorhexidine, and phenolics, but there are new concerns about safety and tolerance, that are putting their effectiveness to the test.[4] As a result, people are more interested in agents that are better and different. Lactic acid is a natural organic acid that works very well to kill germs. It could be a good surface cleaner, but hospitals don't use it enough right now.[5]

Methods

A descriptive, cross-sectional survey was carried out in a 500-bed public hospital in Jhelum, spanning April 2020 to September 2020. The ICU, Surgical ward, acute medical unit (AMU), Obstetrics Unit and consultation clinics were considered as high-risk clinical departments. Departmental heads were consulted and areas that were non-clinical e.g. administrative and service areas were excluded from the study.

Sample size was inferred by WHO formula that calculates one-sample proportion tests (Lwanga & Lemeshow).[6] It was established on a feasibility project that showed 67% prevalence of surface contamination. The confidence interval was 95% with precision of ±5%, the sample size was:

$$n = \frac{Z^2 \times p \times (1-p)}{d^2}$$

$$n = \frac{(1.96)^2 \times 0.67 \times 0.33 \approx 340}{(0.05)^2}$$

363 samples were gathered.

Commonly handled environmental and hospital device surfaces were sampled, for example bed guard rails, cardiac electrocardiogram machines, operating room light handles, ventilator breathing circuit systems, anesthetic instruments and surplus furnishings. In each location, a 10 cm² region was sampled using aseptic cotton applicators that had been impregnated in 5 mL of sterile normal saline (0.9% NaCl). The specimens were carried to bacteriology department of laboratory as soon as possible.

Microbiological Procedures: Swabs were streaked on Blood Agar and MacConkey Agar, respectively which are the reliability tools, incubated aerobically for 24 h at 37°C. Isolates were identified as separate bacterial colonies according to standard biochemical procedures based on CLSI (CLSI, 2020) guidelines.

Disinfectant Susceptibility Testing Four disinfectants were tested in test tubes to check whether they did in fact kill bacteria: They were the active of Chloroxylenol (Dettol), lactic acid (4% with 2% sodium lauryl sulfate.), bleach - 0.1% sodium hypochlorite and 70 percent alcohol. Biological testing revealed that the antibiotics were bactericidal. Fields of bacteria were grown from environmental isolates on agar plate. The plate was subsequently coated with disinfectant. The hot liquid agar was then inoculated with the test organism. The disinfectant solution was inoculated into a hole in the agar with a punch. Agar wells were then filled with 50 μL of each disinfectant. In retardation of development by disinfectant and the functional dose was now large enough to inhibit the germ. The concentration of disinfectant in the solutions was the same as that present around the hole. Plates were incubated at 35 degrees C to 37 degrees C for 18 and 24 h. Resistant emerged as separate zones of resistance from the zone of disinfectant surrounding recordings, and susceptible growths within only the treated area.[7]

Data Analysis The data were recorded and analyzed at Microsoft excel which was our validity tool. Frequencies and percentages were designed for determination of the level of contamination and effectiveness. Using the greatest observed inhibitory zone as a reference, the relative efficacy of disinfectants was also represented as a percentage.

There was no human intervention in this study, it was environmental sampling. Thus there were no ethical considerations or conflicts of interest.

Institutional Review Board (IRB) approval was obtained from the hospital ethical committee.

Result

In the environmental sampling, few high-touch surfaces in hospital settings like anaesthesia machines, bed rails, cardiac monitors, sinks, suction machine, linens and others were sampled. Apart from furniture in outpatient department (OPD) ventilator tubing, work station and some routine touch objects like door handles, tables, keyboards, computer mouses, telephones, slit lamps, blood pressure apparatus, medical emergent trolleys, nebulizer machine, stethoscope, hanging drip, cock, OT light handle, autoclave, handle stair railing and other randomly identified surfaces were also sampled. Out of the 363 swabs collected, 244 (67.2%) showed bacterial growth and 119 (32.8%) did not. Of the 244 isolates, 137 (56.1%) were Gram-positive and 107 (43.9%) were Gram-negative. The most prevalent Gram-positive bacterial species were Staphylococcus aureus (97 isolates, 70.8%), followed by Bacillus spp. and Enterococcus spp. were the least common. Pseudomonas aeruginosa (57, 53.2%) was the most commonly isolated Gram-negative organism and chrysobacterium was least found.

Distribution survey revealed that S. aureus was predominantly present on bedrails of hospital followed by OPD, inpatients wards, labor rooms, emergency room. P. aeruginosa was predominantly detected in suction devices and the most frequent in operating rooms (OT) and ICUs.

Susceptibility tests with disinfectants showed that S. aureus was most susceptible (97%) to sodium hypochlorite, phenol, and 70% alcohol, followed closely by acetic acid (94%; 4% plus 2% sodium lauryl sulfate in distilled water). P. aeruginosa was moderately sensitive to sodium hypochlorite (57%) and lactic acid (57%) whereas, least susceptible to alcohol (55%). The sensitivity of other microorganisms was similar for all disinfectants tested, with the exception of coagulase-negative.

Discussion

This study involved the collection of 363 swabs from high-contact hospital environments, including emergency departments, intensive care units, operating theatres, and outpatient departments. Among these, 244 (67.2%) exhibited bacterial contamination, aligning with prior studies indicating that inanimate hospital surfaces act as reservoirs for healthcare-associated pathogens.[8] High-touch areas, including bed rails, suction machines, and ventilator tubing, exhibited significant contamination, consistent with findings from Nigeria and India, where analogous surfaces were associated with bacterial persistence.[8]

Of the 244 bacterial isolates, Gram-positive organisms were predominant, accounting for 56.1%, with Staphylococcus aureus identified as the most prevalent isolate at 70.8%. This is consistent with the findings of Saka *et al.* who identified S. aureus as the predominant contaminant on aluminum and near-patient surfaces.[8] No MRSA was detected in our setting, in contrast to the findings of Montagna *et al.* where 20 out of 40 S. aureus strains were methicillin-resistant.[9] Gram-negative bacteria constituted 43.8% of isolates, with Pseudomonas aeruginosa identified as the most prevalent, especially from suction machines (53.2%). Similar findings have been observed in European hospitals, where Pseudomonas is frequently associated with water-related equipment.[7] In our study, the less frequently isolated organisms were Chrysobacterium spp. (0.9%), Enterococcus spp. (4.3%), and Bacillus spp. (4.3%).

The disinfectant susceptibility profile indicated that S. aureus exhibited high sensitivity (>94%) to all four tested disinfectants: sodium hypochlorite, phenol, alcohol, and lactic acid. This supports the findings of Prakasam *et al.* who reported significant efficacy of standard hospital disinfectants against Gram-positive cocci.[10] Pseudomonas aeruginosa

exhibited low sensitivity (approximately 3%) to all disinfectants, underscoring its established intrinsic resistance mechanisms.[8]

This is the first time that lactic acid has been proved to destroy germs. As previously documented, lactic acid and surfactants are effective against Gram-negative bacteria and multidrug-resistant Klebsiella pneumoniae biofilms (Boomsma *et al.* 2018),[11] and Bardhan *et al.* who validated its effectiveness against MDR Klebsiella pneumoniae biofilms.[12] Staphylococcus aureus exhibited the maximum sensitivity at 94%, in contrast to Enterobacter, which showed only 3%. Asha and Bhalla discovered that lactic acid was efficacious against resistant strains of Pseudomonas and Staphylococcus aureus.[13] Abriouel *et al*. verified that lactic acid-based disinfectants may eradicate Pseudomonas and Acinetobacter in healthcare settings.[14] Montagna *et al*. discovered that sodium hypochlorite was ineffective against nosocomial bacteria,[9] but our data shows that alcohol and sodium hypochlorite did work. The differences we detect could be because of the types and amounts of disinfectants used or the fact that microbes are resistant to them in different regions. In one of the study by Ejimofor *et al.* in Nigeria, effectiveness of some frequently used disinfectants was tested only against Escherichia coli (E.coli) and S. aureus. The findings revealed that bleach had a stronger impact on S.aureus than E. coli, while ethanol showed the least susceptibility. Phenolics had the highest efficiency against both test species.[15]

Table 1 The frequency of isolated bacteria.

Isolates	N= 244 (67.2%)
Gram negative bacteria	107(43.8%)
Acinetobacter baumannii	16(14.9%)
Pseudomonas aeruginosa	57(53.2%)
Proteus	16(14.9%)
Citrobacter	7(6.5%)
Enterobacter species (spp)	3(2.8%)
Klebsiella oxytoca	7(6.5%)
Chrysobacterium	1(0.9%)
Gram positive bacteria	N=137(56.1%)
Staphylococcus aureus	97(70.8%)
CoNS	28(20.4%)
Bacillus spp	6(4.3%)
Enterococcus (spp)	6(4.3%)

Table 2 Distribution of bacteria on different hospital and environmental surfaces.

Sampling stations	Bacteria, *n* (%)										
	CoNS	*Bacillus spp*	*Enterococcus spp*	*Proteus*	*A. baumannii*	*P. aeruginosa*	*Klebsiella spp*	*Enterob-acter spp*	*Citrob-acter spp*	*Chrysob-acterium spp*	*S.aureus*
Anesthesia machine	2(7.1%)	1(16.6%)	0	0	3(18.7%)	1(1.7%)	1(14.2%)	0(0)	0	0(0)	5(5.1%)
Bed rails	4(14.2%)	1(16.6%)	1(16.6%)	1(6.2%)	1(6.2%)	5(8.7%)	2(28.5%)	0	2(28.5%)	0(0)	18(18.5%)
Environment-ental surfaces*	6(21%)	2(33.3%)	2(33.3%)	2(12.5%)	1(6.2%)	9(15.7%)	1(14.2%)	1(33.3%)	0	0(0)	11(11.3%)
Cardiac Monitor	1(3.5%)	1(16.6%)	0(0)	2(12.5%)	1(6.2%)	4(7.0%)	1(14.2%)	0(0)	0	0(0)	8(8.2%)
Sink	1(3.5%)	0	0(0)	1(6.2%)	0	8(14%)	0	0(0)	0	0(0)	7(7.2%)
Suction Machine	1(3.5%)	0	0(0)	2(12.5%)	4(25%)	13(22.8%)	1(14.2%)	0(0)	1(14.2%)	0(0)	13(1.4%)
Linens	3(10.7%)	0	1(16.6%)	2(12.5%)	1(6.2%)	4(7.0%)	1(14.2%)	1(33.3%)	2(28.5%)	0(0)	8(8.2%)
Furniture	5(17.8%)	0	0(0)	3(18.7%)	0	5(8.7%)	0	0	1(14.2%)	0	9(9.2%)
Ventilator tubings	2(7.1%)	1(16.6%)	0(0)	0	5(31.2%)	3(5.2%)	0	0	1(14.2%)	0	4(4.1%)
Work station	1(3.5%)	1(16.6%)	2(33.3%)	2(12.5%)	0	2(3.5%)	0	1(33.3%)	0(0)	0	6(6.1%)
Miscellaneous	2(7.1%)	0	0(0)	1(6.2%)	0	3(5.2%)	0	0(0)	0(0)	1(100%)	8(8.2%)
Total	**28 (20.4%)**	6	6	16	16	57	7	3	7	1	97

Table 3 Distribution of isolates among different units of hospital.

Bacteria	*ICU %*	*OT%*	*Emergency%*	*Labour Room%*	*Inpatient Block%*	*Outpatient Block%*	*Total*
S. aureus	16	6	13	9	22	31	97
CoNS	8	2	4	5	6	3	28
Bacillus spp	2	2	1	0	1	0	6
Enterococcus spp	3	0	0	1	2	0	6
A. baumannii	11	5	0	0	1	0	16
P. aeruginosa	20	7	11	8	7	4	57
Klebsiella spp	4	1	2	0	0	0	7
Proteus	5	2	2	3	4	0	16
Citrobacter	2	1	1	0	3	0	7
Enterobacter spp	0	0	0	2	1	0	3
Chrysobacterium	1	0	0	0	0	0	1

The study has the similarity with our study in using the common disinfectants and their efficacy against the two bacteria i.e. S.Aureus and E.Coli. The European Chemicals Agency (ECHA) has also approved disinfectants similar to disinfectants used in our study including Lactic acid product type human hygiene.[16] In one of the studies by Fang Liu and associates they analyzed the effects of 3-Phenyllactic acid (PLA) found previously as an antimicrobial compound. They used phenyl lactic acid as disinfectant agent for killing Listeria monocytogenes biofilms and collated with the microbicidal action of lactic acid (LA), and levulinic acid (LVA) with sodium dodecyl sulfate (SDS). It was inferred that 3% PLA and 1% PLA effectively killed the immidiate developed biofilm within a 5-min treatment, while 3% PLA was found to be superior as compared with all others agents, including 1% PLA, 3% LA, 3% LVA and 2% SDS for inactivation of the late mature biofilm after a 5-min treatment.[17] In our study we compared Lactic acid with Sodium hypochlorite, Phenol and Alcohol and lactic acid was found to be equivalent in disinfecting with other disinfectants. In a study by Zhicheng Cai *et al*. they used plasma-activated lactic acid (PALA) to stop the growth of the P. aeruginosa biofilm. PALA halted the proliferation of P. aeruginosa by quickly raising reactive oxygen species (ROS) and free radical skulking enzymes which cannot remove the accumulated ROS at one time leading to decomposition of bacteria.[18] The similarity to our study was the use of Lactic acid to check its effectiveness against P. aeruginosa whereas we checked the efficacy of Lactic acid against other bacteria as well.

Conclusion

Lactic acid showed the same effectiveness as disinfectants that are already in use. It is a safe and cheap way to kill germs. Because it is safe to eat, lactic acid is often used in the food industry as an acidifier, taste enhancer, and preservative. Lactic acid works against a number of bacteria and viruses, including the coronavirus, which is an enveloped virus. It can work better when mixed with other things, such as surfactants, in formulations. In light of emerging resistance to many commonly used disinfectants further broader studies are required to establish Lactic acid as a promising disinfectant agent in future.

Declaration of patient consent Authors certify that they have obtained all appropriate patient consent.

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Conflict of interest No conflict of interest.

Author's contribution

UN: Substantial contribution to conception and study design, acquisition of data and manuscript writing.

QUDK: Substantial contribution to conception and study design, analysis and interpretation of data and revising the manuscript critically.

RI: Substantial contribution to analysis and interpretation of data, drafting the manuscript and revised the manuscript critically.

Every author has given final approval of the manuscript version to be published and agreed to be accountable for all aspects of the work.

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