

## Original Article

# Evaluation of bacterial contamination of dental unit water lines before and after the use of 1% (Lysoformin 3000) disinfectant and awareness of the dental unit waterline disinfection among the practicing dental surgeons of Vadodara city

### ABSTRACT

**Purpose:** The aim is to evaluate the bacterial contamination in dental unit waterlines (DUWLs) before and after the use of the disinfectant 1% (Lysoformin 3000) for its efficacy also assess the awareness about the same among the practicing dental surgeons of Vadodara city.

**Methodology:** A volume of 20 ml water samples from the DUWLs from the clinics of the practicing dental surgeons of Vadodara city was collected. And were asked to answer a validated questionnaire in private. Disinfection of the waterlines was performed twice a week for 2 weeks with 1% disinfectant (Lysoformin 3000) and the samples were again collected. Qualitative and quantitative microbiological analysis was performed for both the predisinfected and postdisinfected samples.

**Results:** Predisinfection samples showed a mean colony count of 670.35 colony forming unit (CFU)/ml which was reduced to 63.90 CFU/ml ( $P < 0.001$ ) postdisinfection. Questionnaire assessment showed that 70% awareness of the participants about DUWL infections and the microorganisms associated. However, only 20% practiced any type of DUWL disinfection.

**Conclusion:** Lysoformin 3000 can be promising to be used a DUWL disinfection solution as an easy to use and readily available solution with great antibacterial properties. However, still more long-term studies are required to check for its efficacy in anaerobic bacteria as well as resistant species of microorganisms.

**Keywords:** 1% Lysoformin 3000, biofilm, dental unit waterline

### INTRODUCTION

Biofilm contamination in dental unit waterlines (DUWLs) remains a considerable problem in dentistry's as the reported bacteria levels in water from DUWLs can often go over the limit exceeding 100,000 colony forming units (CFUs)/ml of water, over 200 times the amount of allowable bacteria in potable water.<sup>[1]</sup> DUWLs in dentistry are used to cool and irrigate tooth surfaces during treatment as well as dental handpieces.<sup>[2]</sup> They includes narrow-bore, plastic tubing, which are highly susceptible to biofilm contamination due to reduced velocity of water at the periphery of the tube.<sup>[2]</sup> The Environmental Protection Agency, American Public Health Association, and American Water Works Association have all set limits for heterotrophic bacteria of  $<500$  CFU/ml.<sup>[3]</sup>

The Centers for Disease Control and Prevention published "Guidelines for infection control in dental health care settings" in 2003, and highlighted that oral *Streptococcus* species, human pathogens such as *Pseudomonas aeruginosa*

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and *Legionella* species, and nontuberculosis *Mycobacterium* species had all been isolated from dental water systems.<sup>[3-5]</sup> In numerous studies, cross contamination between patients has been reported.<sup>[6-8]</sup> Suck-back of oral fluids from a patient, either from protective anti-retraction valve failure, or when handpieces are removed and replaced incorrectly have been the reason for this contamination.<sup>[7,8]</sup> The most efficient means of maintaining good quality dental chair unit (DCU) output water is regular disinfection of DUWLs with a disinfectant or biocide that removes biofilm from the water lines resulting in output water of potable quality.<sup>[9]</sup> A wide variety of commercial waterline cleaning products and systems are available, some of which can be retrofitted to existing DCUs. One such disinfecting agent is Lysoformin 3000, which has an excellent bactericidal and bacteriostatic activity. It is used in various concentrations ranging from 1% to 1.5%. Among which 1% disinfectant (Lysoformin 3000) is a good antiseptic that has been used for disinfection of medical apparatus since a long time and can be used as a DUWL disinfectant. However, very few studies published in the peer-reviewed literature have actually investigated the efficacy of this disinfectant to achieve these desired effects in DCUs and detailed comparative studies have yet to be published.<sup>[9]</sup> Hence, there is a need to study the efficacy of this disinfectant in reducing the microbial load in DUWL. Furthermore, there is an onus on DCU manufacturers to consider the problem of DUWL biofilm contamination when designing DCUs. Hence the aim the study is to evaluate the awareness of the practicing regarding disinfection of DUWLs and to check the efficacy of 1% disinfectant (Lysoformin 3000) in disinfection of DUWLs. The null hypothesis of the study was that there will be no reduction in the load of microorganisms in the DUWL after the use of 1% (Lysoformin 3000) disinfectant.

## METHODOLOGY

Twenty practicing dental surgeons practicing in Vadodara city were selected for the study by lottery method from the Indian Dental Association directory. Dental surgeons having their private practice in the city and consenting for the study were included in the study. All the participants were asked to fill a questionnaire that was content validated and subject validated at 86% (Chi-square test) in private revealing their awareness regarding DUWL disinfection. Twenty milliliters pre-disinfection water samples were then collected in sterile plastic containers (ABDOS P40102) from the three-way syringe of the dental chair. Moreover, the subjected to microbiological evaluation. One percent disinfectant (Lysoformin 3000) was then added to bottle booster system in the dental chair and passed through the waterline into the three-way syringe till a blue colored liquid was seen. It was then allowed to stay in the waterline overnight and then washed away from the

waterline and flushed by distilled water. This procedure was repeated twice a week for 15 days. Then, postdisinfection water samples were collected and also subjected to microbiological analysis.

## Microbiological evaluation

Water samples were then centrifuged at 3000 rpm and the supernatant was discarded, and the centrifuged sample was cultured in a MacConkeys agar without crystal violet (high media) at 33°C for 48 h followed by colony counting. Bacterial identification kit was used for the identification of the bacteria present in the culture.

This data were then subjected to statistical analysis.

## RESULTS

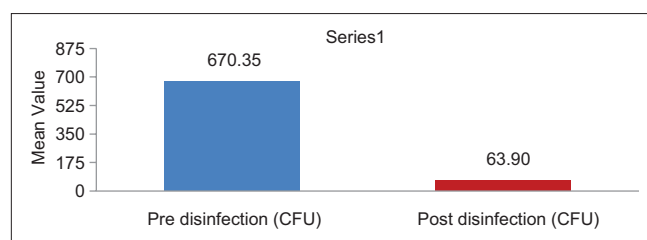
Paired sample *t*-test showed a mean colony count of 670.35 CFU/ml before disinfection which was reduced to the mean of 63.90 CFU/ml ( $P < 0.001$ ) [Table 1 and Graph 1] which was highly statistically significant. Bacterial identification confirmed the presence of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *P. aeruginosa* and postdisinfection left only the traces of *E. coli* and *S. aureus*.

Questionnaire assessment [Table 2] of the validated questionnaire 86% (Chi-square test) showed that 70% of the participants were aware about DUWL infections and the microorganisms associated. However, only 40% knew the evidence-based methods and products regarding DUWL disinfection, whereas only 20% practiced any type of DUWL disinfection protocol regularly and sodium hypochlorite was the material of choice for all.

**Table 1: Paired *t*-test showing mean colony forming unit count pre- and post-disinfection of dental unit waterlines**

	Mean	n	SD	SEM	Mean difference	P
Predisinfection (CFU)	670.35	20	207.31	46.357	-606.45	<0.001
Postdisinfection (CFU)	63.90	20	35.80	8.006		

SD - Standard deviation, SEM - Standard error of mean, CFU - Colony forming units



**Graph 1: Mean colony forming unit count pre- and post-disinfection of dental unit water lines**

**Table 2: Validated questionnaire showing responses of the participants regarding dental unit waterlines disinfection**

Questions	Yes (%)	No (%)
Do you know what is a biofilm?	100	-
Are you aware of the biofilms forming in DUWL?	70	30
Are you aware about the DUWL disinfection?	70	30
Are you informed about the limits set by the CDC, APHA, and AWWA for the heterotrophic bacteria in DUWL?	60	40
Do you know the bacteria associated with the biofilm formed in DUWL?	70	30
Are you aware about the diseases/infections that can be caused by the presence of these bacteria?	70	30
If yes, have you encountered any such infection in any of your patients?	-	100
Do you know the methods for the DUWL disinfection?	40	60
Do you know the disinfectants for the DUWL disinfection?	40	60
Is any method of DUWL disinfection practiced at your dental office?	20	80
Do you perform DUWL disinfection regularly?	20	80

DUWL - Dental unit water lines, CDC - Centers for Disease Control and Prevention, APHA - American Public Health Association, AWWA - American Water Works Association

## DISCUSSION

The result of this study showed a statistically significant reduction in the number of CFUs postdisinfection with the help of 1% disinfectant (Lysoformin 3000). Such major differences in the results obtained are undoubtedly related to the composition of Lysoformin 3000, where, although the main active substance is glutaraldehyde, there are also other compounds considered disinfectants, such as didecyl dimethyl ammonium chloride, isotridecanoethoxylated, and glyoxal.<sup>[10]</sup> Due to the drawbacks and limitations in disinfection of general disinfectants, it is believed that multi-component disinfectants are the optimal solution, as they combine the action of all the ingredients and enhance the properties.<sup>[10]</sup> The combination of glutaraldehyde activity due to the alkaline reactions and the quaternary ammonium compounds in Lysoformin 3000 mark for its antibacterial as well as antifungal effectivity.<sup>[11]</sup> Likewise, studies have reported other disinfectants such as Tegodor and Gigasept Rapid (aldehyde based) achieved a 100% reduction in the biofilm viability but did not remove the biofilm adhered to the tubing surface. This phenomenon is mostly associated with alkaline reactions of glutaraldehyde that are responsible for cell wall lysis of the bacteria rendering the compound to be more effective.<sup>[11]</sup>

The presence of quaternary ammonium compounds summated the efficacy of the disinfectant (Lysoformin 3000). The high efficacy of disinfectant substances tested containing quaternary ammonium salts (QAT) corresponded with results of antibacterial activity of homologous series of alkyl trimethyl ammonium bromides and dialkyl dimethyl ammonium

bromides against the clinical *Salmonella typhimurium* and *P. aeruginosa* strains,<sup>[12]</sup> and one important factor of QAT efficacy was found the length of the alkyl chain.<sup>[12,13]</sup> Similar results were obtained with *E. coli* strains.<sup>[14]</sup> QAT belong to the membrane active compounds binding to the cytoplasmic membrane and influencing cell metabolism.<sup>[15]</sup> Quaternary ammonium compounds gained popularity due to its good antibacterial and antifungal at relatively low concentration within shorter contact time.<sup>[16]</sup> Moreover, the blue color of the solution and a typical odor makes it easy, comfortable and safe for usage as well as cleaning.<sup>[17]</sup>

However, there has been a varied range of disinfection modalities right from the periodic replacement of the waterlines or autoclaving of the waterline units, anti-retraction valves, etc. However, these seem to bit more tedious and non-user-friendly in developing country like ours. Hence, the disinfecting solutions and tablets can make a very comfortable and effective choice for DUWL disinfection for the dental offices in India. Lysoformin 3000, is highly effective against both Gram-positive and Gram-negative bacteria at all concentrations and contact time tested the aldehyde-based disinfectant (Lysoformin 3000). These findings are similar to another study, which showed that 2% Lysoformin 3000 at 30 min was enough for bactericidal and fungicidal activity.<sup>[18]</sup> Lysoformin 3000 was found to be effective against bacterial spores as reported.<sup>[18,19]</sup>

Staniszewska *et al.* stated that glutaraldehyde in the concentration of 1%–2% reduces *Bacillus cereus* spores within 15–30 min by 4 orders of magnitude. The results of the study carried out were different, and demonstrated that Lysoformin 3000 with glutaraldehyde as its main active substance reduces totally the spores of all three strains, namely *B. subtilis* combine the active component with components that have washing properties or are emulgators.<sup>[20]</sup> *Bacillus mycoides*, and *B. cereus* already after 5-min exposure. Hence, 1% disinfectant (Lysoformin 3000) was used for the disinfection.<sup>[21]</sup>

This was followed twice weekly for 15 days. The disinfection protocol was designed accordingly because, In a controlled study, twice a week overnight (15 h) disinfection using either agent reduced the bacterial density to below the American Dental Association recommended the level of 200 CFU/ml.<sup>[9]</sup>

Questionnaire assessment showed that 70% to the participants were aware about DUWL infections and the microorganisms associated. However, only 40% knew the evidence-based methods and products regarding DUWL disinfection, while only 20% practiced any DUWL disinfection protocol regularly and sodium hypochlorite was the material of choice for all.

Hence, the need of the hour is a readily available, easy to use, noncaustic disinfectant that will be effective against a wide range of microorganisms associated with the hospital-acquired infection in a dental office. Lysoformin 3000 can very well be the hero to cometh the moment. However, still more long-term studies are required to check for its efficacy in anaerobic bacteria as well as resistant species of microorganisms.

However, a greater sample size would have given us a better judgment regarding the contamination of DUWL and the awareness about the same among the fellow practicing dental surgeons. A longer and continuous use of disinfectant 1% (Lysoformin 3000) would have enlightened us regarding the long-term efficacy of the disinfectant.

As medical professionals 0% infection is our goal. Hence, fire must be ignited always. Quest for the best must not end.

## CONCLUSION

Within the limitations of this study, 1% disinfectant (Lysoformin 3000) can be promising to be used as a DUWL disinfection solution as an easy to use and readily available solution with great antibacterial properties. However, more long-term studies are required to check for its efficacy in anaerobic bacteria as well as resistant species of microorganisms. Furthermore, there seems to be a need to educate and guide regarding the DUWL disinfection in practicing dentists as well as at the undergraduate and postgraduate levels.

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## Conflicts of interest

There are no conflicts of interest.

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