



COMPARITIVE ANALYSIS OF ACETIC ACID AND CHLORHEXIDINE DRESSINGS ON WOUNDS INFECTED WITH PSEUDOMONAS AERUGINOSA

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ABSTRACT

Objective: To evaluate the effect of chlorhexidine dressing in comparison with acetic acid dressing in the treatment of wounds infected with *Pseudomonas aeruginosa*.

Materials and Methods: Sixty eight patients diagnosed of Grade 1 or Grade 2 ulcer, positive for *P. aeruginosa* were grouped and treated with 1% acetic acid and 0.05% chlorhexidine group as daily dressing material. Wound swab was collected on days 0 and day 7. Bacterial load on both the days were compared and scored as no growth, mild growth, moderate growth and severe growth based on gram staining, number colonies on culture plate.

Results: In acetic acid treatment group, majority of the patients who had mild growth of bacterial load on day 0 (80%) had no growth on day 7, followed by 72.8% who had severe growth on day 0 had no growth and mild growth (36.4% each). Whereas in chlorhexidine group, there was no growth of *P. aeruginosa* as observed in 23 patients on day 0. Overall, the efficacy of topical application of chlorhexidine for its antiseptic activity was comparable with acetic acid in wounds infected with *P. aeruginosa* in diabetes mellitus, Peripheral Vascular Occlusive Disease (PVOD), varicose vein and hypertension cases.

Conclusion: In conclusion, the results of the present study depict that topical application of chlorhexidine on *P. aeruginosa* Infected wounds was at par with acetic acid in terms of its antiseptic activity.

KEYWORDS

Acetic acid, Chlorhexidine, *Pseudomonas aeruginosa*, Infected wound, Antiseptic

INTRODUCTION

Wounds have since long, contributed majority to the health-care burden and is a break in the integrity of the skin or tissues. Indian studies on the epidemiology of chronic wounds have estimated the prevalence at 4.5 per 1000 population. The incidence of acute wounds was more than double at 10.5 per 1000 population.^[1] *Pseudomonas* is one commonly found organism in infected wounds, known to form biofilm and produce various virulence factors leading to its persistence in the wound, continued infection and hence increasing morbidity despite the use of efficient antimicrobials. For the treatment of *Pseudomonas aeruginosa* locally applied acetic acid has now become a standard and practiced frequently. However, in our wards, we found patients not responding to acetic acid.

Aims and objectives

To evaluate the effect of chlorhexidine dressing in comparison with acetic acid dressing in the treatment of wounds infected with *P. aeruginosa*.

MATERIALS AND METHODS

Patients

We conducted a hospital based clinical prospective study over a period of one and a half years. Patients admitted and diagnosed to have Grade 1 or Grade 2 ulcer according to Wagners classification and positive for *P. aeruginosa* at Justice K S Hegde Hospital, Deralakatte. All patients were explained the procedure and signed the written informed consent forms prior to their entry in to the study. Sixty-eight patients between 25 and 75 years of age with Grade 1 or Grade 2 ulcer according to Wagners classification wound positive for *P. aeruginosa* were included in the study. Patients already included in study and came back with reinfection and patients with abscess and osteomyelitis were excluded.

Study design and medication

A total of 68 patients were randomly distributed into acetic acid (n=34) and chlorhexidine group (n=34). Patients in the acetic acid and chlorhexidine groups were treated daily with dressings of 1% acetic acid and 0.05% chlorhexidine dressings respectively. Lesions were swabbed on days 1 and 7, based on gram staining and culture was scored as 0 = no growth, 1 = minimal growth, 2 = moderate growth and 3 = severe growth. End point of the study was considered after 7 days of treatment. Pretested semi structure was used for data collection and variables for conditions that lead to ulcer like diabetes mellitus, PVOD, varicose vein, hypertension was noted.

Statistical analysis

The data collected has been subjected to statistical analysis by using SPSS (statistical package or social sciences) version 16.0. Descriptive statistics was used to calculate percentage, mean and standard deviation. Nominal categorical data between the groups were compared using chi-square goodness-to-fit test. Unpaired or independent t-test was used for comparison of mean value between 2 groups when the data follows normal distribution. Chi-square test was used to investigate whether distributions of categorical variables differ from one another. The p-value less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

Sixty-eight patients were divided into two groups, i.e. 34 patients in acetic acid and 34 patients in chlorhexidine group [Table 1].

Table 1: Distribution of Patients in Acetic Acid and Chlorhexidine Groups

Groups	N	Mean	S. D
Acetic acid	34	58.71	14.169
Chlorhexidine	34	55.79	14.799

In the present study, out of 68 patients, majority were male (77.9%) and only 22.1% were female patients. In acetic acid group, 73.5% male and 26.5% female patients were present. Similarly, 82.4% of male patients and 17.6% of female patients were present in chlorhexidine group [Table 2 and Figure 1].

Table 2: Gender Wise Distribution of Patients in Acetic Acid and Chlorhexidine Groups

Sex	Groups		Total	p value
	Acetic acid	Chlorhexidine		
Male	25	28	53	0.380
	73.50%	82.40%	77.90%	
Female	09	06	15	
	26.50%	17.60%	22.10%	
Total	34	34	68	
	100.00%	100.00%	100.00%	

Figure 1: Gender Wise Distribution of Patients in Acetic Acid and Chlorhexidine Groups

Out of 68 patients, majority of them were in the range of age group between 55-65 years (33.8%), followed by 45-55 years (25%),

followed by 65-75 years (20.6%), followed by >75 years (7.4%) and only 2% were in the range of age group 25-35 years [Table 3 and Figure 2].

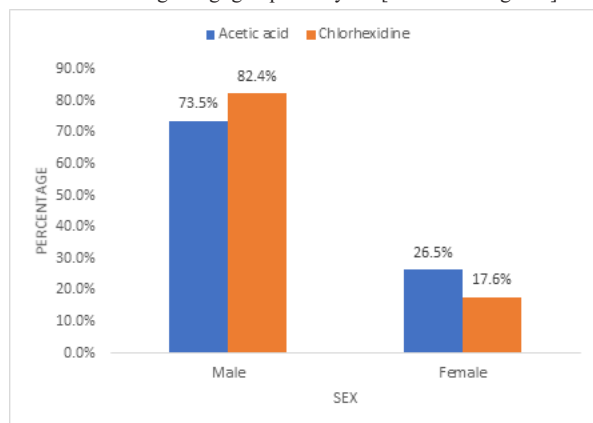


Table 3: Age Wise Distribution of Patients in Acetic Acid and Chlorhexidine Groups

Age (in years)	Groups		Total	p value
	Acetic acid	Chlorhexidine		
16-25	01	03	04	0.687
	02.90%	08.80%	05.90%	
25-35	02	00	02	0.687
	05.90%	00.00%	02.90%	
35-45	01	02	03	0.687
	02.90%	05.90%	04.40%	
45-55	08	09	17	0.687
	23.50%	26.50%	25.00%	
55-65	11	12	23	0.687
	32.40%	35.30%	33.80%	
65-75	08	06	14	0.687
	23.50%	17.60%	20.60%	
>75	03	02	05	0.687
	08.80%	05.90%	07.40%	
Total	34	34	68	0.687
	100.00%	100.00%	100.00%	

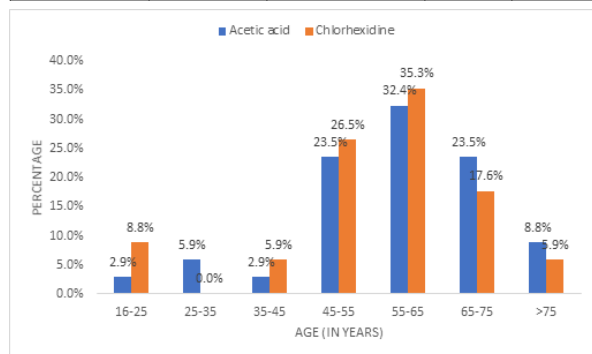


Figure 2: Age Wise Distribution of Patients in Acetic Acid and Chlorhexidine Groups

Out of 68 patients, majority were known case of diabetes 48 (70.6%) and only 20 (29.4%) of patients were non-diabetic. Chi-square test showed that there was no significant association found between the diabetes and groups [Table 4].

Table 4: Distribution of Patients in Acetic Acid and Chlorhexidine Groups According to Diabetes

Diabetic	Groups		Total	p value
	Acetic acid	Chlorhexidine		
Yes	24	24	48	1.000
	70.60%	70.60%	70.60%	
No	10	10	20	1.000
	29.40%	29.40%	29.40%	
Total	34	34	68	
	100.00%	100.00%	100.00%	

In acetic acid group, reduction of bacterial load from mild to no growth was seen in 12 patients and 3 patients had no change in bacterial load. In patients with moderate growth reduction was noted in 05 patients and 02 had no change and in 01 patient bacterial load had increased to severe growth. In 11 patients with severe growth on day 0 reduction in growth was noted in 9 patients and 2 patients had no change. 26 (76%) patients out 34 showed reduction in bacterial load. However, Chi-square test showed that there was no statistically significant reduction in bacterial load on usage of acetic acid ($p = 0.092$) [Table 5].

Table 5: Comparison of Bacterial Load Between Day 0 and Day 7 in Acetic Group

Bacterial load D0	Bacterial load D7				Total	p value
	No Growth	Mild Growth	Moderate Growth	Severe Growth		
Mild Growth	12	03	00	00	15	0.092
	80.00%	20.00%	00.00%	00.00%	100.00%	
Moderate Growth	02	03	02	01	08	0.092
	25.00%	37.50%	25.00%	12.50%	100.00%	
Severe Growth	04	04	01	02	11	0.092
	36.40%	36.40%	9.10%	18.20%	100.00%	
Total	18	10	03	03	34	
	52.90%	29.40%	08.80%	08.80%	100.00%	

In Chlorhexidine group, patients with mild growth on day 0, reduction in bacterial growth was seen in 15 patients and 05 patients had increased growth. In 9 patients with moderate growth on day 0, 08 patients had reduction in bacterial growth and 01 patient had increased bacterial growth. Similarly, in 05 patients with severe growth on day 0, 04 had reduction in bacterial growth. Chi-square test showed that reduction in bacterial growth was not statistically significant with use of chlorhexidine ($p = 0.452$). However, 27 patients out of 34 showed reduction in bacterial load. Out of 24 diabetics who underwent acetic acid dressing, 16 (67%) patients had reduction and 08 (33%) had no reduction in bacterial load [Table 6].

Table 6: Comparison of Bacterial Load Between Day 0 and Day 7 in Chlorhexidine Group

Bacterial load D0	Bacterial load D7				Total	p value
	No Growth	Mild Growth	Moderate Growth	Severe Growth		
Mild Growth	15	03	02	00	20	0.452
	75.00%	15.00%	10.00%	00.00%	100.00%	
Moderate Growth	06	02	00	01	09	0.452
	66.70%	22.20%	00.00%	11.10%	100.00%	
Severe Growth	02	01	01	01	05	0.452
	40.00%	20.00%	20.00%	20.00%	100.00%	
Total	23	06	03	02	34	
	67.60%	17.60%	08.80%	05.90%	100.00%	

Among nondiabetics, 100% had reduction in bacterial load and in diabetic subjects around 67% and 33% had reduction and no reduction in bacterial load in acetic acid treated group. [Table 7].

Table 7: Comparison of Reduction in Bacterial Load in Acetic Acid Group – Diabetic Cases

Diabetic	Reduction in bacterial load		Total	p value
	Yes	No		
Yes	16	08	24	0.037
	66.70%	33.30%	100.00%	
No	10	00	10	0.037
	100.00%	00.00%	100.00%	
Total	26	08	34	
	76.50%	23.50%	100.00%	

Twenty-one (88%) patients with diabetes had reduction in bacterial load and 07 (70%) patients in nondiabetic group had reduction in bacterial load. Even though reduction is not statistically significant 82% patients benefitted from use of chlorhexidine [Table 8].

Table 8: Comparison of Reduction in Bacterial Load in Chlorhexidine Group – Diabetic Cases

Diabetic	Reduction in bacterial load		Total	p value
	Yes	No		
Yes	21	03	24	0.223
	87.50%	12.50%	100.00%	

No	07	03	10	
	70.00%	30.00%	100.00%	
Total	28	06	34	
	82.40%	17.60%	100.00%	

Among the study subjects with PVOD, all of them who had acetic acid dressing had reduction in bacterial load [Figure 3].

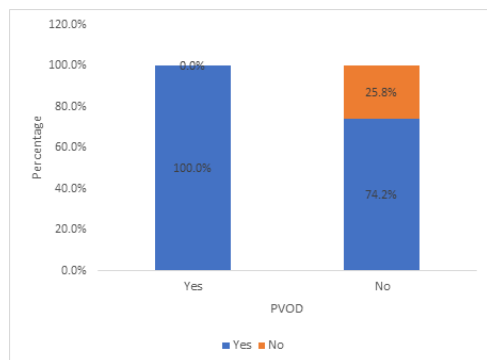


Figure 3: Cross Tabulation of PVOD Cases and Reduction in Bacterial Load in Acetic Group

Among the study subjects with PVOD treated with chlorohexidine, all of them had reduction in bacterial load and among the subjects with no PVOD 81.3% had reduction in bacterial load. [Figure 4].

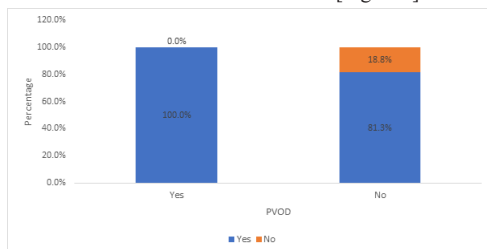


Figure 4: Cross Tabulation of PVOD Cases and Reduction in Bacterial Load in Chlorhexidine Group

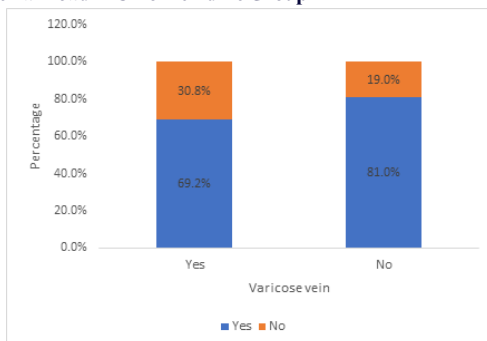


Figure 5: Cross Tabulation of Varicose Vein and Reduction in Bacterial Load in Acetic Acid Group

Among acetic acid group subjects 69.2% and 81% had reduction in bacterial load in patients with and without varicose vein respectively on day 7. [Figure 5]. Whereas, in chlorhexidine group 90% and 79.2% of study subjects had reduction in bacterial load on day 7 with and without varicose vein respectively. [Figure 6].

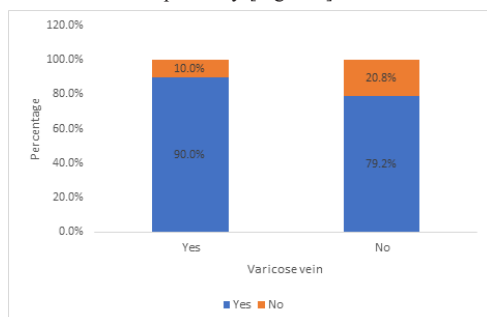


Figure 6: Varicose vein and reduction in bacterial load in Chlorhexidine Group

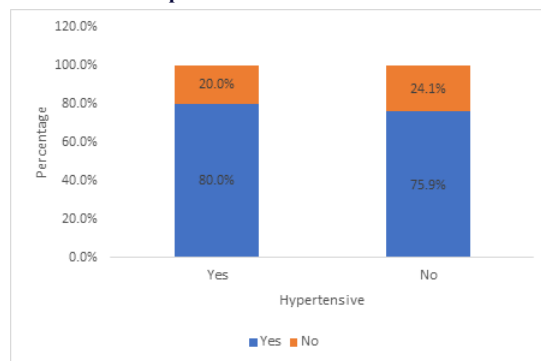


Figure 7: Reduction in Bacterial Load Among Hypertensives in Acetic Acid Group

There was reduction in bacterial load in 80% and 75.9% of hypertensives and non-hypertensives on day 7 respectively in acetic acid group [Figure 7]. Whereas, in Chlorhexidine group there was reduction in bacterial load in 85.7% and 81.5% of hypertension and non-hypertensives respectively [Figure 8].

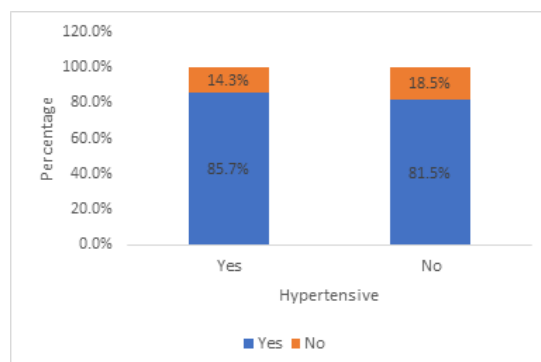


Figure 8: Reduction in Bacterial Load Among Hypertensives in Chlorhexidine Group

DISCUSSION

Pseudomonas aeruginosa, in recent years have gained significant importance as a main source of hospital-acquired infections due to its survival capability in the hospital environment and also its capability in developing resistance to anti-microbial agents. In the hospital environment it can be found everywhere and from various clinical specimens it is the most frequently isolated non-fermentative bacillus. Infections in burn wound, skin and soft tissue are significantly caused by these bacteria. Therefore, present study was designed to assess efficacy of 1% acetic acid in comparison to the chlorhexidine dressings for treating wounds infected by *P. aeruginosa*.

An increased frequency of strains resistant to a number of antimicrobial agents have been evidenced in recent years.^[2,3] Eliminating this nosocomial pathogen from the site of infection is most difficult in spite of continuously introducing powerful antipseudomonal agents. The pseudomonal infection therapy is seriously hampered due to growing resistance of *P. aeruginosa* to anti-microbial agents. In burn units, there is very high incidence of such multiple drug resistant isolates. New antimicrobial agent's emergence in the recent times has aid to decrease the serious effects of many infection types but less satisfying results have been found in the case of nosocomial infections caused by *P. aeruginosa* and are still a serious issue. It is very difficult to treat the infections of burn wound and infections of skin and soft tissues caused by *P. aeruginosa*, in spite of newer antibiotics availability that has broad spectrum of activity. Hence, in patient care, *P. aeruginosa* still continues to create a threat.^[4-8] To accelerate healing of wound it is obvious to the clinician to ultimately aim in reducing the bacterial number in wounds. The antiseptic management has a dichotomous history anchored in tradition and science. In acute as well as chronic wounds management, it is an integral part. The ideal topical therapy is focused mainly on reducing bacterial contamination and removing soluble debris with no adverse effects affecting cellular activities that

are important in healing process of wound. The use of many antiseptics for local wound care as topical agents is a questionable issue with regards to its safety and efficacy. In 1916, a study reported that application of a 1% solution of acetic acid for 2 weeks to purulent war wounds infected with "*Bacillus pyocyaneus*" led to the elimination of the organism.^[9] Previous studies have demonstrated the efficacy of diluted acetic acid using concentrations ranging from 1% to 5%.^[10-11] It was shown to be effective against *P. aeruginosa* from the burn and other skin wounds. Usage of 0.5% acetic acid for wound irrigation to create an unfavourable environment for bacterial growth. This has potentially expanded the spectrum of coverage of acetic acid for wound dressing.

Among antiseptic products for oral and hand washing, the most commonly used biocide is chlorhexidine which is used not only as disinfectant but also as preservative because of its efficacy on broad-spectrum, skin substantivity with lesser irritation. In contrast, reports mentioned about the irritability and that may be product specific.^[12-14] A considerable amount of research has been undertaken on the mechanism of the antimicrobial action of chlorhexidine.^[14] But majority of the time it was praised for its effective action against the deadly bacterial strains.^[15] Regarding its combating on sporostatic and mycobacteriostatic action, antiviral property, effects on protozoa and yeasts, there are extensive data are available. In another study, it was reported that chlorhexidine can be used as bactericidal agent.^[16] Chlorhexidine uptake by *S. aureus* and *E. coli* was very quick and altered on concentration and pH of chlorhexidine. Very recently in another study, it was reported that the uptake action is tremendously quick with in just 20s with a maximum effect with the usage of chlorhexidine gluconate in bacteria and yeasts.^[17] It was also noted that, chlorhexidine can damage the cell layers externally but for the cell death or lysis, it is insufficient. Most probably by passive diffusion, chlorhexidine agent crosses the outer membrane or cell wall and cytoplasm or inner membrane of the bacteria will be combated or the plasma membrane of yeast.^[18] Partition of cell wall, plasma membrane and cytoplasm of cells takes place in yeasts by the chlorhexidine actions.^[19] By using suitable techniques, semipermeable membrane damage, followed by intracellular constituents leakage can be measured. Due to leakage, immediately cell death will happen due to the cellular inactivation consequences.^[20] In intracellular constituents' coagulation will be happened in higher concentrations of chlorhexidine by which cytoplasm becomes clotted with subsequent decrease in leakage. As a result, permeability of membrane faces biphasic effect. As the chlorhexidine concentration increases, initially there will be rise in leakage at higher rate, but it will be subsequently reduced due the cytosol coagulation at higher concentrations of biocide.^[21] In a review article, chlorhexidine was found to be safe with little effect on wound healing.^[22] In another research study reported the healing rate of wound was improved following topical application of 5% chlorhexidine.^[23] Wu et al conducted a pilot trial on use of chlorhexidine patch at pin site to reduce the local morbidity and results revealed that use of chlorhexidine impregnated polyurethane dressings applied to pin sites for external fixation devices reduces the pin tract infections significantly.^[24] An *in-vitro* study evaluated the antimicrobial efficacy of a 1% silver sulfadiazine and 0.2% chlorhexidine digluconate cream, 1% silver sulfadiazine cream and a silver coated dressing. The results depicted that the combination of 1% silver sulfadiazine and 0.2% chlorhexidine digluconate (Silvazine) was found to most effective in killing of all bacteria.^[25] There was substantial literature evidence available on the use chlorhexidine as an antiseptic agent proved to be helpful in faster wound healing processes. In the wound management, digluconate is most frequently used among diacetate, and dihydrochloride. Because it has very rapid acting bactericidal against wider spectrum of bacteria which are non-sporing by destructing outer cell layers and the semi-permeable cytoplasmic membrane to allow leakage of cellular components. Depending on concentration, it will also cause intracellular constituents' coagulation.^[26] With this agent, bactericidal activity against *P. aeruginosa*, *S. aureus* and range of different clinical isolated has been well documented in the literature.^[27] In the treatment of *P. aeruginosa* infected superficial wound, Philips et al reported first usage of acetic acid as one of the topical agents.^[28] Concentration of acetic acid between 0.5 percent and 5 percent as topical agent was used to target *P. aeruginosa* from the burn and soft tissue wounds and they observed that all *P. aeruginosa* strains showed a minimum inhibition at the concentration of 2 percent *in-vitro*. Within 2 weeks of treatment, authors successfully inhibited and eliminated *P. aeruginosa* from 14 patients out of 16 patients. Acetic acid was implied to be an economical

and effective agent for combating *P. aeruginosa* for burn and soft tissue wounds.^[29] The effect of one percent acetic acid for burn wounds in eliminating of *P. aeruginosa* infection was conducted by Al-Ibran E and Khan M. Authors found that acetic acid application for 10 to 14 days eliminated *P. aeruginosa* in 90 percent of cases.^[30] However, a study reported acetic acid at 0.25%, 0.5% and 1.00% concentration were found to be bactericidal against most gram-positive and gram-negative organisms, including *Pseudomonas aeruginosa* with some disadvantages like *in-vitro* cytotoxicity has been reported and limited activity against biofilm.^[30] In our study, chlorhexidine dressings have changed the scene of our dressing, suggesting the reduction of wound infection which may provide benefit in terms of the development of pathological infection. Meanwhile drug resistance does not create any panic as the dressing works with the same effect without the need of systemic antibiotic therapy.

CONCLUSIONS

In conclusion, the results of the present study depict that topical application of chlorhexidine on *Pseudomonas aeruginosa* infected wound was at par with acetic acid in terms of its antiseptic activity. Furthermore, Topical application of chlorhexidine was also comparable to acetic acid in terms it's antiseptic effects in the treatment of chronic wounds like diabetic foot ulcers and aggravated wounds due to varicose vein and hypertension.

Limitations

The sample size of the present study was very small. A randomized clinical trial with larger sample sized could be conducted to ascertain the antiseptic effect chlorhexidine dressings for the treatment of wounds infected with *P. aeruginosa*.

Declarations

The authors certify that they have obtained informed written consent from the participants prior enrollment to the study.

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