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Assessment of effectiveness of the sodium perborate cleansing tablet on the dentures of new and long-term wearers

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Abstract

Aim: The aim of this investigation was to quantify the microbial changes from the surface of new and long-term wearer of maxillary complete dentures using Sodium Perborate denture cleansing tablet.

Materials and methods: Thirty (Fourteen males and Sixteen females) patients, who were rehabilitated with acrylic complete dentures were enrolled in this study. The patients were instructed to use water and mechanical cleansing using toothbrush for denture maintenance. The efficacy of cleansing tablet (Densive, Group Pharma Ltd, India) was evaluated against two organisms E. faecalis and C. albicans as well as total bacterial CFU at 15 min, 30 min, and 45 minutes. At the end of each phase microbiological analysis and ATP quantity were estimated using standardized microbiological processing and bio luminometer respectively.

Results: A statistically significant reduction of microorganisms after 15 minutes of denture immersion into cleansing tablet was observed for both new and long term used dentures; and after 45 minutes the reduction for long term used dentures was like new dentures. Analysis of ATP also showed a reduction in the overall bacterial load.

Conclusions: The removal of microbial load from the dentures were proved to be effective with 15 minutes of immersion into denture cleansing tablet.

Keywords: Denture cleansing tablet; Enterococcus faecalis; Candida albicans; Denture.

Introduction

Partial or complete dentures can amass plaque, stains, and calculus much like natural teeth. Failure to clean dentures adequately can lead to the formation of biofilm, potentially contributing to denture stomatitis. Many surveys suggest that a significant portion of the denture-wearing population neglected proper denture hygiene [1-3]. Beyond aesthetic considerations, maintaining good denture hygiene plays a crucial role in preventing denture stomatitis [3]. The prevalence of denture stomatitis varies, affecting up to two-thirds of elderly denture wearers. Despite understanding of role of denture hygiene, the etiology of denture stomatitis remains unclear. Complete dentures, yeast proliferation between the maxillary denture and palatal mucosa may lead to denture stomatitis [1,4].

The association between denture cleanliness and inflammation was explored by Budtz-Jorgensen and Bertram [5]. A statistically significant correlation between poor denture cleanliness and severe inflammation was also reported in their study [6]. In a comprehensive assessment of materials and methods for denture cleaning, it was concluded that bacterial and yeast plaque on dentures could cause infections. Consequently, it was recommended that denture wearers be educated and motivated to brush their dentures [7-10]. The American College of Prosthodontists (ACP) advocates daily cleaning of dentures through soaking and brushing, employing an effective, nonabrasive denture cleanser to diminish biofilm and mitigate the presence of harmful bacteria and fungi [11]. It is essential to treat dentures akin to natural teeth, cleaning

them daily to eliminate food particles, bacteria, and prevent permanent staining [11].

The first step in denture cleaning involves rinsing away loose food particles and detaching any denture adhesive. Subsequently, a commercial denture cleanser designed for removable dentures, available in various forms such as tablets, creams, pastes, gels, and solutions, should be used [11]. Denture cleanser tablets are to be used as per manufacturer's instruction. Generally, they are placed in warm water to generate an effervescent solution in which the denture is submerged. Soaking dentures in this cleaning solution aids in reducing the bacterial load, potentially minimizing denture-related odor [12]. Literature review shows microbial contamination exhibits a linear increase with prolonged usage, potentially leading to persistent changes that contribute to the formation of plaque having significant pathogenic microorganisms among denture users [12].

Densive Denture Cleanser is a unique color changing denture cleaning tablet, which has Sodium Perborate Monohydrate ($NaBO_3.H2_O$) as one of the active ingredients. With its strong oxidizing property, it penetrates the denture, ensuring a fast, simple, and effective cleanse killing 99.9% of odor-causing bacteria and fungi within 15 minutes. The present study was designed with an aim to assess antimicrobial efficacy of denture cleaning tablet (Densive Tablet) applied to new and old acrylic (Heat cure Denture base material, Dental Products India Ltd, India., containing Poly Methyl Methacrylate (PMMA) Polymer and the Methyl Methacrylate (MMA) monomer) dentures across varied usage intervals evaluated through ATP-driven bioluminescence and bacterial plating method. The null hypothesis of this study was that there will be no significant difference in the antimicrobial efficacy of the denture cleanser at different time intervals.

Methodology

Study design and participants: An ex vivo, comparative study was conducted at the study setting of Special Interest Group (SIG) in Dental Cariology, Jagadguru Sri Shivarathreeshwara Academy of Higher Education & Research (JSS AHER) and Department of Prosthodontics and Crown and Bridge, JSS Dental College and Hospital, Mysuru. A purposive sample size of 30 participants per group based on the earlier studies was required to ensure a power of 80% for rejecting the null hypothesis.

Ethics: The protocol of this study was approved by the Institutional ethics committee (JSSDCH IEC Research Protocol No 12/2022). This study was performed in compliance with the Declaration of Helsinki.

Selection of study participants: A total 30 participants; 15 participants who have been newly rehabilitated with complete dentures and 15 participants who were already been long term users of dentures for more than 3 years from single institution from the Department of Prosthodontics and Crown and Bridge, JSS Dental College and Hospital, were included in the study based on following selection criteria due to small sample size, stratification was not done according to the age, gender and other demographics.

Sample selection criteria

Inclusion criteria: Participants aged 55 to 75 years wearing complete denture for first time (New Denture user Group) or were inserted at least 3 years prior to the study (Long-Term Denture user Group). Participants wearing denture made from heat-polymerized acrylic resin and willing to take part in the study. Participants who have not used denture cleansers/ toothpaste or other antiseptics and who were not suffering with any systemic disease.

Exclusion criteria: Patients unwilling to take part in the study, already on antifungal therapy or participants who have used antibiotics or antifungal agents for any medical or dental condition within 1 month prior to screening, and who were immune compromised because of medications or condition. Those who had been using denture hygiene products like mouthwash or toothpaste besides washing with plain water.

Withdrawal criteria: Subjects who could not complete the follow ups and subjects who wanted to withdraw from the study research, on his/her own will, at any given time.

Sampling of all those subjects who consented and fulfilled the inclusion criteria were included in the respective groups.

Study protocol

At base line the biofilm samples for ATP and microbiological evaluation were collected from the tissue surface of each complete upper denture from patients reporting at 11 am (2 hours of continuous wearing the dentures after morning breakfast). The denture was then soaked in the warm water solution having denture cleanser tablet used according to the manufacturer's guidelines (Figure 1 a-d). After 15, 30 and 45 minutes of immersion biofilm samples for ATP and microbiological evaluation were collected at all these timepoints based on Uludamar et al. with a modified time interval and chemical method were followed [20].

ATP assay: The BacTiter-Glo™ Microbial Cell Viability Assay (Promega corporation, USA) was used which provides a method for deciding the number of viable microbial cells in culture based on quantitation of the ATP present.

Reagent Preparation: For convenience, the Buffer was thawed and stored at room temperature for up to 48 hours before use. Ten milliliters buffer was then transferred into the amber bottle having substrate to reconstitute the lyophilized substrate mixture to form the reagent. Before use, the reagent was thawed and gently vortexed, to obtain a homogeneous solution.

Measurement of ATP: All the steps were performed at room temperature (22-25°C). The reaction mixture was prepared by mixing a 10 µl of collected samples with 10 µl reagent. The control tube was prepared with Millipore water and reagent to obtain a value for background luminescence. The tubes were mixed briefly on an orbital shaker and incubated for five minutes, following which luminescence was recorded. An integration time of 0.25-1 second per tube served as a guideline.

ATP standard curve: All steps were performed at room temperature (22-25°C). The standards were prepared by treating a solution of the pure substance of known concentration with reagents. 10-fold serial dilutions of ATP in culture medium (1 µM to 10 pM; 100 µl) were also prepared. A multi-well plate with varying concentrations of standard ATP solution in 100 µl medium was set. Reagent was added to ATP standard present in each well (1:1 ratio). Contents were mixed on an orbital shaker and incubated for one minute, following which luminescence was recorded.

Microbiological sample processing: The clinical samples were cultured on Sheep Blood Agar (Hi Media, India) and Sabouraud's Dextrose Agar with Chloramphenicol (Microxpress, India) and incubated aerobically at 37°C for 24-48 hours. The cultures were examined at 24 hours and at 48 hours for the growth of tiny translucent colonies suggestive of Enterococcus species and cream-colored pasty colonies suggestive of Candida species. The tiny translucent, smooth, alpha, beta or non-hemolytic colonies on blood agar suggestive of Enterococcus species was further confirmed by Gram stain and esculin hydrolysis. The colony smear was made on the slide and stained with crystal violet for one minute followed by Gram's iodine for another one minute. Decolorization was done by ethyl alcohol for 30 seconds and counter stained by saffranine stain [13]. The slides were observed in oil immersion objective for the presence of Gram-Positive cocci in pairs showing spectacle appearance. The colony was inoculated onto Bile esculin agar and incubated in ambient air at 35°C for 24 hours. Enterococcus species ferment esculin in the presence of 4% bile to form black colored colonies. The cream-colored pasty colonies suggestive of Candida species were further identified by performing the Gram stain and Germ Tube Test. The Gram stain was performed as above and observed in oil immersion objective for the presence of Gram-Positive budding yeast cells.

Germ tube test: A single colony was inoculated into 0.5ml of human serum and incubated at 37°C. After 2-4 hours, a wet mount was prepared and examined under the microscope for the presence of germ tube. Those strains which produced germ tubes were identified as Candida albicans.

Statistical methods: The data obtained were statistically analyzed using IBM SPSS Statistics for Windows, Version 23.0 (Released 2015; IBM Corp., Armonk, New York, United States). The significance level was maintained at 5% (α=0.05). Normality and Homgeneity of the data was checked by using Shapirowilk test. Repeated measures of ANOVA and Tukey HSD (honestly significant difference) tests were used to analyze the difference in reduction of colony counts using ATP analysis and denture cleansing tablets at different time intervals (α≤0.05).

Results

ATP Evaluation

Comparative evaluation of new and old dentures: The average ATP values (Tables 1 and 2) showed a notable and statistically significant decline in bacterial load compared to the baseline after 15 minutes of immersing new dentures in a denture tablet solution. In contrast, long term used dentures, while demonstrating a significant reduction from their initially high ATP values at both 15 and 30 minutes, only showed a noteworthy reduction at 45 minutes of immersion, resembling the reduction observed in new dentures.

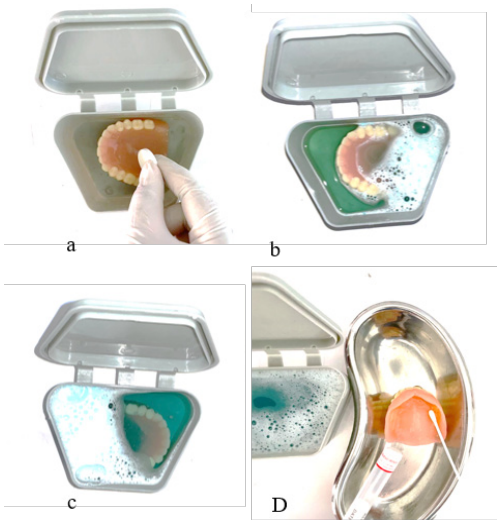

Figure 1: (a) Denture tablet in denture case with warm water, **(b)** Green color at 0 minute of denture insertion, **(c)** Blue color after 15 minute of insersion and **(d)** Biofim swab sample for microbial and ATP evaualtion.

Table 1: Comparison of reduction of ATP, Total Bacterial Count, Candida Albicans (CA) and E Faecalis (EF).

Groups			N	Mean	SD	Std. error	F value	P value
New denture	Total CFU	Baseline	30	2092.33	344.89	62.96812	1094.029	0.001***
		15 min	30	21.00	5.48	1.00000		
		30 min	30	4.40	1.59	.29007		
		45 min	30	2.67	1.45	.26407		
	Total ATP	Baseline	30	35266.67	4410.44	805.23193	940.068	0.001***
		15 min	30	3861.00	789.63	144.16653		
		30 min	30	403.00	76.12	13.89741		
		45 min	30	4.53	1.87	.34151		
	CA-CFU	Baseline	30	147.67	20.94	3.82290	1424.745	0.001***
		15 min	30	3.10	1.58	.28907		
		30 min	30	2.90	1.58	.28907		
		45 min	30	1.43	.68	.12395		
	EF-CFU	Baseline	30	211.73	39.90	7.28436	823.006	0.001***
		15 min	30	2.93	1.48	.27094		
		30 min	30	3.13	1.46	.26580		
		45 min	30	1.33	.48	.08754		
Long-term used denture	Total CFU	Baseline	30	2468.33	431.81	78.83794	940.068	0.001***
		15 min	30	75.47	11.96	2.18392		
		30 min	30	33.00	9.88	1.80357		
		45 min	30	39.73	8.31	1.51766		

	Total ATP	Baseline	30	246036.90	39028.64	7125.62284	1189.735	0.001***
		15 min	30	761.67	145.46	26.55761		
		30 min	30	3.10	1.63	.29692		
		45 min	30	1.43	.57	.10376		
	CA-CFU	Baseline	30	1854.43	221.85	40.50357	989.091	0.001***
		15 min	30	781.97	167.39	30.56128		
		30 min	30	321.83	40.53	7.40023		
		45 min	30	10.10	5.91	1.07890		
	EF-CFU	Baseline	30	2262.33	151.59	27.67664	3432.836	0.001***
		15 min	30	1191.60	77.92	14.22614		
		30 min	30	549.53	56.45	10.30642		
		45 min	30	25.87	13.28	2.42484		

On Repeated Measures of ANOVA used on Total Bacterial count (Total CFU), Candida Albicans (CA) and E Faecalis (FE).
*** Highly Significant.

Table 2: Tukey HSD test for comparison of reduction in ATP and microbiological analysis.

Groups	Parameter	Time interval	Mean difference	Std. error	Sig.
New denture	Total CFU	Baseline -15 min	2071.33	44.53167	.000***
		Baseline -30 min	2087.93	44.53167	.000***
		Baseline -45 min	2089.67	44.53167	.000***
		15 min-30 min	16.60	44.53167	.982
		15 min-45 min	18.33	44.53167	.976
		30 min to 45 min	1.73	44.53167	1.000
	Total ATP	Baseline -15 min	31405.67	578.52213	.000***
		Baseline -30 min	34863.67	578.52213	.000***
		Baseline -45 min	35262.13	578.52213	.000***
		15 min-30 min	3458.00	578.52213	.000***
		15 min-45 min	3856.47	578.52213	.000***
		30 min to 45 min	398.47	578.52213	.901
	CA-CFU	Baseline -15 min	144.57	2.72002	.000***
		Baseline -30 min	144.77	2.72002	.000***
		Baseline -45 min	146.23	2.72002	.000***
		15 min-30 min	0.20	2.72002	1.000
		15 min-45 min	1.67	2.72002	.928
		30 min to 45 min	1.47	2.72002	.949
	EF-CFU	Baseline -15 min	208.80	5.15818	.000***
		Baseline -30 min	208.60	5.15818	.000***
		Baseline -45 min	210.40	5.15818	.000***
		15 min-30 min	0.20	5.15818	1.000
		15 min-45 min	1.60	5.15818	.990
		30 min to 45 min	1.80	5.15818	.985
Long-term used denture	Total CFU	Baseline -15 min	2392.87	55.79313	.000***
		Baseline -30 min	2435.33	55.79313	.000***
		Baseline -45 min	2428.60	55.79313	.000***
		15 min-30 min	42.47	55.79313	.872
		15 min-45 min	35.73	55.79313	.919
		30 min to 45 min	6.73	55.79313	.999
	Total ATP	Baseline -15 min	245275.23	5038.61123	.000***
		Baseline -30 min	246033.80	5038.61123	.000***
		Baseline -45 min	246035.47	5038.61123	.000***
		15 min-30 min	758.57	5038.61123	.999
		15 min-45 min	760.23	5038.61123	.999
		30 min to 45 min	1.67	5038.61123	1.000
	CA CFU	Baseline -15 min	1072.47	36.26609	.000***
		Baseline -30 min	1532.60	36.26609	.000***
		Baseline -45 min	1844.33	36.26609	.000***
		15 min-30 min	460.13	36.26609	.000***

		15 min-45 min	771.87	36.26609	.000***
		30 min to 45 min	311.73	36.26609	.000***
	EF CFU	Baseline -15 min	1070.73	23.24308	.000***
		Baseline -30 min	1712.80	23.24308	.000***
		Baseline -45 min	2236.47	23.24308	.000***
		15 min-30 min	642.07	23.24308	.000***
		15 min-45 min	1165.73	23.24308	.000***
		30 min to 45 min	523.67*	23.24308	.000***

On evaluation the long term used dentures group showed higher mean ATP values (2,46,036.90 RLU) at the baseline indicating presence of higher microbial load and slower reduction in microbial load at all the time points tested compared to the ATP values of the new Dentures group (35,266.67 RLU). The total microbial load was reduced significantly, from base line, in both the groups up to 45 minutes of intervention.

Microbial evaluation

Comparative evaluation of new and old dentures: Similar, to the ATP values, the data from microbial counts (Tables 1 and 2) also revealed that the long-term used dentures exhibited a higher mean CFU count at baseline (2468 CFU) compared to new dentures group (2092 CFU), indicating a higher microbial load. The long-term used dentures group also demonstrated a slower reduction in CFU count at all specified time points when compared to new dentures. The mean CFU counts (Table 1) exhibited a significant and drastic decrease in bacterial CFU from baseline after 15 minutes of immersing dentures into a denture tablet solution for new dentures. In contrast, long-term used dentures, while also showing a significant reduction from a very high baseline CFU count at 15 and 30 minutes, demonstrated a significant reduction similar to new dentures at 45 minutes of immersion.

Furthermore, compared to baseline, the total microbial count significantly decreased in both groups up to 45 minutes of intervention. Additionally, individual evaluations of reduction in Candida albicans counts were observed from baseline to 15 minutes (New denture group: 147 CFU to 3 CFU; long-term used denture group: 1854 CFU to 781 CFU) and E. faecalis (New denture group: 211 CFU to 2.9 CFU; Long-term used denture group: 2262 CFU to 1191 CFU) through culture plate methods showed a statistically significant reduction (p=0.001).

At 45 minutes, CFU counts of Candida albicans further reduced to 1 CFU in the new dentures group and to 10 CFU in the long-term used denture group. Additionally, E. faecalis at 45 minutes in the new denture group reduced to 1 CFU, while the long-term used denture group reduced to 25 CFU, both statistically significant at p=0.000.

Discussion

Edentulism interferes with mastication, speech, and esthetics. Providing dentures can overcome the problem of edentulousness and improve the quality of life but may be potentially harmful when it is not properly cared [14]. The phenomenon of initial adherence representing the first step in the colonization process is plaque formation that leads from the formation of a thin biofilm, followed by a multilayer, which culminates into denture plaque [15]. The process by which dentures accumulate plaqueand calculus is similar to the process, which takes place on natural teeth. Microbial plaque, which forms on the tissue-fitting surface of the denture is probablyof greatest clinical significance. A variety of soft tissue changes manifest themselves as a series of related symptom complexes, which include denture stomatitis, inflammatory hyperplasia, and chronic candidiasis.

Candida albicans as an opportunistic pathogen exploits the host immune system and causes a variety of life-threatening infections [16]. The polymorphic nature of this fungus gives it tremendous advantage to breach mucosal barriers and cause oral and disseminated infections (REF). Similar to C. albicans, Enterococcus faecalis is a major opportunistic pathogen, which is of critical concern in immunocompromised patients [16]. There is increasing evidence that E. faecalis co-exists with C. albicans in the human body in disease samples [16].

Enterococcus faecalis, a Gram-positive bacterium, is known to cause several infections including endocarditis, bacteremia, abdominal abscesses, burn wound sepsis, meningitis, urinary tract infections, and various nosocomial infections, particularly in immunocompromised patients. This organism holds a certain clinical relevance as it is resistant to multiple antibiotics [17].

During the contact points of 15, 30, 45 of immersion into densify cleansing tablet, the cleanser had different levels of effectiveness in reducing the 2 main types of microorganisms. Densive tablet was effective in reducing overall bacterial growth, C. albicans and E faecalis in both new and old denture wearers evaluated both by ATP and microbiological analysis. In contrast, the reduction in the number of both species were significantly greater in new denture wearers than old denture wearers but was not statistically significant. The significant difference in reduction in Candida and E. faecalis is related the variability in the number of CFUs of Candida and E. faecalis spp. in both old and new denture wearers (Table 2). The effects of a cleanser in vivo are constantly challenged by the daily ingestion of food, which may explain at least part of the variability between duration of usage of dentures and subjects.

The significant reduction in the number of microorganisms observed in this study suggests that the use of denture cleansers is a suitable method for cleaning dentures in the geriatric and hospitalized population. This conclusion is supported by the finding of Chan and others that brushing alone with a denture abrasive was less effective than cleanser use for maintaining good denture hygiene. Authors of this study suggested that cleanser use may be particularly appropriate for elderly people who lack manual dexterity [17]. Further studies are needed to determine if daily use of a cleanser can reduce the high prevalence of denture stomatitis or if it might cause mucosal irritation and allergy [18].

The standard culture-based plate counting method has conventionally been used to quantify microflora; however, this process is time-consuming and labor-intensive. A rapid oral bacteria detection apparatus using BacTiter-Glo™ method can be used to detect total oral bacteria numbers from the tissue surface of the denture within 5 minutes at chair-side and to know the effectiveness of commercially available denture cleansing tablet.

In the present study, the number of times a participant brushed their dentures, the time at which individuals brushed was standardized. Thus, further study is needed to determine the potential effects of these biases. The validity of the results of this study relates to methodology. Two trained Dentists (N and M), following the same protocol to decrease bias among groups, applied the treatments. Individual patients were not informed about the study hypotheses, and the microbiologist who cultured swab samples was blinded to treatment. Further, to have a good agreement among the 3 dentists microbiological scores were recalculated for 10 percent of the samples to validate the calibration made under the supervision the microbiologist to reduce inter operator subjectivity.

The antimicrobial property of saliva could contribute to the decreased adhesion of microorganisms, which was not considered in the present study. Depending on the duration of usage of dentures, the immersion period varies. However, for individuals who practice good denture hygiene frequently, the actual immersion time may differ and in the present study the usage of denture cleaning tablet showed antimicrobial activity by immersion for 15 minutes guided by the color change to complete the treatment without under or over extended time of immersion. Future studies should focus on evaluating the role of an acquired salivary pellicle in microbial adhesion and the efficacy of denture cleansers.

In summary, the Bac Titer-Glo™ swab test values demonstrated a correlation with those obtained from the bacterial count assay. The findings of this study propose that the subjective assessment of oral hygiene could be substituted with the ATP swab test, and as was found to be comparable to the bacterial count assay. In the study by Baba et al have assessed microbial count through ATP assay and microbiological analysis to gauge the efficacy of only saline and found ATP assay to be reliable, while other study has evaluated effects of alkaline peroxides, mouthwashes on dentures hygiene using only microbial assays [19,20].

Conclusions

From the present study clinicians might consider recommending the use of color changing denture cleansing tablets for a minimum of 15 minutes for new dentures while an immersion period exceeding 45 minutes for long-term used dentures to achieve effective antimicrobial action initially and subsequently to follow 15 minutes immersion for denture cleansing. Denture hygiene by denture cleansing tablets for the geriatric age group for selfcare and care by their caretaker/ nurses at care homes or during hospitalization is an economical and practicable method for daily basis. The evidence from this study suggests that the existing trends of using hygiene by cleaning only by mechanical methods have shown high retention of bacterial colonies as seen at the baseline from the old dentures wearers group who were practicing only mechanical cleaning using brushes and water. Hence from the findings of the present study, use of denture cleansing tablet for acrylic dentures hygiene need to be recommended for the wellbeing of the patients.

Declarations

Author contributions: Conceptualization, N, M, RS, R; Methodology, N, M RS, R; software- N, RS. Validation- N, M, RS, SO; Investigation, N, M, RS, SO; writing-original draft preparation- M, N, RS; Writing-review and editing- N, M, RS, SO.; Project administration, -N, M, R.; Funding acquisition, N. All authors have read and agreed to the published version of the manuscript.

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Institutional review board statement: Applicable.

Informed consent statement: Applicable.

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Conflicts of interest: The authors declare no conflicts of interest.

Clinical relevance: Can be practiced easily effectively and regularly for denture hygiene by selfcare as well as by the caretakers for inmates at care homes and to those hospitalized.

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