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Research Article

IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY TESTING OF PATHOGENIC MICRO-ORGANISM FROM DENTAL PATIENTS

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ABSTRACT

Objective: To isolate and identify aerobic microbes present in the periodontal infected patients and to evaluate the choice of antibiotics in the management of periodontal diseases.

Methods: In this study, these patients have not been treated previously for their conditions. An informed consent was obtained from these patients before collection of an oral swab. This study was approved by the Institutional Ethical Committee. The details of the patient's age, sex, and clinical details were recorded on a per forma meant for this study. The following methodologies were adopted for the isolation and identification of the micro-organisms from these cases.

Results: In this study out of 50 oral samples, culture positivity was recorded in 43 (86%) cases and no growth in 7 (14%) cases. Antibiotic susceptibility test using to identified as resistant, sensitive, intermediate of pathogenicity of oral microbes. Such antibiotics were methicillin, ceftazidime, clindamycin, amikacin, cloxacillin, and cefotaxime. This study should be kept in mind when a local application of antibacterial compounds is used in the therapy of periodontal disease.

Conclusion: This study highlights the different organisms involved in the different types of dental infections. The antibiotic pattern shown in this work will be a guide to the clinician in the selection of proper antibiotics for the treatment of these infections. Hence in this study, the limitations were time and the number of patients. For better outcomes, a larger study population for a longer period of time should be undertaken to know the bacteriology and to the select the effective drugs of choice for dental infections. A comparative study of bacteriology and mycology and its antimicrobial property would be very fruitful in the future.

Keywords: Dental, Periodontal, Bacteria, Antibiotics.

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INTRODUCTION

Teeth is a hard, resistant structure occurring on the jaws in or around the mouth and pharynx areas of vertebrates used for catching and masticating food, for defense, and for other specialized purposes [1]. A tooth consists of a crown and one or more roots. The crown is the functional part that is visible above the gum [2]. The root is the unseen portion that supports and fastens the tooth in the jawbone [2]. The microbial floras of the oral cavity are rich and extremely diverse. This reflects the abundant nutrients and moisture, hospitable temperature, and the availability of surfaces on which bacterial populations can develop. Over 500 bacterial species have been identified from the human oral cavity [3] but with the exception of a limited number of pathogens the majority of them are considered to be benign or beneficial. Micro-organism such as *Streptococcus*, *Actinomyces*, *Neisseria*, and *Veillonella* present as primary colonizers while *Fusobacterium nucleatum* and *Capnocytophaga* species occur as secondary colonizers [4,5]. The main objective of the current study is to isolate and identify aerobic microbes present in patients with different dental infections and to evaluate the choice of antibiotics in the management of these dental infections. The results of this study will be useful for the clinician in the selection of appropriate antibiotics for treatment and prevent the phenomena of drug resistance in the micro-organism.

METHODS

Sample collection

Over a period of 3-month from January 8, 2013, to April 8, 2013, a total of 50 patients presenting in the outpatient unit of Nataraja Dental College, Komarapalayam, Namakkal, Tamil Nadu, were enrolled in this study. These patients have not been treated previously for their conditions. An informed consent was obtained from these patients before the collection of an oral swab. This study was approved by the Institutional Ethical Committee for the enrollment of human subjects. The patient's details - namely age, sex, and clinical details - were recorded on a per forma exclusively meant for this study. The following methodologies were adopted for the isolation and identification of the micro-organisms from these cases. Two swabs were taken for each patient from the Kumaramangalam region by the medical doctor.

Isolation and identification of pathogens

The oral swabs were inoculated onto the plate of nutrient agar and Sabouraud dextrose agar plate. The bacterial plates were incubated at 37°C for 24 hrs. The fungal plates were incubated at 27°C to 30°C for 48-72 hrs. Significant growth of the culture was interpreted as positive for microscopy with the growth of the same organism in culture in more than one medium. The identification of the bacterial isolates was based on the standard methods [6].

Antibiotic susceptibility test

The antibiotic susceptibility tests of the bacterial isolates were done following the method of Kirby-Bauer disc diffusion method [7]. The sterilized nutrient broths were prepared, and the bacterial isolates were inoculated and incubated at 37°C for 1-2 hrs. The bacterial culture was made into a thin suspension and was plated onto plates of Muller-Hinton agar using a sterile cotton swab and spreading it in all the directions over the surface of the agar to obtain a uniform growth. The antibiotic disc was placed over the agar and incubated at 37°C for 24 hrs. The diameter of the zone of inhibition was measured around each disc and interpreted using the standard chart.

RESULTS

Over a period of 3-month from January 2013 to April 2013, a total of 50 oral swab samples were collected from the patients presenting at the outpatient unit of Nataraja Dental College, Komarapalayam, Namakkal, Tamil Nadu, India. The oral swabs were taken to the medical laboratory and investigated for both bacteria and fungi. Out of 50 oral samples, culture positivity was recorded in 43 (86%) cases and no growth in 7 (14%) cases. There was a mixed bacterial infection in 11 (22%) cases. Along with bacterial isolates, yeast species were also identified in 10 (20%) cases. Of the 43 culture positive cases, Gram-positive bacteria were found to be high 38 (88.37%) isolates, followed by Gram-negative bacteria as 17 (39.53%) isolates and yeast for 10 (23.25%) cases which were speciated by Hi-chrome *Candida* agar. The patient's demographic details were tabulated in Table 1.

Distribution of gender

Among 50 patients, 27 (54%) were males and 23 (46%) were females. The male:female ratio was found to be 1.17:1. Hence, male patients were highly susceptible to oral infections than females.

Distribution of types of dental infections

The pattern of patient's distribution of different types of dental infection included 34 (68%) patients with periodontal infections followed by carries 10 (20%) patients with mild to moderate condition, 3 (6%) patients who had undergone root canal treatment, pulpitis, malocclusions, and root stump patients with each 1 case (2%), respectively. In this distribution, it was found that patients with periodontal infection were found to be higher than any other oral infections (Fig. 1).

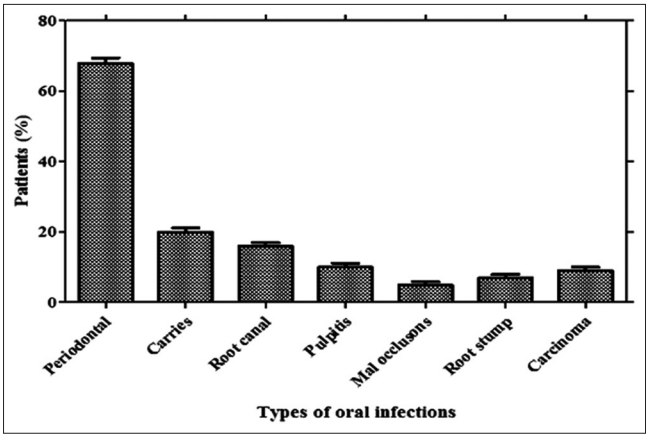

Fig. 1: Distribution of types of dental infections

Age wise distribution pattern of dental infections

The most predominant age group was 21-30 years accounting for 16 (32%) cases, followed by the age group of 31-40 years. The major reason attributing to such a cause is improper tooth care, smoking and usage of carbonated drinks (Fig. 2).

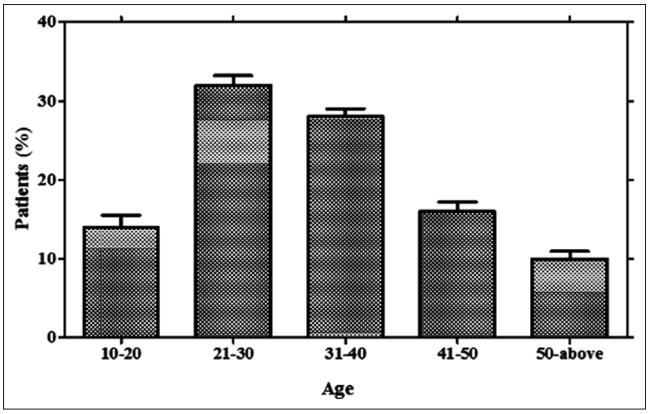

Fig. 2: Age wise distribution pattern of dental infections

Growth pattern of bacterial isolates in dental infections

The Gram-positive cocci accounting for 22 (41%) cases showed a higher predominance followed by Gram-negative bacilli which was found as 17 (29%) cases (Fig. 3).

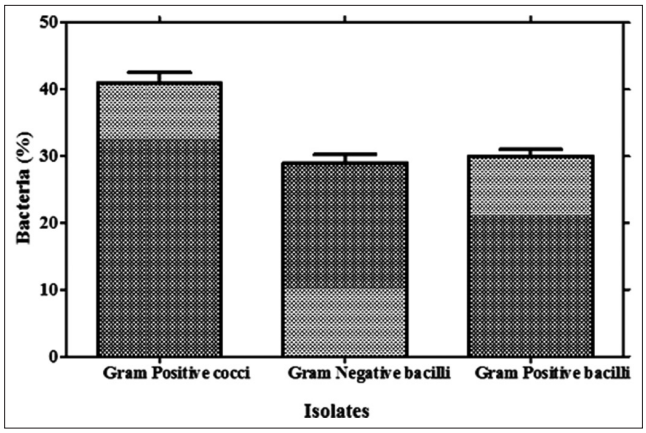

Fig. 3: Growth patterns of bacterial isolates in oral infections

Bacterial distribution among dental infections

Altogether, 55 bacterial isolates were obtained from 50 samples of different oral infections. The most frequent and predominant bacteria associated with the oral infections were *Bacillus* spp. in 16 (29%) cases, followed by *Micrococcus* spp. in 12 (22%) cases. The predominant Gram-negative organism was *Pseudomonas aeruginosa* in 9 (16%) cases, followed by *Klebsiella* spp. in 7 (13%) cases (Fig. 4).

Fungal distribution among dental infections

Along the fungal isolates, *Candida* spp. was isolated in 10 (20%) cases. Of the various *candida* species, *Candida albicans* was the predominant one isolated and confirmed in this study.

Antibiotic sensitivity pattern

The antimicrobial susceptibility pattern showed *Staphylococcus aureus* as 100% sensitive to methicillin, ceftazidime, clindamycin, amikacin, cloxacillin, and cefotaxime, whereas *Bacillus* spp. was resistant to cloxacillin. *Micrococcus* spp. showed resistant to both cloxacillin and clindamycin. Gram-negative isolates, *Klebsiella* spp. and *Pseudomonas* spp., were highly resistant to clindamycin, cloxacillin, and tetracycline but sensitive to amikacin (Table 2).

DISCUSSION

Oral infections (dental caries, periodontal disease, and gingivitis) were the most common chronic oral disease in the world. Bacterial dental plaque was considered to be the primary etiological factor in the development of dental caries, gingivitis, and periodontitis. Since the level of individual oral hygiene was directly related to the amount of plaque buildup on the teeth, it was reasonable to predict that the level of oral hygiene in a population was positively correlated with the prevalence and severity of periodontal disease and dental caries [8]. Hence, an understanding of the regional etiological agents was important in the management of this disease [9]. This study was undertaken to identify the both bacterial and fungal an etiology and the *in vitro* antimicrobial susceptibility pattern of the pathogen to commonly used antimicrobial agents. Polymicrobial nature of these dental infections has been brought out in this study.

The microbiological investigations were carried out, and a total of 55 bacterial and 10 fungal isolates were isolated from the infected

Table 1: Demographic details, clinical types of dental infections and isolated microbes

Sample No.	Date	Name	Patient No	Symptomatic oral infections	Age	Sex	Identified microorganisms
1	1-2-2013	Vengadesh	091	Gingivitis	31	M	*Bacillus* sp., *Micrococcus* sp.
2	1-2-2013	Kalavathi	2404	Carries	45	F	*Staphylococcus aureus*, *Micrococcus* sp.
3	1-2-2013	Soundarya	581	RTC	19	F	*Bacillus* sp.
4	1-2-2013	Ramayee	98	Carcinoma	60	F	*Micrococcus* sp.
5	1-2-2013	Mathivanan	102	Carries	28	M	*Bacillus* sp., *Micrococcus* sp.
6	1-2-2013	Murugan	99	Gingivitis	30	M	*Bacillus* sp.
7	1-2-2013	Lakshmi	105	Carries	55	F	*Klebsiella* sp.
8	1-2-2013	Dharshni	107	Carries	12	F	*Listeria* sp.
9	1-2-2013	Jothi	108	Carries	27	F	*Bacillus* sp.
10	1-2-2013	Sekar	116	Carries	53	M	*S. aureus*
11	8-2-2013	Muthu	389	Carries	40	M	*Klebsiella* sp
12	8-2-2013	Kannammal	2809	Chronic periodontitis	40	F	*Micrococcus* sp., *Lactobacillus* sp.
13	8-2-2013	Tamilselvi	2396	Chronic periodontitis	26	F	*S. aureus*
14	8-2-2013	Prathab Kumar	2340	Carries	20	M	No growth
15	8-2-2013	Kuttiraj	827	Carries	40	M	*P. aeruginosa*
16	8-2-2013	Sakthivel	798	Chronic periodontitis	32	M	*Micrococcus* sp.
17	8-2-2013	Murugan	99	Chronic periodontitis	30	M	*Micrococcus* sp.
18	8-2-2013	Kathavarayan	833	Periodontitis	27	M	*P. aeruginosa*
19	8-2-2013	Annadurai	834	Chronic periodontitis	40	M	No growth
20	8-2-2013	Selvaraj	835	Pericornlitis	42	M	*P. aeruginosa*
21	15-2-2013	Pushpa	1206	Chronic periodontitis	25	F	*Micrococcus* sp.
22	15-2-2013	Abdhul Rahim	1645	Periodontitis	22	M	*No growth*
23	15-2-2013	Latha	1620	Root stump	38	F	*Bacillus* sp.
24	15-2-2013	Kathavarayan	833	Periodontitis	27	M	*Bacillus sp.*
25	15-2-2013	Manikandan	1647	Periodontitis	60	M	*Bacillus* sp.
26	15-2-2013	Ayisha Yashmin	2201	Chronic periodontitis	33	F	*Micrococcus* sp.
27	15-2-2013	Mahalakshmi	1183	Apical periodontitis	36	F	*P. aeruginosa*, *Micrococcus* sp.
28	15-2-2013	Priya Darsini	1648	Apical periodontitis	30	F	*S. aureus*
29	15-2-2013	Bhuvaneshwaran	816	Gingivitis	23	M	*Bacillus* sp.
30	15-2-2013	Muthusamy	8422	Mol occlusion	24	M	*P. aeruginosa*, *Micrcoccus* sp.
31	15-2-2013	Sivakumar	942	Gingivitis	29	M	*S. epidermidis*
32	15-2-2013	Selvi	539	RTC	48	F	*Klebsiella* sp., *Bacillus* sp.
33	22-2-2013	Nafila	1437	Periodontitis	24	F	No growth
34	22-2-2013	Nagaraj	2176	Periodontitis	42	M	*P. aeruginosa*
35	22-2-2013	Vijayalakshmi	3160	Periodontitis	40	F	*S. aureus*
36	22-2-2013	Arunkumar	2467	Gingivitis	21	M	*Klebsiella* sp., *Bacillus* sp.
37	22-2-2013	Logabharani	457	Periapical periodontitis	15	F	*S. aureus*
38	22-2-2013	Victoriya	2665/1/13	Periodontitis	32	F	No growth
39	22-2-2013	Padmavathi	2477	Pulpitis	42	F	*P. aeruginosa*, *S. epidermidis*
40	22-2-2013	Annaporani	1995	Periodontitis	39	F	No growth
41	22-2-2013	Vadivel	2461	Periodontitis	43	M	*P. aeruginosa*, *Micrococcus* sp.
42	22-2-2013	Dhanapriya	1506	Periapical periodontitis	16	F	*Bacillus* sp.
43	1-3-2013	Sabitha	589	Periodontitis	20	F	*Klebsiella* sp., *Bacillus* sp.
44	1-3-2013	Poongodi	3279	Apical periodontitis	36	F	No growth
45	1-3-2013	Ganeshan	92	Generalized periodontitis	57	M	*Klebsiella* sp.

(Contd...)

Table 1: ***(Continued)***

Sample No.	Date	Name	Patient No	Symptomatic oral infections	Age	Sex	Identified microorganisms
46	1-3-2013	Rajendran	94	Generalized periodontitis	50	M	*S. aureus*
47	1-3-2013	Chandra Sekar	1655	Gingivitis	22	M	*S. epidermidis*
48	1-3-2013	Kathirvel	2380	Periodontitis	46	M	*Bacillus* sp.
49	1-3-2013	Ananda Kumar	1031	Apical periodontitis	20	M	*Bacillus* sp.
50	1-3-2013	Raja	3255	Carris	38	M	*Klebsiella* sp., *P. aeruginosa*

S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, S. epidermidis: Staphylococcus epidermidis, RTC: Randomized controlled trial

Table 2: Antibiotic sensitivity test

S. No	Name of the isolates	Number of bacterial isolates	Number of sensitivity																			
			Methicillin (M)			Cefotoxime (Ce)			Tetracyclin (T)			Ceftazidime (CA)			Clindamycim (CD)			Cloxacillin (CX)			Amikacin (AK)		
			R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
1	*S. aureus*	7	-	-	7	-	-	7	-	-	-	-	-	7	-	-	7	-	-	7	-	-	7
2	*S. epidermidis*	3	-	-	3	-	-	3	-	-	3	-	-	3	-	-	3	-	-	3	-	-	3
3	*Micrococcus*	12	8	-	4	-	-	12	-	-	12	-	-	12	2	-	10	2	-	10	-	-	12
4	*Bacillus* sp.	16	10	-	6	-	-	16	-	-	16	2	-	14	-	-	16	1	-	15	-	-	16
5	*Klebsiella* species	7	4	-	3	-	-	7	1	-	6	-	-	7	1	-	6	1	-	6	-	-	7
6	*P. aeruginosa*	9	3	1	5	7	-	2	3	-	4	-	-	9	4	-	5	2	-	7	-	-	9
7	*Listeria* spp.	1	1	-	-		1	-	-	-	1	-	-	1	-	-	1	1	-	-	1	-	1

S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, S. epidermidis: Staphylococcus epidermidis

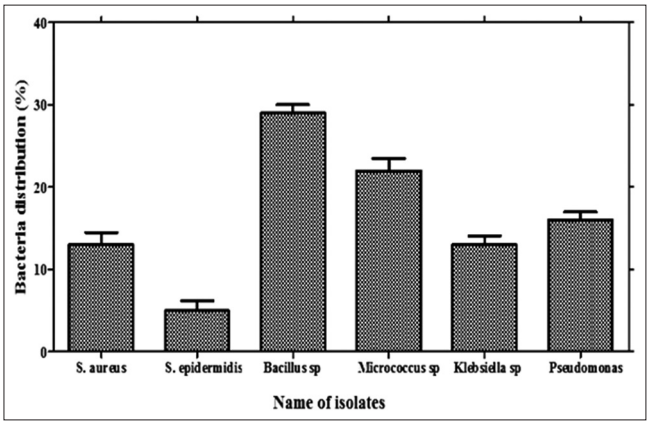

Fig. 4: Bacterial distribution among dental infections

oral patients [10]. Among 50 patients, 27 (54%) were males and 23 (46%) were females. Hence, the male showed a high predominance of dental infections than females. This was because of tobacco smoking was significantly more incident in men than females which was a possible explanation of the detected differences. On the other hand, no specific gender differences in the incidence or severity of gingival and periodontal diseases were detected [11]. Besides carries, gingivitis and periodontitis are the most frequently diagnosed oral diseases in hospitals. A total of 50 patients with oral infected patients of which, 34 (68%) patients had periodontal, 10 (20%) had carries lesions, 2 (4%) had root canal, 1 (2%) had pulpitis, 1 (2%) had malocclusions, 1 (2%) had root stump, and the remaining 1 (2%) had carcinoma of oral infections. The percentage of periodontal infection was found to be higher than that of other oral infection [12,13]. The prevalence of 21-25% of conditions including pulpitis, dent alveolar abscess, periodontitis, and each other accounts for <5%.

Age seems to play an important role in the pathology of oral diseases. Out of 50 patients, 7 (14%) were between the age of 10 and 20 years, 16 (32%) were between the age of 21 and 30 years. From 31 to 40 years, 14 (28%) patients were infected, 8 (16%) were between 41 and 50 years, and remaining 5 (10%) were between above 50. In this study, the patients above the age group of 21-30 years were most significantly infected with oral infections. That the risk of suffering from oral diseases increases with age from 14.5% at 10-19 years to 100% at 50 years and above [14,15]. Oral microorganisms are generally organized in biofilms that are, based on the location relative to the gum line, divided into supragingival and subgingival plaque [16]. The periopathogenic bacteria of the subgingival plaque were considered to be the primary etiological factor of periodontitis. Among 55 bacterial isolates, 22 (41%) were Gram-positive cocci, 17 (29%) were Gram-negative bacilli, and the remaining 16 (30%) were Gram-positive bacilli. Hence, the percentage of Gram-positive cocci was predominately higher than that of Gram-negative bacilli and Gram-positive bacilli [17]. Normal flora was predominated by Gram-positive cocci followed by Gram-positive bacilli. The most frequent bacteria associated with the oral infections were *Bacillus* 16 (29%) followed by *Micrococcus* spp. 12 (22%), *Klebsiella* spp. 7 (13%), *S. aureus* 7 (13%), *Staphylococcus epidermidis* 3 (5%), and *Listeria* spp. 1 (1.8%). Hence, *Bacillus* spp. were predominately present in the oral infections. *S. epidermidis, C. albicans, S. aureus,* and *P. aeruginosa* to be the predominant sub gingival microorganisms associated with acute exacerbations of chronic periodontal disease. The standard treatment of periodontal disease was centered on the mechanical removal of dental plaque, as well as calculus and other contaminants from tooth and root surfaces. However, this mechanical treatment was not always effective [8,13,18].

Hence, the antimicrobial sensitivity pattern varies from community to community. This was because of the resistant strains as a result of indiscriminate use of antibiotics. The antimicrobial susceptibility test for *S. aureus* showed 100% sensitive to methicillin, ceftazidime, clindamycin, amikacin, cloxacillin, and cefotaxime but *Bacillus* spp. was resistant to cloxacillin and *Micrococcus* spp. showed resistant to both cloxacillin and clindamycin. Gram-negative isolates, *Klebsiella* spp. and *Pseudomonas* spp., were highly resistant to clindamycin, cloxacillin, and tetramycin but sensitive to amikacin.

CONCLUSION

This study is a pilot study conducted over a period of 3-month from January 15, 2013, to April 8, 2013, at JKKK Nataraja Dental College, Komarapalayam, Namakkal, Tamil Nadu, India. In this study, the patients with different types of dental problems were screened for their microbiological profile. Their antibiotic pattern was also ascertained. In this study, there was a higher predominance of Gram-positive cocci accounting for 22 (41%) cases followed by Gram-negative bacilli accounting for 17 (29%) cases. In this study, male showed a high predominance of oral infections than females. The pattern of patient's distribution of different types of dental infection showed that patients with periodontal infection were found to be higher than that of any other oral infection. The age distributions of patients showed that patients in the age group of 21-30 years were more affected by dental problems followed by the age group of 31-40 years. The major reason attributing to such a cause is improper tooth care, smoking, and usage of carbonated drinks. The most frequent and predominant bacteria associated with dental infections were *Bacillus* spp. followed by *Micrococcus* spp. and *Klebsiella* spp. The antibiotic sensitivity pattern showed *S. aureus* sensitive to methicillin, ceftazidime, clindamycin, amikacin, cloxacillin, and cefotaxime, while Gram-negative isolates like *Klebsiella* spp. and *Pseudomonas* spp. were highly resistant to clindamycin, cloxacillin, and tetracycline but sensitive to amikacin. In conclusion, this study highlights the different organisms involved in the different types of dental infections. The antibiotic pattern shown in this work will be a guide to the clinician in the selection of proper antibiotics for the treatment of these infections. Hence in this study, the limitations were time and the number of patients. For better outcomes, a larger study population for a longer period of time should be undertaken to know the bacteriology and to the select the effective drugs of choice for dental infections. A comparative study of bacteriology and mycology and its antimicrobial property would be very fruitful in the future.

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