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Each water outlet is a unique ecological niche for *Legionella pneumophila*

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SUMMARY

We determined the natural history of the colonization of our hospital's potable water by culturing water approximately biweekly from 20 sites throughout the hospital for 4 years. Overall, 545 (24·7%) of the 2200 samples grew *Legionella pneumophila*. During hyperchlorination, 11·7% of the samples were positive while 41·6% were positive in the absence of chlorination. There was no seasonal trend towards positivity, but there was marked inter-site variation in the semi-quantitative culture results. However, a single strain of legionella (as defined by plasmid profiling) tended to persist at a site. Such a site was a unique ecological niche in that different sites in the same wing were populated by distinct strains.

The two wings of our hospital had a significantly different distribution of strains of legionella – plasmid profile type III predominated in the Victoria Wing while types II and VI predominated in Centennial Wing. Twenty-four of our 28 cases of nosocomial Legionnaires' disease occurred in the Centennial Wing. Three of the four cases in the Victoria Wing were caused by plasmid profile type III while 18 of the 24 isolates from patients who acquired their infection in the Centennial Wing were type II. We conclude that each water outlet serves as its own ecological niche of *L. pneumophila*.

INTRODUCTION

Nosocomial Legionnaires' disease is an important problem in some hospitals [1]. Contamination of the potable water of such hospitals has been linked epidemiologically to cases of Legionnaires' disease [2]. However, legionellae are ubiquitous contaminants of water and their presence in a hospital's water supply does not always result in disease in patients [3].

A number of studies of factors influencing colonization of the potable water of a hospital have been carried out [4–6], however little information is available on the natural history of colonization of a hospital's water supply system by legionella. Cases of nosocomial Legionnaires' disease at our hospital [7] prompted us to investigate our water supply as the source of the legionella. Detailed

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epidemiological investigations led us to conclude that drinking contaminated potable water was the source of the legionella. Hyperchlorination eradicated legionella from the potable water and resulted in cessation of cases of nosocomial legionellosis. Subsequent plumbing leaks due to hyperchlorination led us to abandon this measure. We then sampled the water biweekly for legionella and this gave us an opportunity to describe the natural history of legionella in our hospital's water supply.

METHODS

The hospital

The Victoria General Hospital is a 710-bed, tertiary-care referral centre. It consists of two wings – the Victoria Wing built in 1948 and the Centennial Wing built in 1967. There are 352 beds in the Victoria Wing and 358 in the Centennial Wing. The potable water plumbing in Centennial Wing consists of copper only, while in the Victoria Wing the risers are brass and 90% of the distribution pipes are copper and 10% are brass. Many dead ends are present in the Victoria Wing while there are none in the Centennial Wing. The hot-water heaters are of the recirculating type without storage tanks. The hot-water temperature at different taps on the same day ranged from 44–50 °C. The circulating hot water had a temperature of 60 °C. The pH of the water ranged from 7·8 to 9·0.

Continuous hyperchlorination (5 parts per million) of the water was carried out from 6 February 1986 until 8 October 1986. Pulse chlorination at 10 p.p.m. was carried out on 24–25 January 1987; 14–25 March 1987; 1–3 May 1987; 24–26 August 1987; 24–25 January 1988; 12–15 August 1988. Chlorination was discontinued on 13 September 1988 on the advice of our engineers because of many leaks in the pipes.

The water was superheated for 1 week (14–20 March 1987). The water was heated to 80 °C centrally with temperatures ranging from 71–76 °C at the taps. This resulted in the rupture of one of the risers in Victoria Wing.

Sampling of potable water

Twenty water outlets, 10 from each building, were randomly selected for sampling from 6 February 1986 to 8 May 1990. Water was collected at approximately 2-week intervals. Water samples were obtained by turning on the hot and cold water taps so that the water flowed slowly. The hot and cold water systems were not sampled separately. The cold water was cultured as it enters the hospital. Two hundred millilitres of water was then collected into a sterile bottle containing 0·1 ml of 10% solution sodium thiosulphite. Water samples were collected on the same day of the week at each sampling time between 10.00 and 12.00 hs. Fourteen outlets were taps and six were shower heads.

Culture of water for legionella

Water samples (50 ml) were centrifuged at 3000 rpm for 20 min. The supernatant was removed, leaving approximately 10% of the original volume in which the sediment was resuspended. A sterile cotton tipped swab (Salam Manufacturing

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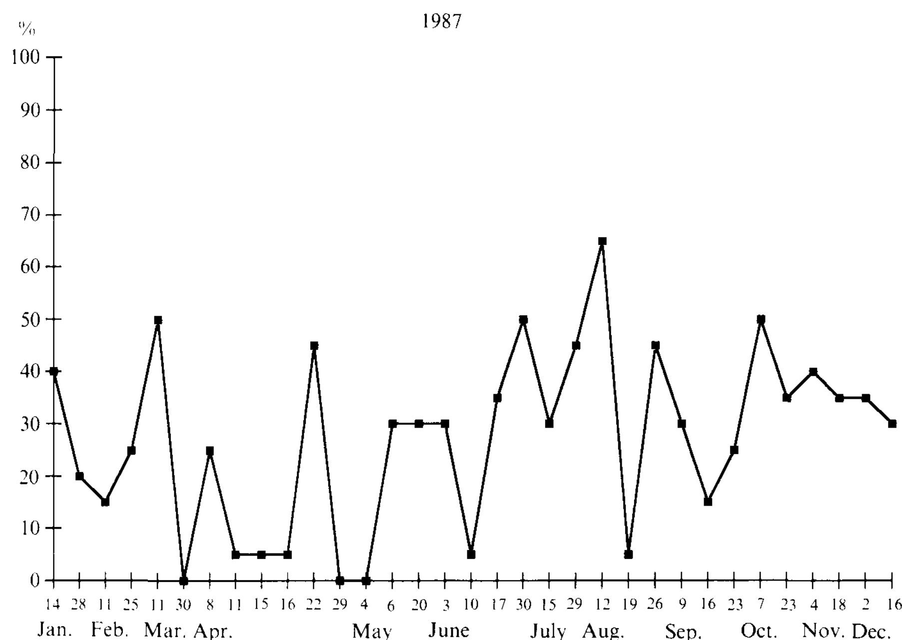

Fig. 1. Percent of water samples positive for *Legionella pneumophila* at each sampling time during 1987. Twenty water samples were collected from the same sites at each sampling time. Pulse chlorination at 10 p.p.m. for 48 h was carried out on these dates: 24–25 January; 14–25 March; 1–3 May; 24–26 August. Superheating to 70 °C was carried out during 14–20 March.

Company, Salam, MN) was then used to inoculate the surface of the following media: 5% sheep blood agar; buffered charcoal yeast extract agar (BCYE) containing 0·1% α-ketoglutarate (Gibco Laboratories, Madison, WI) [8]. Early in the course of this study we also used BCYE agar with 0·1% α-ketoglutarate, cefamandole, polymyxin B, anisomycin, and BCYE agar with 0·1% α-ketoglutarate, polymyxin B, anisomycin, and vancomycin (Gibco Laboratories, Madison, WI). These additional plates did not increase our yield over the use of only BCYE agar containing 0·1% α-ketoglutarate. All plates were incubated at 37 °C, in a humidified atmosphere containing 5% carbon dioxide, for 7 days and were examined daily. Colonies that morphologically resembled legionella were cultured onto blood and BCYE agar. Those that failed to grow on blood agar were examined by a direct fluorescent antibody technique [9] using *L. pneumophila* serogroup 1 antisera (Centers for Disease Control, Atlanta, GA).

In a separate set of experiments we found that when a single swab was used to inoculate two plates the results were identical to plating 0·1 ml of sediment on each plate.

Plasmid profiles

Portions of the growth achieved after 48 h incubation of the isolates on BCYE agar were suspended in 0·5 ml of TE buffer (0·5 M Tris–HCl pH 8·0, 0·02 M–EDTA). After pelleting and resuspending in 25 μl of TE, plasmid DNA was extracted from the cells using a modified alkaline SDS procedure [10]. The contents of the extracts

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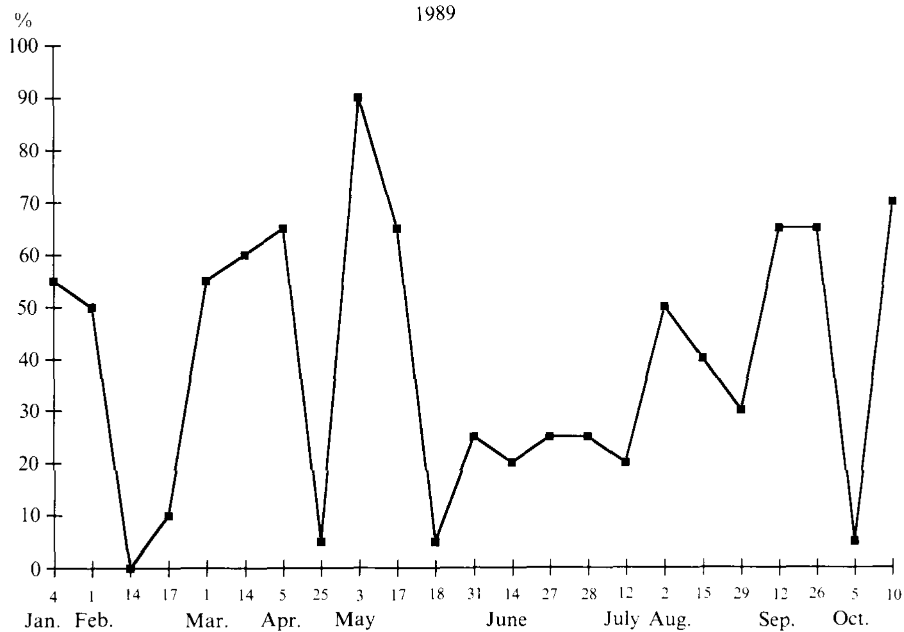

Fig. 2. Percent of water samples positive for *Legionella pneumophila* at each sampling time during 1989. Twenty water samples were collected from the same sites at each sampling time.

Table 1. *Number and percent of water samples positive for* Legionella pneumophila *serogroup I. The number within [square brackets] indicates the number of times this site was sampled that year*

	Site	Shower (S) or tap (T)	1986 [41] No. (%)	1987 [31] No. (%)	1988 [17] No. (%)	1989 [20] No. (%)
1	2A	T	9 (22)	11 (35)	6 (35)	13 (65)
2	NICU	T	2 (5)	15 (48)	3 (18)	8 (40)
3	3A	T	3 (7)	16 (52)	8 (47)	9 (45)
4	4A	T	14 (34)	14 (45)	7 (41)	12 (60)
5	5B	T	9 (22)	18 (58)	11 (65)	10 (50)
6	6A	S	2 (5)	12 (39)	7 (41)	15 (75)
7	7B	T	5 (12)	7 (23)	5 (29)	8 (40)
8	8A	S	0 (0)	11 (35)	6 (35)	5 (25)
9	9B	T	2 (5)	12 (39)	7 (41)	8 (40)
10	EMERG	T	4 (10)	6 (19)	8 (47)	13 (65)
11	4S	T	2 (5)	4 (13)	3 (18)	6 (30)
12	5N	T	12 (29)	9 (29)	4 (24)	5 (25)
13	6W	S	5 (12)	0 (0)	2 (12)	11 (55)
14	7N	T	1 (2)	2 (6)	2 (12)	6 (30)
15	7S	T	2 (5)	2 (6)	0 (0)	6 (30)
16	8W	T	8 (20)	5 (16)	4 (24)	9 (45)
17	8NTU	T	3 (7)	9 (29)	3 (18)	9 (45)
18	9V	T	3 (7)	5 (16)	2 (12)	7 (35)
19	10V	S	3 (7)	9 (29)	2 (12)	9 (45)
20	11V	S	7 (17)	11 (35)	6 (35)	6 (30)
	Overall		96 (11.7)	178 (28·7)	96 (28·2)	175 (41·6)

Sites numbered 1–10 are in the Centennial Wing; those numbered 11–20 are in the Victoria Wing.

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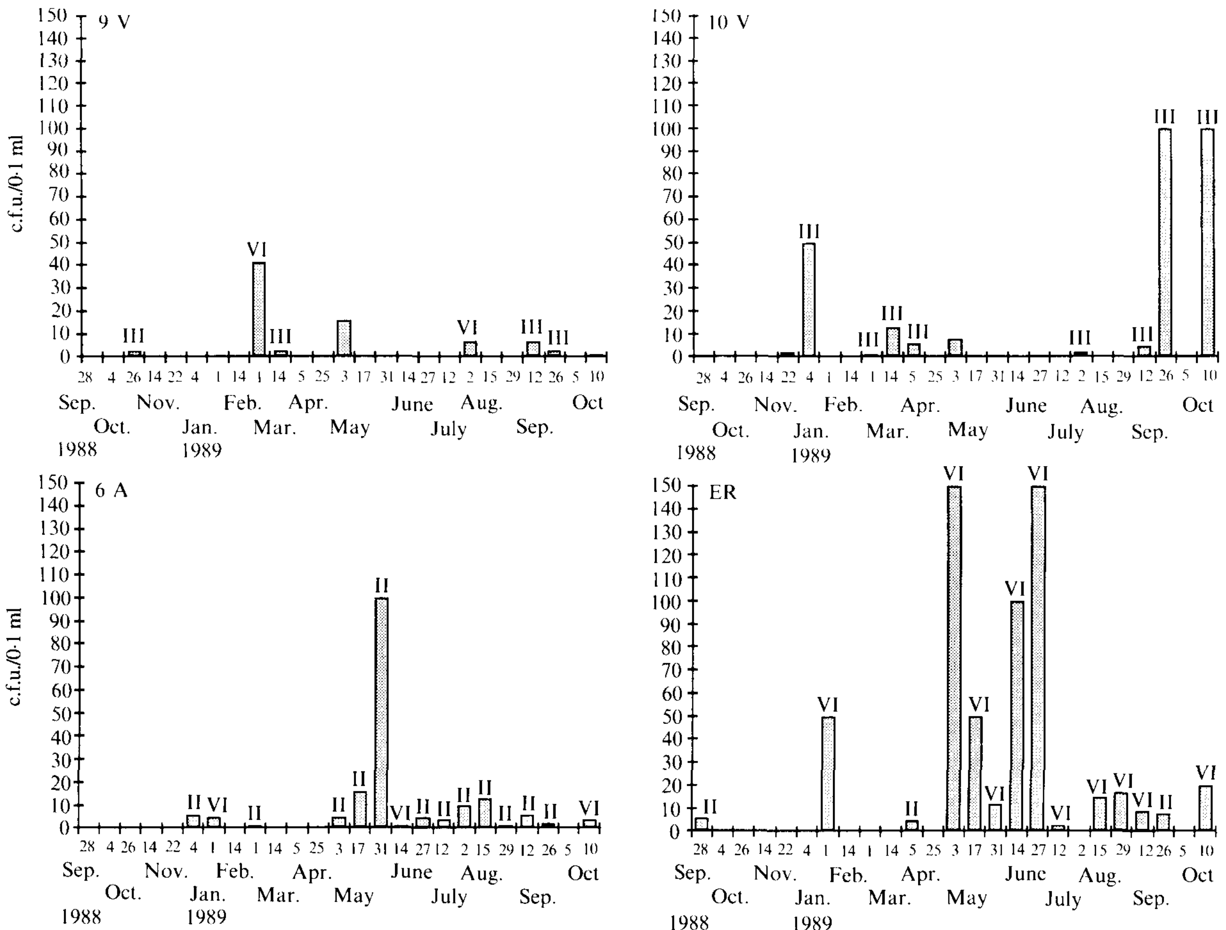

Fig. 3. Semi-quantitative culture results – number c.f.u. of *Legionella pneumophila* at four different sampling sites (9V, 10V, 6A and ER) at the times indicated. 9V, 10V, are in the Victoria Wing, 6A, and ER are in the Centennial Wing. Note that there is no inter-site correlation. The Roman numeral above each bar indicates the plasmid profile of that isolate of *L. pneumophila*. Note, however, the relative constancy of the type of legionella at each site, that is, there is intra-site constancy.

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Table 2. *A comparison of the rate of water cultures positive for* Legionella pneumophila *for each of two wings of the Victoria General Hospital*

Year	Centennial Wing No. positive/ no. sampled	Victoria Wing No. positive/ no. sampled	*P*
1986*	51/410	46/410	N.S.
1987	122/310	56/310	< 0·00000
1988	61/170	26/170	< 0·00002
1989	101/200	74/200	< 0.008

* Hyperchlorination was carried out during 1986.

were determined by electrophoresis in vertical 0·75% agarose gels followed by eithidium bromide staining. The plasmid profiles were: 0, no plasmid; II, 20 MDa plasmid; III, 96 and 72 MDa plasmids; IV, 70 MDa plasmid; V, 89 MDa plasmid; VI, 100 MDa plasmid; VII, 58 and 103 MDa plasmids. Generally one colony per plate was selected for plasmid profiling. Up to ten colonies were selected on occasion. Only one plasmid type was found each time.

RESULTS

The cold water (sampled six times) as it entered our hospital was never positive for legionella. Over the 4 years of the study 2200 water specimens from the 20 sites were cultured – 545 (24·7%) grew *Legionella pneumophila* serogroup 1. No other serogroups or species of legionella was isolated. The percentage of samples positive on a yearly basis ranged from a low of 11·7% during 1986 when hyperchlorination was carried out for 8 months to a high of 41·6% during 1989 when no chlorination was carried out. Figs. 1 and 2 show the percent of samples with detectable *Legionella pneumophila* at each sampling time for the years 1987 and 1989. Note the marked variation and the lack of any seasonal trend. Table 1 shows the percent of samples positive at each site, for each of the 4 years of the study. Considerable variation in the percentage of samples positive is evident at each site. Fig. 3 shows the number of colony-forming units (c.f.u.) per 0·1 ml of water sediment isolated from four separate areas and the plasmid profile of some of these isolates. Two of the areas (9V, 10V) are in the Victoria Wing, 6A and ER are in the Centennial Wing. Note that there is no inter-site correlation either qualitatively or quantitatively, but there is intra-site constancy – the same plasmid profile tends to persist and a site tends to have the same range of c.f.u. of legionella recovered (see 6A and ER, Fig. 3). Analysis of semi-quantitative culture results at other sites showed no trend for higher counts to occur in one of the two wings. However, the rate of positivity was higher in the Centennial Wing for 1987, 1988, 1989 (Table 2).

Three predominant plasmid profiles were observed among isolates from the potable water: type II containing a single 20 MDa plasmid, type III composed of 72 and 96 MDa plasmids and type VI with a 100 MDa plasmid (Table 3). Two additional plasmid types were isolated once only, types V and VII. There was a difference in the distribution of the various plasmid types of legionella between the two wings – types II and VI predominated in Centennial Wing while type III

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Table 3. *Distribution of plasmid profiles among environmental isolates of* Legionella pneumophila *according to the site of isolation – Centennial or Victoria Wings*

Plasmid profile:	No. with this profile: Centennial Wing	Victoria Wing	P
Water isolates			
II	87	29	< 0·00000
III	12	60	< 0·00000
V	1	0	
VI	43	12	< 0·001
VII	1	0	
Total no. of isolates	144	101	
Patient isolates			
II	18	1	
III	2	3	
V	1	0	
VI	3	0	
Total no. of isolates	24	4	

predominated in Victoria Wing. Four of our 28 cases of nosocomial Legionnaires' disease occurred in the Victoria Wing while 24 cases occurred in the Centennial Wing. The plasmid profiles of the patient isolates mirrored those of the predominant water isolates in each wing (Table 3). Thus 3 of the 4 patients who acquired their legionella in the Victoria Wing had plasmid profile III, while 18 of the 24 (75%) patients whose legionella was acquired in Centennial Wing has plasmid profile type II isolated.

DISCUSSION

Our study shows that once established, legionella may persist in the water supply of an institution. Even allowing for the limits of detection of our semi-quantitative technique it is evident that marked variations in the number of organisms occur at each sampling site (Fig. 3). We sampled each site between 10.00 and 12.00 hs to avoid the high counts that may have resulted from the outlet not being used overnight. There was no intra- or inter-site consistency in numbers of legionella discovered. Likewise, when the system as a whole was considered, marked fluctuations in the positivity rate were evident (Figs 1, 2). However, there was consistency in the plasmid complements of the strains recovered from three of the sites studied in detail. Moreover the predominant type varied with each site. Thus, individual isolates showed an intra-site consistency.

A large number of studies have elucidated some of the factors that are associated with the presence of legionella in a hospital's water supply. The general consensus is that it enters in low numbers from mains water and multiplies in warm conditions of 20–45 °C [6]. Once in the system the temperature of the water, $\leqslant$ 60 °C [11], presence of amoebae [12], turbidity, organic carbon, zinc, copper and algae [13] are all important factors in maintaining growth. Various components of plumbing systems are more readily colonized by legionella than others [14, 15].

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Neoprene washers in particular enhance growth of this microorganism [15]. Despite physical cleaning and chemical sterilization of taps and replacement of washers with approved brands, eradication of the organism from the plumbing system is often not achieved [5]. Schulze-Robbecke and colleagues [16] have shown that especially the peripheral areas of the hot water system are colonized by legionellae. The plumbing systems in our two wings differ in that all the risers in the Victoria Wing are brass. Also there are dead ends in the Victoria Wing – however the colonization of the plumbing system is higher in Centennial Wing. Also most of the cases of Legionnaires' disease occurred in Centennial Wing.

The water supply to both wings of our hospital is from the same source, and all sites sampled in both wings grew *Legionella pneumophila* serogroup 1 at some time. However, several differences emerged: the rate of isolation was significantly higher in the Centennial Wing; plasmid types II and VI were more likely to be found in Victoria Wing. These plasmid types were reflected in the isolates from patients who acquired Legionnaires' disease in these wings (Table 3). Most of the cases, 24 of 28, of nosocomial Legionnaires' disease occurred in the Centennial Wing. These findings are similar to those reported by Plouffe and colleagues [17] in that two strains of *L. pneumophila* were isolated from the potable water of their hospital. The plasmidless isolates found in the water of one building caused 24 of the 25 cases of nosocomial Legionnaires' disease at their centre. No disease occurred in the building with the plasmid-containing strain. These investigators in a subsequent study showed that the plasmidless strain was more virulent than the plasmid-containing strain. Other studies which have used plasmid profiling of clinical and environmental isolates of *Legionella pneumophila* have shown that isolates with a variety of plasmids can cause disease and the dominant isolate in the environment was also the dominant clinical isolate.

This study was not designed to determine the factors responsible for the higher rate of isolation of legionella from patients in the Centennial Wing. In a 4-year prospective study of nosocomial pneumonia we found that 2·35 times as many cases of pneumonia occurred in Centennial Wing, despite the fact that the bed capacity of the two wings is almost identical. Thus the rate of nosocomial Legionnaires' disease should be 2·35 times more in Centennial Wing. The observed rate of seven times higher suggests that the plasmid type of legionella may indeed play a role in this higher attack rate.

We recognize that other factors such as patient susceptibility and the number of patients at risk needs to be considered. The latter factor is reflected in the higher rate of nosocomial pneumonia in the Centennial Wing.

We were unable to control nosocomial Legionnaires' disease in our hospital by hyperchlorination and intermittent superheating of the water because of difficulties with our plumbing system. The hyperchlorination led to leaks and the superheating resulted in rupture of one of the risers. We did not try to maintain the temperature of the water at the outlets at 55 °C, a measure that others have reported to have eradicated legionella [5]. Instead, we have controlled nosocomial Legionnaires' disease at our hospital by providing sterile water to patients (for drinking, brushing teeth, and taking medications) who are receiving corticosteroid therapy on units where cases have previously occurred. In addition these patients are advised not to shower.

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We conclude that each water outlet represents a unique ecological niche for *L. pneumophila* and that further studies are necessary to define the factors that make each outlet unique.

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