

## Crab meat: a novel vehicle for *E. coli* O157 identified in an outbreak in South West England, August 2011

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### SUMMARY

In August 2011, we investigated an outbreak of *Escherichia coli* O157 in Plymouth, England, utilizing a case-control study and food traceback. Nine cases, eight laboratory-confirmed with *E. coli* O157 phage type 21/28 verocytotoxin 2 and one epidemiologically linked, had onsets from 30 July 2011 to 15 August 2011. We compared cases ( $n=8$ ) with controls ( $n=28$ ) of similar age and sex (median age 61 vs. 55 years, females 75% vs. 61%). Cases were 58 times more likely to have eaten crab (88% vs. 11%; odds ratio 58, 95% confidence interval 4–2700). Eight cases consumed crab sourced from the same supplier who was not registered with the local authority. This outbreak pointed to crab as a possible vehicle of *E. coli* O157 infection. We ensured the withdrawal of crab meat sourced from unregistered suppliers from food venues by 25 August 2011. We also emphasized the importance of only using registered suppliers to the food venues. Since then no further associated cases have been reported.

**Key words:** Case-control study, crab, *E. coli* O157, outbreak, traceback, vehicle.

### INTRODUCTION

Verocytotoxin-producing *Escherichia coli* (VTEC) serotype O157 is an important cause of gastrointestinal infection in humans and has the potential to cause haemolytic uraemic syndrome and death [1]. The incubation period generally ranges from 6 h to 10 days, with a median of 3–4 days [1, 2]. The major reservoirs of *E. coli* O157 are cattle and other farm ruminants

that shed the pathogen transiently in their faeces. The infection is mainly acquired by ingestion of contaminated food derived from infected animals or cross-contaminated during the preparation process [2]. It can be also transmitted by direct contact with infected animals and their environment, or by contact with untreated and recreational water [2–4]. Secondary spread by the faecal–oral route from infected individuals is common.

Most *E. coli* O157 infections are sporadic. In England and Wales the Health Protection Agency (HPA) electronic Foodborne and Non-foodborne Gastrointestinal Outbreak Surveillance System

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(eFOSS) reported from 10 to 17 VTEC O157 outbreaks annually between 2006 and 2010 [5]. National enhanced VTEC surveillance, introduced in England in 2008, collects a standard minimum dataset for each VTEC case in order to identify linkages and improve outbreak recognition [6]. The HPA recommends that local microbiologists send all presumptive VTEC O157 isolates for confirmation and typing to the HPA Gastrointestinal, Emerging and Zoonotic Infections Reference Laboratory (GEZI-RL) in Colindale where isolates are distinguished mainly by phage typing (PT), selected virulence typing [genes for verocytotoxins (VT)] and multi-locus variable-number tandem-repeat (VNTR) Analysis (MLVA).

In this paper we present a small outbreak of *E. coli* O157 that occurred in Plymouth in summer 2011. Plymouth, situated on the south west coast of England, is mostly rural and the incidence rates of *E. coli* O157 are higher than the national English average. From 2007 to 2011 the incidence rates ranged from 2.23 to 3.05/100000 inhabitants of the South West (South) Health Protection Unit [SW(S) HPU] area compared to 1.50 to 2.15/100000 inhabitants of England. Identification of clusters and outbreaks of *E. coli* may be difficult and relies on regular systematic reviews of enhanced VTEC surveillance and laboratory data to identify possible links or common exposures. When we were alerted to four reports of *E. coli* O157 in one week we investigated with the aims of determining whether it was an outbreak, to identify and control the source of the infection, and to prevent further spread.

## METHODS

### Detection of the outbreak

Between 5 August and 11 August 2011 laboratory staff at a hospital in Plymouth reported four females with *E. coli* O157 infection to the SW(S) HPU; the women were aged between 57 and 69 years and lived locally. In completed surveillance questionnaires we noted that three of the reported individuals ate shellfish (two mentioned crab) outside the home at different food venues in Plymouth. All four isolates were *E. coli* O157 PT21/28 VT2 with an indistinguishable MLVA pattern, confirmed at GEZI-RL. On 15 August 2011 we received reports of two adult males who visited Plymouth during their holidays and developed diarrhoea after consumption of a shared crab sandwich purchased at a food venue in Plymouth. They were

subsequently confirmed to have *E. coli* O157 with the same phage type and MLVA pattern.

On 16 August 2011 we convened an outbreak control team (OCT) to identify and control the source of the infection and to prevent further spread. The OCT was composed of multi-agency representatives, both local and regional. We agreed on active case-finding, an epidemiological analytical study, environmental and microbiological investigations, communication strategy, and institute-relevant control measures.

### Case definition and case-finding

We defined a case as an individual with onset of diarrhoea on or after 20 July 2011, residing in or visiting Plymouth in the 10 days before illness, who had either laboratory diagnosis of *E. coli* O157 PT21/28 VT2 with the same MLVA pattern (confirmed case) or had an epidemiological link to a laboratory-confirmed case (epidemiologically linked case).

We searched for further cases by (a) alerting general practitioners (GPs) and microbiologists in Plymouth and adjacent areas and asking them to report any suspected cases to us; (b) requesting the GPs to search retrospectively in clinical records for any patients with an illness compatible with *E. coli* infection; (c) reviewing information from VTEC surveillance questionnaires and the laboratory database for the South West region since mid-July 2012 to look for common exposures and possible links; (d) reviewing, in collaboration with colleagues at GEZI-RL and the Gastrointestinal Division at HPA Colindale, the national VTEC database, to identify possibly linked cases reported from other regions in England and Wales. As part of the wider communication we alerted health protection units and environmental health departments across England.

### Case-control study

We conducted a case-control study to test the hypothesis that infection with *E. coli* O157 PT21/28 VT2 was associated with eating crab obtained from food venues (restaurants, cafes, pubs, etc.) in Plymouth in the 10 days prior to developing diarrhoea.

We used the case definition stated above and defined controls as individuals aged  $\geq 18$  years, residing in Plymouth. We excluded cases and controls who travelled outside the UK or who had a close contact with other individuals suffering from diarrhoea or vomiting in the 10 days before symptom onset of

cases, and before the interview day of controls. We aimed to recruit three healthy controls for each case from people who had had the opportunity to eat out in Plymouth, in order to detect odds ratios (ORs) above 15 as significant at the 5% level. We asked each case to nominate controls from adult family members, friends or acquaintances, not sharing the same household. Where case nomination was not appropriate (e.g. cases who visited Plymouth) or possible, controls were selected through voluntary staff at the local government and health commissioning office.

We designed a questionnaire asking about consumption of seafood, meat and other food items, such as salad leaves and sprouts that might be consumed with fish or meat. We separated food history into two sections, first asking about food eaten out/away from home including takeaways, and second, about food prepared and eaten at home. We also collected information on some other known risk factors for *E. coli* O157, e.g. animal exposure. Questionnaires were completed over the telephone or self-administered by email following instructions given by members of the OCT.

Data from questionnaires were entered into an EpiData file and analysed. We summarized personal characteristics of cases and controls, such as age and sex, and compared them using the Wilcoxon rank-sum test. We analysed exposures between cases and controls to the various food items and calculated ORs and 95% confidence intervals (CIs) for all exposures to describe the magnitude of any possible association between the food item and illness. Fisher's exact test was conducted because of small cell sizes. Analysis was performed in Stata v. 10.0 (StataCorp LP, USA). Exposures with an estimated OR > 1 and  $P < 0.1$  were deemed eligible for inclusion in the multivariable analysis. Further, we analysed exposures between cases and controls, separately for controls that were case-nominated and controls that were recruited from local staff.

### Environmental and microbiological investigations

In conjunction with Plymouth City Council we undertook hygiene inspections of the food venues mentioned by cases. We also visited other Plymouth food venues with crab on the menu and a major shellfish processor in Plymouth that were not associated with our cases.

As there was no leftover crab meat available from the dishes eaten by cases at the time of inspections, we

sampled supplies of fresh crab meat at two venues mentioned by cases where supplied meat was available. Subsequently we sampled supplies of crab meat, both frozen and fresh, at another three Plymouth venues that were not implicated in the outbreak but had reported a similar origin of crab meat as the implicated places. Further, we took environmental swabs and crab meat samples from the Plymouth shellfish processor. All samples and swabs were examined at Bristol HPA Laboratory.

Cases had obtained crab from a number of different food venues. We interviewed the owners of these venues in order to identify and trace the source of crab meat and other shellfish.

## RESULTS

### Case-finding

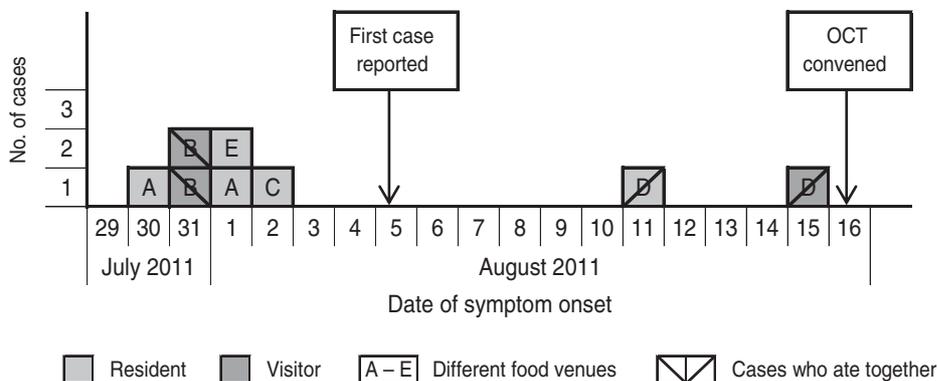
A total of nine cases of *E. coli* O157 were identified in this outbreak. Eight cases, aged between 35 and 69 years (seven females), were confirmed with infection by *E. coli* PT21/28 VT2, with an undistinguishable MLVA pattern. The ninth case, without microbiological confirmation, was epidemiologically linked by time and place to one of the confirmed cases. Six were residents of and three visitors to Plymouth. Symptom onset of confirmed cases ranged from 30 July to 15 August 2011 (Fig. 1), seven reported bloody diarrhoea and two required hospitalization.

Cases had eaten shellfish purchased from food venues in Plymouth in the 10 days prior to developing diarrhoea. Eight ate crab and one had prawns. As shown in (Fig. 2), cases obtained the shellfish at five different food venues. Some cases were linked in time and place, attending the same food venue at the same time.

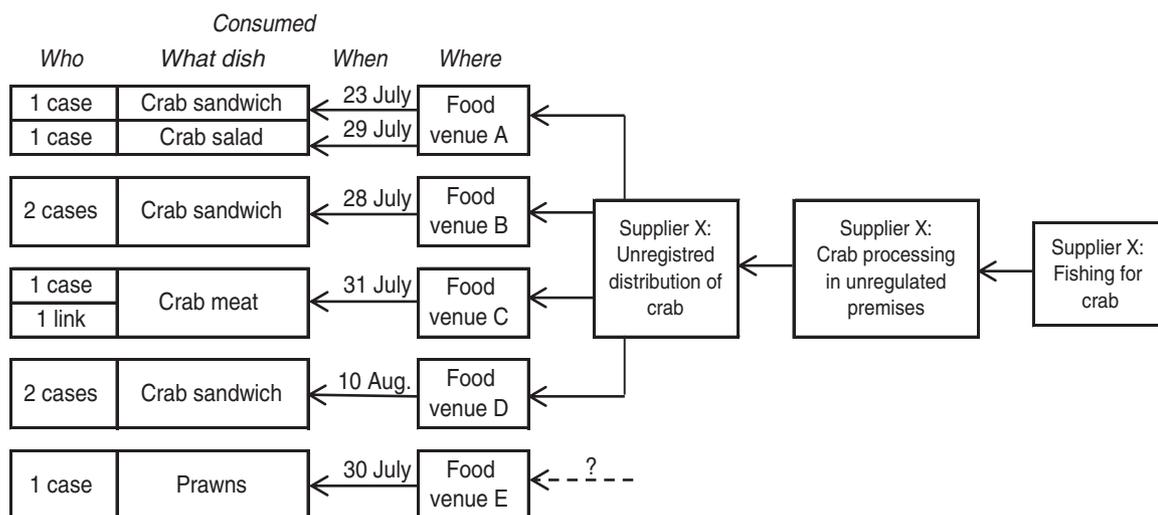
### Case-control study

We included only eight confirmed cases as the epidemiologically linked case was not able to be contacted. Cases nominated a total of six controls. Twenty-two (79%) additional controls were recruited from staff at the local government and health commissioning office in Plymouth.

Age and sex distribution of cases: median age 61 years [interquartile range (IQR) 47–68], 75% females, compared to controls: median age 55 years (IQR 43–62), 61% females, was not statistically significantly different ( $P = 0.32$  and  $0.46$ , respectively).



**Fig. 1.** Onset dates of confirmed cases of *E. coli* O157 PT21/28 VT2, according to resident/visitor status and sharing of meals, Plymouth, UK, July–August 2011 ( $n=8$ ). OCT, Outbreak control team.



**Fig. 2.** Traceback of crab meat, from cases ( $n=9$ ) to the food venues where the shellfish were obtained, and from the venues to the source supplier X, *E. coli* O157 outbreak, Plymouth, UK, July–August 2011.

Cases were more likely to have eaten crab outside the home than controls (88% vs. 11%; unmatched OR 58, 95% CI 4–2725). Multivariable analysis was not undertaken as there were no other exposures with an OR > 1 and  $P < 0.1$  (Table 1).

In analyses performed separately for case-nominated controls and controls recruited from local staff, we still found a statistically significant association between infection and eating crab. Cases were more likely to have eaten crab outside the home than case-nominated controls [7/8 (88%) vs. 2/6 (33%), OR 14, 95% CI 1–785], as well as staff controls [7/8 (88%) vs. 1/22 (5%), OR 147, 95% CI 6–6901].

**Environmental and microbiological investigations**

During the inspection of food venues mentioned by cases we found minor hygiene concerns and poor

practices of food handlers at one venue. These concerns were dealt with formally, serving Hygiene Improvements Notices and emphasizing the need for training of food handlers. Following visits to the other Plymouth food venues with crab on the menu and the major shellfish processor in Plymouth we did not identify any hygiene issues.

Following the interviews with the owners of the food venues we discovered that the crab meat in four of the five implicated food venues was supplied by one crab supplier (supplier X) who was not registered with the local authority (LA), i.e. unapproved in legal terms as a supplier, as well as unregulated under LA procedures, e.g. inspections and food safety checks (Fig. 2).

Supplier X was interviewed under formal caution on suspicion of supplying food unfit for human consumption and for supplying food from an

Table 1. Frequency of exposures in cases ( $n=8$ ) and control ( $n=28$ ), case-control study, *E. coli* O157 outbreak, Plymouth, UK, July–August 2011

Exposure*	Cases	Controls	OR	95% CI	P value
	Exposed (%)	Exposed (%)			
Crab (out)	7 (87.5)	3 (10.7)	58.3	4.2–2725.8	0.000
Pepper (out)	2 (25.0)	1 (3.6)	9.0	0.4–549.8	0.118
Other seafood (out)	2 (25.0)	2 (7.1)	4.3	0.3–67.8	0.207
Steak (out)	1 (12.5)	0 (0.0)	–	0.0–0.0	0.222
Hot fish (home)	5 (62.5)	10 (35.7)	3.0	0.5–22.8	0.236
Hot fish (out)	0 (0.0)	6 (21.4)	0.0	0.0–2.0	0.302
Prawn (home)	0 (0.0)	6 (21.4)	0.0	0.0–2.0	0.302
Beef mince (out)	2 (25.0)	3 (10.7)	2.8	0.2–29.5	0.305
Other beef (out)	2 (25.0)	3 (10.7)	2.8	0.2–29.5	0.305
Pork (out)	2 (25.0)	3 (10.7)	2.8	0.2–29.5	0.305
Herb (home)	1 (12.5)	9 (32.1)	0.3	0.0–3.1	0.397
Pork (home)	1 (12.5)	9 (32.1)	0.3	0.0–3.1	0.397
Cold roast beef (home)	1 (12.5)	1 (3.6)	3.9	0.0–313.7	0.400
Crabstick (home)	1 (12.5)	1 (3.6)	3.9	0.0–313.7	0.400
Cabbage (home)	2 (25.0)	12 (42.9)	0.4	0.0–3.2	0.441
Non-domestic animal contact	1 (12.5)	2 (7.1)	1.9	0.0–40.0	0.541
Turkey (out)	1 (12.5)	2 (7.1)	1.9	0.0–40.0	0.541

OR, Odds ratio; CI, 95% confidence interval.

\* Exposure: 'out' refers to food eaten out/away from home including takeaways; 'home' refer to food prepared and eaten at home.

unapproved establishment, illegally. It was the legal-basis under which a prosecution could be brought.

Supplier X fished for crabs in the sea locally using his own boat. We identified a number of domestic addresses as sites where the crabs were processed. Crab processing usually includes boiling or pasteurization, followed by hand-picking of the boiled meat and packing it for distribution. The process should be a subject of standard food safety control measures, regulated by hazard analysis and critical control point (HACCP) principles. These domestic addresses were not registered or approved for food production and processing, and were not regulated. We were not allowed to access them for inspection.

The distribution of the processed crab meat to food outlets was based on local acquaintances, agreed by word of mouth. The area of distribution of the supplied crab was limited to Plymouth and the last distribution occurred on 15 August 2011.

The food samples of supplied crab meat and environmental swabs that we took at different food venues and at the major shellfish processor did not show any microbiological evidence of *E. coli* O157. However, some samples revealed microbiological

contamination of *E. coli* of a different serogroup and other pathogens (Table 2).

### Control measures

During the interview, under caution, the unregistered supplier was formally warned of the consequences of continuing to supply crab processed in unapproved premises thereby committing offences under the Food Safety Act. We have no evidence to suggest that this supplier is still supplying food businesses with crab meat. We also warned the caterers who sourced their crab meat from the unregistered supplier about potential adverse public health consequences of such action.

By 19 August 2011 we had most crab meat sourced from the unregistered supplier removed from the food venues, by agreement with the managers of the food venues or if necessary by seizure using legislative powers. Some additional crab meat that we identified later at another food venue not implicated in the outbreak was seized on 25 August 2011.

We provided written information to all food retail outlets in Plymouth emphasizing the importance of

Table 2. Environmental and food samples taken for microbiological testing and the results, *E. coli* O157 outbreak, Plymouth, UK, July–August 2011

Sample date	Sample place	Sample origin	Result
10 Aug.	Venue A	Crab sandwich	Negative for <i>E. coli</i> , Enterobacteriaceae and aerobic colony count
16 Aug.	Venue A	Coleslaw Fresh crab	Positive for <i>E. coli</i> (not O157) Positive for Enterobacteriaceae and positive aerobic colony count
		Unopened fresh crab in packet 6 × fresh crab meat	Positive for <i>E. coli</i> (not O157) Negative for <i>E. coli</i> O157
17 Aug.	Registered processor*	Swabs and fresh crab samples	Negative for <i>E. coli</i> O157
18 Aug.	Venue D Venue Z†	Fresh (previously frozen) crab meat Fresh (previously frozen) crab meat	Negative for <i>E. coli</i> O157 Positive aerobic colony count
23 Aug.	Venue Y†	2 × chilled (previously frozen) crab meat	Borderline <i>E. coli</i> (not O157) and Enterobacteriaceae
25 Aug.	Venue W†	5 × frozen crab meat	Positive for <i>Listeria</i>

\* Major shellfish processor in Plymouth.

† Reportedly these venues received meat from supplier X, no cases were associated with them.

only buying food and ingredients from approved, registered suppliers.

No further cases of *E. coli* O157 associated with the outbreak have been reported.

## DISCUSSION

We identified crab meat as a likely vehicle for infection in a community outbreak of *E. coli* O157 in South West England. We showed a large, statistically significant association between the infection and eating crab meat (OR 58). In traceback we identified a linkage to a supplier who was not registered with the LA for food processing and distribution. For all cases who ate crab meat we traced it back to the unregistered supplier. The outbreak strain was characterized by MLVA and in each confirmed case the strain was indistinguishable. While PT21/28 VT2 is not that unusual, the MLVA pattern has a high degree of discrimination [7] and provided microbiological evidence of a link between cases. Nevertheless we did not isolate the outbreak strain of *E. coli* from any food or environmental samples available at the time of investigation and there was no leftover crab meat available from the dishes eaten by cases for microbiological testing.

In our investigation we did not obtain sufficient information on crab fishing, its handling and processing before distribution. Supplier X did not provide a stool sample for microbiological testing. With respect to how the crab meat may have been

contaminated it seems unlikely that the crab was cross-contaminated at food venues before serving as a number of venues were implicated and food items were obtained at different times. Contamination or cross-contamination was likely to occur at some point(s) before the crab meat was distributed.

Crabs could have been infected at the outset in their sea habitat as the coastal water can be polluted by faeces from domesticated animals, by influx of water from field streams or sewage overflow [4, 8–10]. Use of a personal boat with no toilet and hand-hygiene facilities could have contributed to contamination during transportation. Processing of the crabs, including boiling, hand-picking and handling of the boiled meat, at domestic addresses was not regulated according to HACCP principles, with no records on storage temperatures, boiling lengths and temperatures, personnel hygiene, etc. Supplier X could have been a healthy carrier of *E. coli* O157. Contamination of fish and shellfish with *E. coli* due to poor hygiene and sanitary conditions has been documented in the past [11]. However, due to lack of information on handling crabs prior to distribution, and microbiological evidence, we cannot conclude where or how the crab meat became infected with *E. coli* O157.

A variety of different food items have been implicated in VTEC outbreaks in the past, such as undercooked meat (especially beef), unpasteurized milk or cheese, apple cider, melons, vegetables (lettuce, cabbage, celery, spinach), and different sprouts (alfalfa, cress, radish sprouts) [12–25]. Faecal

pollution from domesticated animals in the shellfish culture environment and *E. coli* O157 contamination of shellfish harvested at the coast have been reported [8–10]. To our knowledge this is the first time that human *E. coli* O157 infection has been reported in association with the consumption of crab. This emphasizes the need to take into consideration novel, unknown food items as possible sources or vehicles for *E. coli* infection during outbreak investigations. Further, in this outbreak as in many others, such as the recent large German outbreak of *E. coli* O104:H4 [14], thorough food traceback is essential to identify the source and prevent further spread.

We investigated a relatively small outbreak identified through various enhanced surveillance. As crab dishes tend to be popular in the coastal Plymouth area, particularly in summer, we might have expected more cases if the infected crab meat was distributed on a larger scale. The unregistered supplier was supplying a small number of food venues only in Plymouth and it seems plausible that the population at risk from that distribution chain was also small. However, we identified some food venues that reportedly sourced crab meat from the unregistered supplier and were not associated with any cases. It is possible that some cases may have escaped our case-finding as they had mild symptoms or were asymptomatic.

The small study sample size could potentially under-power the detection of the association with food exposures more prevalent in the general population or with a hidden vehicle such as salad vegetables or other accompaniment. However, there were no other likely exposures identified through the trawling questionnaires and no common accompaniment reported. Five different food venues provided our cases with shellfish dishes, and four of them provided crab sourced from the unregistered supplier. As the implicated dishes were obtained over time ranging from 23 July to 10 August 2011, it appears unlikely that the infection would be associated with any other common food item or garnish.

Some cases were visitors to Plymouth and for these individuals nomination of controls was not appropriate. Therefore we had to choose a different approach. Our aim was to identify a control group that would be representative of the population cases, i.e. having an opportunity to eat out in restaurants and cafes in Plymouth. Taking into consideration the fact that Plymouth is a deprived area [26], people who were in an employment may have been more likely

to dine out than the general Plymouth population. We sought controls among employees at the local government and health commissioning office as they may be more similar to cases in socioeconomic terms. If the staff-recruited controls were similar to cases in this regard then the strength of the association we found might have been artificially reduced compared to what we may have found if we had randomly selected controls from across the city.

On the other hand case-nominated controls might have been even more similar to cases in respect of the exposure. We analysed exposures separately, first for cases and case-nominated controls, and second for cases and staff-recruited controls. We found a statistically significant association between the infection and eating crab in both analyses, with a larger effect when the staff controls were considered.

Our investigations identified the likely crab meat vehicle and ensured its swift removal from retail outlets. The legal consequences of committing Food Safety Act offences were highlighted. We warned the catering sector by alerting food venues about potential adverse public health consequences of using unregistered food sources. The actions taken prevented further spread and will hopefully prevent similar future outbreaks.

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## DECLARATION OF INTEREST

None.

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