Microbiological detection of bacteria in animal products seized in baggage of international air passengers to Brazil

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ABSTRACT

Airline travel favours the transmission of diseases, given the short time it takes to travel long distances. In this study, animal products without health certificates seized in international air passengers’ baggage at Guarulhos (GRU) and Galeão (GIG) airports in Brazil underwent a microbiological evaluation. Analyses (1610) were carried out on 322 seizures to test for the presence of total and thermotolerant coliforms, as well as Staphylococcus aureus counts and the presence of Listeria monocytogenes and Salmonella. Most seizures analysed showed coliform contamination and coliforms were present above acceptable limits in 83.4% (40/48) of the products that had some type of contamination. The second most prevalent microorganism found was L. monocytogenes in 22.9% (11/48) and S. aureus was cultivated in 14.58% (7/48) of seizures. Among the items seized in the present work, Salmonella was found in one seizure of pig sausage. Contamination of animal products with microbiological pathogens of importance to public health and indicators of the bad quality of the food were shown in the present study.

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1. Introduction

Infections caused by bacteria in humans are often dangerous and may be occasionally lethal. Salmonella are enteric microorganisms and clinical symptoms may vary from asymptomatic carriage to life-threatening systemic infections (Elad, 2013). Among the food-borne illness,
Salmonellosis is the most prevalent worldwide (Funk et al., 2005).

Listeria monocytogenes can cause sporadic meningitis in humans, before being recognised as an opportunistic, food-borne pathogen of human, cattle and wild animals. Listeriosis can constitute a life-threatening disease in the elderly and in immunocompromised patients; in pregnant women, can cause still-birth or frequently lethal neonatal infections (Cossart and Lebreton, 2014). Escherichia coli can cause diarrhoea, urinary tract infections, respiratory illness and pneumonia, as well as other symptoms in humans, while Staphylococcus aureus are important bacteria that cause infections in a wide range of conditions in humans and animals, from mild skin infections to life-threatening bacteremia, economic losses in both terms of animal and human health (Leonard and Markey, 2008; Williams et al., 2013). Over time, the transport and dissemination of pathogenic bacteria in food has caused health problems over the world (Yilmaz et al., 2009; CDC, 2014).

Animal products in baggage introduced illegally do not follow any specific sanitary standard, thereby creating a risk to public health. Air travel can lead to a quick global dissemination of bacteria and airports are currently areas of high flow of individuals coming from various parts of the world, where diseases may be introduced and disseminated (Wilder-Smith et al., 2003; Hartnett et al., 2007; Schneider, 2011).

The International Airport of São Paulo (Guarulhos – GRU Airport) is the busiest airport in Brazil, and it also has the second highest number of international flights in the Southern Hemisphere, behind Sydney International Airport. In terms of cargo, it is the largest in Latin America and 66th in the world. The International Airport of Rio de Janeiro (Galeão Airport – GIG) is the second busiest in Brazil for international passenger flights. It is the main gateway to Brazil, considering that about 40% of foreign tourists choose Rio de Janeiro city as their destination (Infraero, 2014).

Brazil will also be the host of upcoming major sporting events, such as the Olympics and Paralympics in 2016, which will increase passenger movement, as well as the risk of introduction of strains of infectious agents through airports. Therefore, strategic measures need to be taken to restrict the entrance of infectious agents into the country in baggage of international air passengers.

Brazilian authorities work to study and expand veterinary surveillance in international airports to evaluate the introduction of agents that may compromise public health. The objective of this study was to analyse microbiologically seized of animal products in air passenger baggage on international flights, for faecal coliforms and S. aureus (as indicators of the degree of safety of the products) and, L. monocytogenes and Salmonella under the perspective of public health.

2. Materials and methods

2.1. Products seized at airports and transport to the laboratory

Animal products without health certificates in international air passenger baggage were seized at Guarulhos (GRU) and Galeão (GIG) Airports in Brazil. The minimum sample size was calculated according Thrushfield (2004), in accordance with a confidence level of 95%, desired absolute precision of 5% and an observed prevalence of 10%. Previous studies on observed prevalence in animal products showed an observed prevalence of approximately 10% (range of 9.1–11.9%) based on studies by Jonnalagadda and Bhat (2004) – Salmonella spp. in 11% shrimp samples in India; Busani et al. (2005) – Salmonella and Listeria was detected in 10.3% in raw pork in Italy; de Boer et al. (2009) – S. aureus in 10.6% (cattle beef) and 10.7% in pork; Lee et al. (2009) – E. coli contamination in fresh beef, poultry, and pork resulting in an overall isolation rate of 9.1% in Korea, and Di Pinto et al. (2010) – L. monocytogenes in 105/1045 (10%) ready-to-eat (RTE) foods from supermarkets in Southern Italy. Therefore, 138 seizures per airport were determined as the minimum number. Nevertheless, a higher number was effectively collected (322 items – dairy and meat) were seized in the two airports for laboratory analysis.

The seizures were carried out on twelve occasions from April 23, 2010 to August 19, 2011 (six missions in each Airport) to obtain the illegal products in international air passenger baggage. Together with the International Agricultural Surveillance of the Ministry of Agriculture, Livestock and Supply (VIGIAGRO/MAPA), following routine procedures, baggage was inspected using a non-invasive scanning equipment. Those with organic products were intercepted, and those products of animal origin were seized for the laboratory analyzes. Other products, such as those of plant origin, that were not of interest for the present work, were destroyed using the standard protocols at each airport.

Passengers from 119 international flights of 35 air companies were intercepted by the official service in these two airports, according to Brazilian standard protocols (Brasil, 2006). After seizure, no packaging was violated to avoid contamination and all products remained under refrigeration until analysis. All seizures were catalogued and packaged in biosecurity boxes (BVQ/Bureau Veritas Certification, ISO 9001:2008) according to IATA (2010) for air transport of dangerous cargo, monitored by the project coordinator and authorised by the Brazilian Official Veterinary Service. These were transported immediately by air to the National Agricultural Laboratory (LANAGRO-MG/MAPA) in Minas Gerais, Brazil, to perform bacteriological analyses, following protocols listed below and with official permission of the General Coordination of Laboratory Support (CGAL/MAPA).

2.2. Application of tests in the laboratory

We determined total and thermotolerant coliforms, S. aureus, L. monocytogenes and Salmonella. These bacteria were chosen because laboratory analyzes for these pathogens were well established in LANAGRO-MG/MAPA and had undergone accreditation criteria according to ISO 17025/2005 (INMETRO, 2005). The tests could not be changed to provide reliable laboratory analyzes for all products, maintaining sensitivity and specificity. In LANAGRO-MG/MAPA, a total of 1610 analyzes were performed on the 322 products to verify the presence of...
pathogens. The samples were opened only after arrival at the laboratory to avoid external contamination. Analyses began immediately after the arrival at the laboratory. Before opening the sample, asepsis of the packing was carried out using cotton wool embedded in a disinfecting solution and 70% ethanol. Samples from various points (surface and interior) of the seized product were cut (using sterilised tweezers, scissors and scalpels) and weighed (25 ± 0.2 g), transferred into plastic bags and this pool homogenised in a stomacher. All microorganism determinations were carried out according to Brasil (2003) official procedures.

2.3. S. aureus, faecal and thermotolerant coliform counts

S. aureus count was carried out by inoculating desired dilutions of the samples in Baird-Parker Agar. The composition of this agar evidences the ability of the microorganism to grow in the presence of 0.01–0.05% potassium telluride in combination with 0.2–0.5% lithium chloride and 0.12 to 1.26% glycine. Baird-Parker Agar was supplemented with egg yolk solution to verify proteolytic and lipolytic activities of S. aureus through the appearance of a transparent halo and precipitation around the colony, respectively. S. aureus ATCC 25923 (ATCC®, Manassas, VA, USA) was used as a positive control, and results were expressed as a power of 10 × 10⁹ colony forming units/gram – CFU/g.

We determine total and thermotolerant coliforms count using a presumptive test based on the inoculation of the desired dilutions of the samples under neutral crystal Violet Red Bile Agar (VRBA) and subsequent counting was performed on suspicious colonies. For faecal coliforms, a confirmatory test was performed and the confirmed presence of total coliform performed by inoculation of suspected colonies on brilliant green bile broth with 2% lactose and subsequent incubation at 36 ± 1 °C. The confirmation of the presence of faecal coliforms was performed by inoculation of suspect colonies in EC broth and further incubation at temperature of 45 ± 0.2 °C in a water bath with agitation and the results were expressed as CFU/g.

2.4. L. monocytogenes detection and the presence of Salmonella

L. monocytogenes detection was performed by biochemical analyzes in the sample pool verifying catalase production, observation of the morphological and staining characteristics, verification of typical growth in semi-solid media and by testing the inability to reduce nitrate and checking the positive reactions of methyl red and Voges-Proskauer. The L. monocytogenes strain ATCC 19112 (ATCC®, Manassas, VA, USA) was used as a positive control and the results were expressed as presence or absence of L. monocytogenes/25 g.

The presence of Salmonella was tested using a pre-enrichment step based on incubation at 36 ± 1 °C for 16–20 h on 25 ± 0.2 g or 25 ± 0.2 mL of the sample (sample pool) added to 225 mL of buffered saline peptone water. In a second stage, we cultured this with Rappaport-Vassiliadis and Tetrathionate Broth. Isolation and selection were based on the selection of colonies of Salmonella in two solid media, Brilliant Green Phenol Red Lactose Sucrose (GLP) and Rambach Agar and subsequent biochemical identification. Finally, the agglutination test, based on antigen-antibody reaction with the antigen agglutination against the polyvalent Salmonella “O” antiserum was performed. As a positive control, the strain S. typhimurium ATCC 14028 (ATCC®, Manassas, Virginia, USA) was used and results were expressed as presence or absence of Salmonella spp./25 g.

2.5. Statistical analysis

All analyses were carried out using SAS v.9.2 (Statistical Analysis System INC, Cary, NC, USA). Total coliform (TC), thermotolerant coliform count (TCC) and S. aureus counts were transformed on positive counts using logarithm. An analysis of variance was carried out using general linear model (PROC GLIMMIX) to see if levels of bacteria were higher in dairy or meat, and within dairy to see if there was a difference between products from sheep or cattle, as well as region of origin. Salmonella was not analysed as there was only a single positive sample. L. monocytogenes was analysed for the presence (1) and the absence (0) in a chi squared test adjusted for small numbers using Fisher’s exact test.

2.6. Ethical aspects and special governmental authorizations

The present study had its technical and ethical procedures approved by the Brazilian National Council for Scientific and Technological Development (CNPq) through process number 578255/2008-1 and special permits were obtained from the General Coordination of International Agricultural Surveillance of the Ministry of Agriculture, Livestock and Supply (VIGIAGRO/MAPA – number 294/2010) as well as the Customs (number 00571/2009). No information that could violate privacy of passengers was obtained or used in this study. Following standard procedures, all passengers signed a notice of seizure of illegal products in their baggage. Also, passengers who had illegal products seized were released and did not pay any fines or fee.

3. Results and discussion

Table 1 shows the mean (non-transformed) bacteria colony forming units and proportion of products positive for bacteria seized in Brazilian airports. Total coliform count was associated with the type of product (higher in meat than dairy), and species of animal (higher in other products such as duck compared with cattle, sheep and pigs). The count was also higher in products that originated in Asia. S. aureus and thermotolerant coliform count were not seen to be associated with any of these factors. Although some tendencies were seen, the low number of samples may have affected this outcome.

Considering that these bacteria are mainly indicator of the degree of safety of the product and that in Brazil the acceptable number for S. aureus is lower that 100 (1 × 10²) CFU/g or mL and for total and thermotolerant coliform the maximum tolerated number is equal to 100.
Table 1
Mean number bacteria colony forming units for positive samples seized in Brazilian airports.

<table>
<thead>
<tr>
<th>Type</th>
<th>TCC Mean (10^2 CFU/g or mL)</th>
<th>TC Mean</th>
<th>S. aureus Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Dairy</td>
<td>115,000</td>
<td>4</td>
<td>5021</td>
</tr>
<tr>
<td>Meat</td>
<td>70,986</td>
<td>36</td>
<td>30,000</td>
</tr>
<tr>
<td>Species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>60,534</td>
<td>29</td>
<td>5782</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>150,000</td>
<td>2</td>
<td>150,000</td>
</tr>
<tr>
<td>Sheep</td>
<td>86,125</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Swine</td>
<td>10,000</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asia</td>
<td>150,000</td>
<td>3</td>
<td>100,000</td>
</tr>
<tr>
<td>Europe</td>
<td>68,152</td>
<td>29</td>
<td>4318</td>
</tr>
<tr>
<td>NI</td>
<td>4100</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>South America</td>
<td>83,571</td>
<td>7</td>
<td>4889</td>
</tr>
</tbody>
</table>

N – number of positive samples; NI – not informed. TCC – thermotolerant coliform count; TC – total coliforms. Numbers followed by the different letters in the same column differ significantly by the Chi square test (p < 0.05).

(1 × 10^2) CFU/g or mL in samples of feedstock, food and feed (Brasil, 2003), the results of the present study demonstrate contamination levels far above from the limits established that by law, mainly for total coliforms count: ham (cattle), meat (duck tongue) and sausage (swine), cheese (cattle and sheep); thermotolerant coliforms count: meat (duck tongue) and cheese (cattle and sheep); count of S. aureus: meat (cattle) and cheese (cattle).

No significant effects were found for the presence of L. monocytogenes per region, type of product (dairy or milk) but was affected by species (P < 0.05) with a greater risk from products of origin other than cattle (Odds ratio = 6.34, CI = 1.48–27.22) (Table 2). Only one sample was positive for Salmonella so no statistical analyses were carried out. Salmonella was cultured from a seizure of swine sausage (seizure 439) from Italy, which occurred at the Galeão Airport (GIG) in Rio de Janeiro. According to what is described in the methodology, the agglutination test, based on antigen-antibody reaction with the antigen agglutination against the polyvalent Salmonella “O” antiserum was performed. As a positive control, the strain S. typhimurium ATCC 14028 (ATCC®), Manassas, VA, USA was used. In fact, one positive sample represents a serious risk for public health.

L. monocytogenes and Salmonella spp. have a public health importance by themselves. In Brazil, the tolerance for the presence of L. monocytogenes and Salmonella in dairy and meat products is zero and the research results are expressed as presence or absence of L. monocytogenes/25 g and the presence or absence of Salmonella spp./25 g. L. monocytogenes was detected in meat (cattle), sausage (swine) and cheese (cattle and sheep). Salmonella was detected in sausage (swine). The detection of L. monocytogenes and Salmonella in the seizures reinforces the danger that these products may pose to public health.

Several types of animal products were intercepted and seized in the baggage including dairy (cheese) and several meat types such as ham, bologna and sausages in general. The seizures were from cattle, buffalo, goat, chicken, llama, rabbit, kudu, sheep, pig and unidentified species origins, when packages were in languages not identified by staff (such as indigenous dialects). Illegal animal products were intercepted from 119 international flights of 35 air companies in these two airports and the focus of the present study was microbiological analysis. In another study we analysed and describe the profile of the passengers considered as offenders (de Melo et al., 2014).

The seizures were from 48 countries (South Africa, Angola, Egypt, Morocco; Canada, Costa Rica, Cuba, United States, Mexico, Panama, Puerto Rico; Argentina, Bolivia, Chile, Colombia, Peru, Uruguay, Venezuela; China, Korea, India, Japan, Taiwan; Spain, France, Italy, Portugal; Romania, Turkey; Australia; Germany, Holland, Hungary, Norway, Poland, United Kingdom, Switzerland, Qatar, United Arab Emirates, Israel, Iran, Iraq, Israel, Lebanon, Turkey; Russia and its borders Lithuania and Ukraine).

In the 10 contaminated meat samples, total coliforms were present in samples from South Korea, China and England. Thermotolerant coliforms were found in samples from China and L. monocytogenes from Spain, Argentina, England and France. Salmonella was found in a sample from Italy and it was not possible to identify the origin of S. aureus.

In the cheese seizures, total coliforms were from Italy, Bolivia, Portugal (16 seizures), France, Spain, Turkey and Peru. As for thermotolerant coliforms, these were detected in samples from Peru, France and Portugal, while S. aureus was found in samples from Peru and Bolivia. The presence (in 25 g) of L. monocytogenes was seen in samples from England and Portugal. Positive S. aureus counts were observed in dairy/cheese from cattle, while L. monocytogenes were present in seizures of both cattle and sheep products.

No specific country of origin pattern was seen for bacteria distribution, but cheeses from Portugal stood out. In total, bacteria were detected in cheeses and meat from 11 countries (France, Italy, Bolivia, Spain, England, Portugal, Turkey, South Korea, Argentina and China). Italian cheeses had a high total coliform count indicating possible hygiene problems during manipulation. Swine sausage from Italy was seen to be contaminated with Salmonella, as the paper from Busani et al. (2005), who detected Salmonella...
Enterica and *L. monocytogenes* contamination in food of animal origin in Italy. Di Pinto et al. (2010) detected *L. monocytogenes* in ready-to-eat food from supermarkets in Southern Italy. Lee et al. (2009) isolated *E. coli* from fresh beef, poultry, and pork in Korea. Our study also found *E. coli* in cattle meat (ham) from Korea.

In a study carried out in 2012 and 2013 by Schoder et al. (2014), 5% (30/600) contamination was seen in confiscated animal products from baggage in Vienna International Airport (VIE airport) – Austria, where *Salmonella* spp., *Campylobacter* spp., verotoxigenic *E. coli* and *L. monocytogenes* were isolated. Considering the intense airline traffic from Africa, Asia, Russia, China and Turkey, the problems presented in VIE airport are different from those observed in our case because the seized products mainly originated from South America (Peru, Bolivia, Argentina and Uruguay) or Latin European countries (France, Italy and Portugal).

Nevertheless, products of Chinese origin were similar in both papers. Difficulties in protecting borders and awareness of the population about the health dangers of carrying products without health certificates remain a major challenge in both studies.

While dairy products had significantly (*P* < 0.05) a higher proportion of thermodurable coliforms and *S. aureus*, meat had a higher proportion of thermodurable coliforms, *Salmonella* and *L. monocytogenes*. Within dairy products there was no significant difference (*P* > 0.05) between cattle and sheep for the level of infection. Nevertheless, only cattle products had thermodurable coliforms and *S. aureus*.

There were 40 products contaminated with faecal coliforms and coliforms were present in 83.3% (40/48) of the products that had some type of contamination. The second most common type of microorganism found in contaminated products was *L. monocytogenes* (11 contaminated products). The presence of *S. aureus* was seen in 14.6% (7/48) of products collected and *Salmonella* was found in one seizure of swine sausage.

In relation to the seizures, it is important to note that 79.2% of the products were of dairy origin and 20.8% was meat from various animal species. In the present study, of the seizures that were contaminated by *L. monocytogenes*, 63.6% were dairy products. Recent studies have suggested cheese can represent a significant source of *L. monocytogenes* and potential health risk for listeriosis in Italy (Lomonaco et al., 2012) and another large study reviewed the presence of *L. monocytogenes* in cheese on a worldwide scale, especially those associated with high value artisanal production like soft cheeses (Todd and Notermans, 2011).

Three seizures from Peru (two rabbit meat and one sheep meat) could not be analysed because they were in a state of putrefaction, which demonstrates that these products are usually transported in inadequate conditions and without refrigeration on the flight.

The animal products seized were stored in diverse types of packaging and some had a label indicating that the product was supposedly industrialised, but this does not guarantee the safety of the food since we cannot vouch for the veracity of the packaging and proof of its actual content. There were various other types of product packaging without any identification of the content or origin. These were mostly handmade products, being produced and marketed under inferior hygienic conditions, without refrigeration, and were often packaged in newspapers and underwear, probably in an attempt to fool the authorities. Labelling and packing could not be considered reliable as no international health certificate was produced and may be falsified. Product certification of animal product in international transit is essential as it proves that the product meets security and quality requirements agreed internationally (Pastoret and Chaisemartin, 2011).

There has been growing concern about the importance of meat products as potential carriers of *Salmonella* and *L. monocytogenes*, especially those that are consumed without any post-processing heat treatment (Dong et al., 2014). In our study, 20.8% of the seized positive products were meat. Meat is highly perishable due to its biological characteristics and may be responsible for the transmission of pathogenic bacteria to man (Zhou et al., 2010).

Previous studies have demonstrated the importance of intercepting illegal animal products in airports. In Roissy-Charles de Gaulle Airport, Chaber et al. (2010) estimated the nature and volume of animal products entering Europe illegally via Paris airport, carried by passengers who boarded planes in various parts of the African continent. Most of these passengers had health certificates, which were not legally valid for the majority of livestock products and especially for fresh meat.

There is a great need for scientific studies in airports, mainly in periods preceding major world sporting events. de Melo et al. (2014) identified associations between possession of illegal animal products in baggage and demographic characteristics of the passengers, as well as

### Table 2

Presence of *Listeria monocytogenes* in samples of dairy and meat seized in Brazilian airports with Bayesian binomial confidence intervals.

<table>
<thead>
<tr>
<th>Type</th>
<th>Absence</th>
<th>Presence</th>
<th>Proportion (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy</td>
<td>31</td>
<td>7</td>
<td>0.184 (0.093–0.335)</td>
</tr>
<tr>
<td>Meat</td>
<td>6</td>
<td>4</td>
<td>0.400 (0.167–0.692)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Absence</th>
<th>Presence</th>
<th>Proportion (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29</td>
<td>4</td>
<td>0.121 (0.049–0.274)</td>
</tr>
<tr>
<td>Others&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2</td>
<td>0</td>
<td>0.000 (0.000–0.708)</td>
</tr>
<tr>
<td>Sheep&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>4</td>
<td>0.500 (0.212–0.788)</td>
</tr>
<tr>
<td>Swine&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>3</td>
<td>0.600 (0.223–0.882)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Region</th>
<th>Absence</th>
<th>Presence</th>
<th>Proportion (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td>3</td>
<td>0</td>
<td>0.000 (0.000–0.602)</td>
</tr>
<tr>
<td>Europe</td>
<td>25</td>
<td>9</td>
<td>0.265 (0.146–0.433)</td>
</tr>
<tr>
<td>NI</td>
<td>1</td>
<td>1</td>
<td>0.500 (0.094–0.906)</td>
</tr>
<tr>
<td>South America</td>
<td>8</td>
<td>1</td>
<td>0.111 (0.025–0.445)</td>
</tr>
</tbody>
</table>

Names followed by the different letters in the same column differ significantly by the chi square test (*p* < 0.05).
characteristics of their travel plans in the two main Brazilian international airports. The International Agricultural Surveillance should maintain alert and be improved in Brazilian airports, considering the increase of passengers worldwide and that can inadvertently endanger public health, by attempting to introduce contaminated food of animal origin.

4. Conclusions

Illegal animal products in baggage of international air passengers from the two largest airports in Brazil showed contamination with bacteria that are important to public health, such as L. monocytogenes and Salmonella. Also, illegal animal products seized in the airports showed S. aureus and E. coli, important indicators of the degree of safety of the product.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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