

Analysis of microbiological hazards in turmeric tamarind traditional drink products

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Abstract: This study aimed to determine the presence of bacterial contaminants, specifically *Escherichia coli*, *Salmonella sp.*, and *Staphylococcus aureus*, in turmeric tamarind traditional drink products according to the Indonesian National Standard (SNI 2019). The research was conducted using samples from 11 different locations in Yogyakarta. The samples were analyzed using Chromocult Coliform Agar (CCA), *Salmonella Shigella* Agar (SSA), and Baird Parker Agar (BPA) to isolate the specific bacteria. Further biochemical tests, including IMViC, Urease, and sugar fermentation tests, as well as confirmation using API 20E and API STAPH tests, were performed on the suspected isolates. The findings reveal that *Escherichia coli*, *Salmonella sp.*, and *Staphylococcus aureus* were not detected in any of the samples. Thus, the traditional turmeric tamarind drink is considered safe for consumption according to the tested parameters.

Keywords: *turmeric tamarind, microbiological hazard, Klebsiella pneumoniae, Staphylococcus aureus, E. coli*

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INTRODUCTION

Turmeric tamarind drink is a traditional Indonesian product developed for generations (A'yunin *et al.*, 2019). This drink is highly regarded by individuals spanning different age groups, including students and college-goers, and also gained popularity among local tourists visiting Yogyakarta. Furthermore, it has a mildly tart flavor, complemented by a unique sweetness from brown sugar, and served chilled or with ice cubes, resulting in a refreshing taste. The drink is readily available in numerous public settings, including markets, terminals, schools, and campuses with an affordable price point that accommodates individuals from all strata of society.

Traditional turmeric tamarind drink is made from turmeric and tamarind with a traditional manufacturing process using hands (A'yunin *et al.*, 2019). This drink is made using the tuber parts of the turmeric, then washed and steamed for 30 minutes. Turmeric that has been steamed is added to water, brown sugar, and tamarind processed with a mixture of formulations, and equipment (A'yunin *et al.*, 2019; Yusuf & Nurkhasanah, 2015). Due to the simplicity of its raw materials, equipment, and manual processes, this beverage may be susceptible to bacterial contamination, which can lead to health issues, such as diarrhea (Priyandina *et al.*, 2017).

Sholichah's study (2012) found *E. coli* contaminant bacteria in turmeric tamarind drinks in Merbung Village, Klaten regency, with TPC numbers between 4.8×10^6 - 2.8×10^7 CFU/mL and Tivani *et al.* (2019) reported *E. coli* in Tegal. Similar results were also found by Yolanda

et al. (2021) in Jimbaran and Kedonganan Bali, with an average TPC count of $>10^5$ CFU/mL. Tango *et al.* (2015) reported *Staphylococcus aureus* in food products that occurred due to hand contact. According to Wen *et al.* (2020), an estimated 50% of diseases are caused by contaminated water. Bacterial contamination depends on the amount and type of contaminants causing health problems and various diseases.

Based on numerous results, traditional drink products often exhibit high levels of contamination, above the established limits set by the Indonesian National Standard (SNI). These products have been found to exceed the permissible levels for *E. coli*, registering at 1.8 APM/100 mL, as well as surpassing the Total Plate Numbers (TPN) for powdered drinks, reaching up to 106 colonies/g (Lukito, 2019). Monitoring the presence of bacterial contaminants, specifically those exceeding the SNI limits, is imperative to ensure food safety and protect public health. Therefore, this study ascertains the presence or absence of specific bacteria, including Coliform *Salmonella sp.*, and *Escherichia coli*, within traditional turmeric tamarind drinks, and assesses their compliance with the established standards set by Indonesia.

METHOD

The number of samples used was taken from 11 different locations of traditional turmeric tamarind drink vendors selling in Yogyakarta. The samples were tested at the Industrial Biotechnology Laboratory of Duta Wacana Christian University, Yogyakarta from March 25 to

Each sample analyzed was repeated twice and 10 mL was inoculated into 90 mL Buffered Peptone Water (BPW) (Naratama & Santoso, 2020; Nethathe *et al.*, 2023) to provide an opportunity for bacterial cells (Budiarso *et al.*, 2021). A total of 1 mL healthy cell culture on BPW medium was taken to make a dilution series in 0.1% peptone water medium of 9 mL up to 10^{-5} . The cell culture was homogenized using a vortex, and then 0.1 mL was taken to grow on Chromocult Coliform Agar (CCA) medium (Beshiru *et al.*, 2022) and Baird Parker Agar (BPA) (Duvenage *et al.*, 2021; Johny *et al.*, 2021). Subsequently, suspected colonies were grown on CCA and BPA medium in streak plates to obtain single isolates. Single colonies were grown on Brain Heart Infusion Agar (BHIA) medium to be assimilated as a collection of isolates. In addition, isolates collected from the CCA medium were tested referring to biochemical characters according to Bergey's manual using Indole, Methyl Red, Voges-Proskauer, Citrate, Motility, TSIA, and Urease tests to obtain *E. coli* and *Salmonella sp.* (Brenner & Farmer III, 2015). Biochemical tests on isolates obtained from BPA medium use Indole, Methyl Red, Voges-Proskauer, Citrate, Mannitol Salt Agar (MSA), Glucose, Lactose, Sucrose, Maltose, and Urease tests to obtain *S. aureus* (El-Hadedy & Abu El-Nour, 2012; Götz *et al.*, 2006).

The isolate selection from biochemical tests led to members of the Enterobacteriaceae family being re-grown on CCA medium. The growing colonies were taken using an ose and included in 5 mL NaCl 0.85% physiological agar medium and the cell culture was standardized with a cell turbidity level equivalent to McFarland 0.5. The cell culture was inoculated in 20 wells of API 20E kit using a sterile pipette, and at the bottom of the kit was given sterile distilled water to maintain moisture during incubation. Meanwhile, API 20E kit in wells ADH, LDC, ODC, H₂S, and URE with the bottom line code was inoculated with a cell suspension of half the tube well's height, and mineral oil was added to the cupule. In CIT, VP, and GEL wells, the cell suspension was inoculated until the cupule was full. Wells without a special code was inoculated at half the well height or only up to the tube. The inoculation of the API 20E kit was at 37°C for 24 hours.

The test results were observed to be positive or negative, according to the standard control Table. Readings on special results of IND and TDA wells were added with 1 drop of James and TDA reagents. VP was added with 1 drop of VP1 and VP2 reagents, while Nit 1 and Nit 2 were added to the GLU wells for NO₂ testing, with a yellow negative result of Zn powder for N₂ gas testing. All readings were obtained on the API test sheet and confirmed using API web software (Harada *et al.*, 2018; Khalifa *et al.*, 2016). The procedure for verifying suspected *Staphylococcus* sp isolates was almost similar to testing on members of Enterobacteriaceae, but the kit used API STAPH (Sutejo *et al.*, 2017; Veliev & Nakipoglu, 2022). In API Staph, colonies are taken in an agar medium before putting in 5cc vials of sterile saline at the bottom of the kit to maintain humidity. The cell suspension solution was added up to half the tube, while ADH and URE were given mineral oil before incubating at 37 °C for 24 hours. NIT, NIT1, NIT2, PAL, ZYM A, ZYM B, VP, VP1, and VP2 were dripped, and incubated at 37 °C for 24 hours. Positive or negative results were compared with the control Tables from the color changes obtained on the STAPH Fire, which were then confirmed with web API software (Sutejo *et al.*, 2017).

FINDINGS AND DISCUSSION

Enumeration and isolation of bacteria. Detection of the presence or absence of coliform, *E. coli*, and *S. aureus* in turmeric tamarind started with enumeration into a differential selective medium. The process was carried out on 11 samples obtained from drink sellers at different locations in Yogyakarta. Before enumerating the bacteria, cell resuscitation was performed using a BPW medium to recover bacterial cells injured due to the processing method of traditional drinks. The enumeration and isolation process for coliform bacteria and *E. coli* used a CCA chromogenic medium for growing groups of bacteria from the Enterobacteriaceae family (Turner *et al.*, 2000). According to the enumeration of suspected *S. aureus* bacteria, cell cultures were raised in a BPA medium (Thaker *et al.*, 2013). The results of the total coliform colony count are shown in Table 1.

Table 1 shows that all samples of turmeric tamarind drink contain coliform contaminant bacteria with more than 10⁵ CFU/mL after being grown on CCA medium. These results indicate that all samples exceeded the plate count threshold of more than 10³ CFU/mL (Lukito, 2019).

Typical colonies of suspected coliform bacteria growing on CCA medium are characterized by a red colony color for suspects of the genus *Citrobacter*, *Enterobacter*, and *Klebsiella*, while *E. coli* gives a dark blue appearance. Subsequently, suspected *Salmonella* sp colonies are characterized by bright blue or white colonies (Budiarso *et al.*, 2021). Suspected *S. aureus* on BPA medium yields colonies surrounded by an opaque zone with a black center (Sutejo *et al.*, 2017). The appearance of colonies growing on CCA and BPA medium is shown in Figure 1.

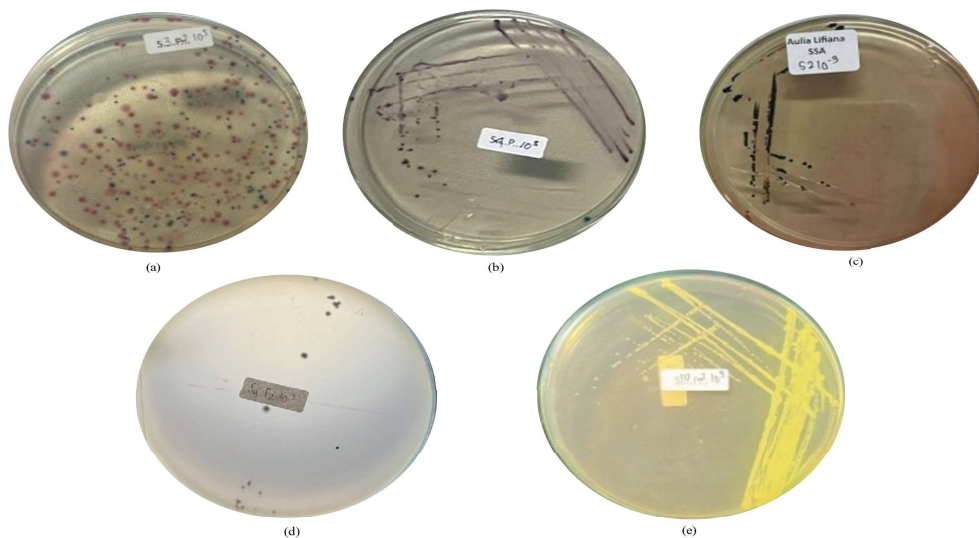
After the enumeration stage, the colony isolation was carried out to obtain a single isolate by streak plate on the CCA and BPA medium. Dark blue and red colonies were suspected coliform and *E. coli* grown on a CCA medium to obtain pure isolates. Selection for white and light blue colonies as suspected *Salmonella* sp was grown on *Salmonella Shigella* Agar (SSA) medium. The results found the presence of typical colonies characterized by black or brown centers. *Salmonella* sp colonies possessed similar appearance as *Proteus* sp, hence biochemical testing is needed to distinguish both. To test *S. aureus* isolates, the suspected colonies were grown in streak plates on MSA medium to test the resistance of 7.5% (Nocera *et al.*, 2022).

Table 1
Enumeration on CCA and BPA mediums

Sample	Average total bacterial colonies (CFU/mL)	
	Medium CCA	Medium BPA
S1	4.1×10^6	1.8×10^5
S2	2.7×10^6	2.1×10^5
S3	2.8×10^7	2.2×10^5
S4	3.0×10^5	1.0×10^4
S5	1.1×10^6	9.3×10^4
S6	5.9×10^6	1.2×10^5
S7	9.7×10^4	4.2×10^4
S8	1.8×10^5	5.7×10^4
S9	2.9×10^7	3.6×10^3
S10	8.1×10^6	5.3×10^4
S11	1.3×10^5	2.0×10^3

Description: S1: Jl. Monjali S5: Jl. Sengkan Joho S9: Jl. Wonosari
 S2: Jl. Affandi S6: Jl. Masjid S10: Jl. Ambarbinangun
 S3: Jl. Argo S7: Jl. Hayam Wuruk S11: Jl. Kadipiro
 S4: Jl. Terban S8: Jl. Kaliurang

Figure 1. Enumeration and purification results of suspected coliform and staphylococcus isolates. Description (a) typical Coliform colonies on CCA medium, (b) streak plate suspected E.coli on CCA, (c) Salmonella suspected streak plate on SSA medium, (d) typical Stap colonies on BPA medium and (e) Streak plate on MSA medium



Isolates purified from CCA, SSA, and MSA are stored in a BHIA medium for biochemical testing. The total selected from 10 samples of traditional turmeric tamarind drink is 17 isolates derived from yellow colonies, 43 from red, purple, and dark blue colonies, and 2 from black center colonies.

According to Hamida *et al.* (2019), positive results for *E.coli* on CCA medium growth were marked by a violet-blue color on a single colony. In the study, a violet-blue color was found on a single colony. Members of Enterobacteriaceae had pathogenic properties that were unable to use β -galactosidase and β -glucuronidase substrates with white or bright blue results, namely *Salmonella*, *Shigella*, and *Yersinia* colors in CCA medium (Budiarso *et al.*, 2021). The results showed the presence of bright blue and white colors on the CCA medium, which was used as suspected *Salmonella*, *Shigella*, and *Yersinia* bacteria. Suspected *Salmonella* sp colonies that appeared as a typical black center color on SSA medium were reinforced by previous studies (Assefa & Girma, 2019).

The BPA medium was given the addition of egg yolk tellurite to distinguish suspected *S. aureus* colonies from others (Götz *et al.*, 2006). Typical colonies using BPA medium will be round black with a clear edge zone around the colony (Yennie *et al.*, 2022). According to (Cunha *et al.*, 2004), the results achieved for *Staphylococcus aureus* in MSA medium exhibited positive outcomes characterized by a yellow appearance. The results from an investigation using MSA medium also presented a yellow appearance. In addition, when using Brain-Heart Infusion Agar (BPA) medium, black colonies with well-defined perimeters were observed, raising suspicions of their identity as *Staphylococcus aureus*.

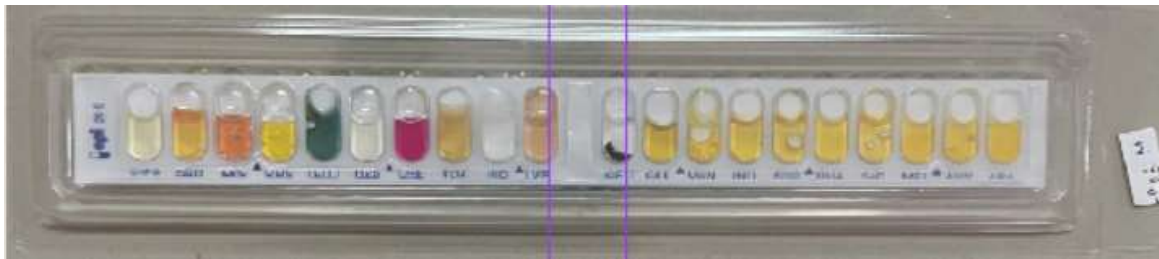
Identification of enteropathogenic bacteria. At the identification of enteropathogenic bacteria, biochemical testing was carried out on isolates previously selected by grouping by color. Isolates of coliform bacteria, namely dark blue, purple, red, and black colors, were continued in the biochemical test.

In the study, four species were identified as *Citrobacter murlinae*, *Citrobacter freundii*, *Klebsiella oxytoca*, and *Staphylococcus aureus*, which have some similarities (Brenner & Farmer III, 2015). *Citrobacter freundii* colonies exhibited positive results for the Methyl red, Citrate, Motility, and Voges-Proskauer tests, while yielding negative results for the Indole test. In contrast, *Citrobacter murlinae* reported positive results for the Indole, Methyl red, Citrate, and Motility tests, and registered negative outcomes for the Voges-Proskauer test. *Klebsiella oxytoca* exhibited positive outcomes for the Indole, Voges-Proskauer, and Citrate tests, but produced negative results for the Methyl red and Motility tests (Brenner & Farmer III, 2015). Suspected colonies for *S. aureus* showed negative results and citrate, lactose, sucrose, and glucose, produced positive results (Muruhan *et al.*, 2012). However, in the study, not all suspected isolates had similar biochemical properties with *S. aureus*. Isolates that led to suspicion of *Coliform*, *E.coli*, and *S.aureus* were confirmed with API 20E and API Staph (Sutejo *et al.*, 2017; Budiarso *et al.*, 2021).

The identification stage used biochemical testing and advanced stages by confirming the enteropathogenic bacteria using API 20 E. In the previous test, 13 isolates were identified and the genus level was taken as a representative for the API 20E test. The confirmation results using API 20E are shown in Figure 2 and Table 2.

The results of red and purple coliform colony isolates in API 20 E were identified as *Klebsiella pneumoniae ssp pneumoniae* 1 with an ID percentage of 97.3%. Red colonies previously in the biochemical testing were identified as *Klebsiella oxytoca*. However, API 20 E was identified

Figure 2. Isolate confirmation results using API 20E



GOOD IDENTIFICATION			
Strip	API 20 E V5.0		
Profile	5 2 1 5 7 7 3		
Note	POSSIBILITY OF <i>Raoultella planticola</i>		
Significant taxa	% ID	T	Tests against
<i>Klebsiella pneumoniae ssp pneumoniae 1</i>	97.3	1.0	
Next taxon	% ID	T	Tests against
<i>Klebsiella oxytoca</i>	1.7	0.72	IND 99%
Complementary test(s)	5KG	METHYL RED	
<i>Klebsiella pneumoniae ssp pneumoniae</i>	2%	9%	
<i>Raoultella terrigena</i>	91%	60%	
<i>Raoultella planticola</i>	98%	100%	

Table 2
Results of enteropathogenic bacteria using API 20E

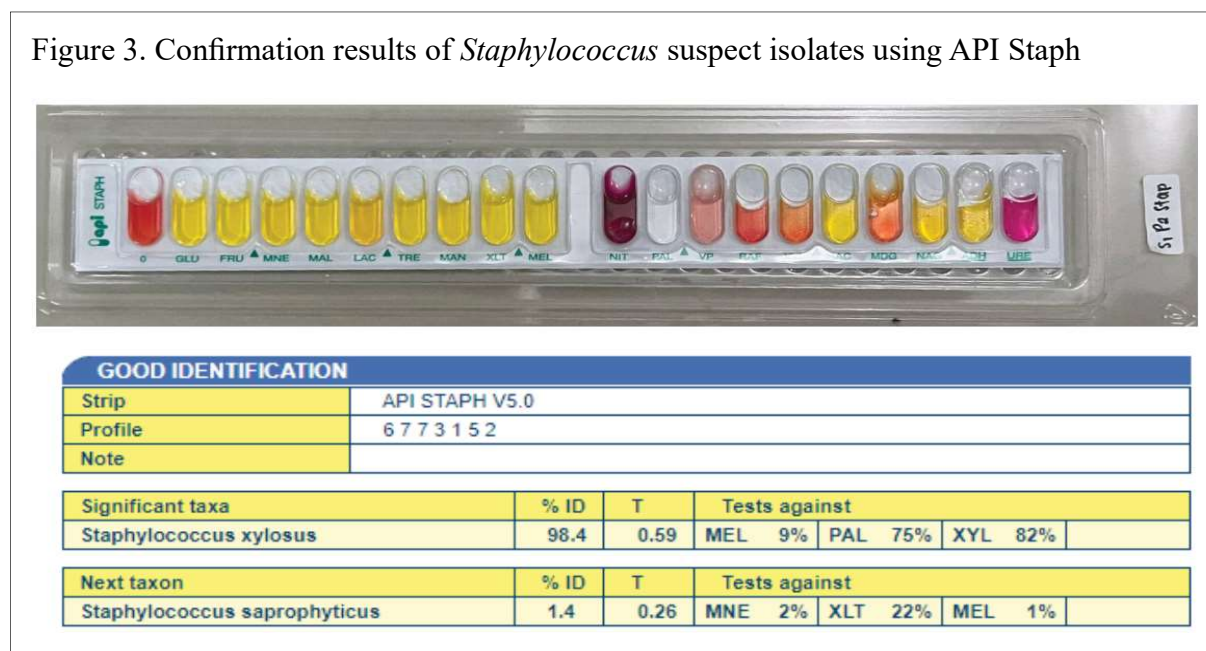
Isolate Code	Identified	%ID
S4P1 U	<i>Klebsiella pneumoniae ssp pneumoniae 1</i>	97.3
S9P2 M	<i>Klebsiella pneumoniae ssp pneumoniae 1</i>	97.3
S3P2 M	<i>Klebsiella pneumoniae ssp pneumoniae 1</i>	97.3
S2P1 H	<i>Citrobacter freundii</i>	89.9

as *Klebsiella pneumoniae ssp pneumoniae 1*, and purple colonies in the results of biochemical testing were identified as *Klebsiella oxytoca*. Black coliform colony isolates on biochemical testing were identified as *Citrobacter murlinae*, but on API 20 E was identified as *Citrobacter freundii* with a percentage of 89.9%. Based on the results, only 2 isolates were found, namely *Klebsiella pneumoniae ssp pneumoniae 1* and *Citrobacter freundii*. This bacterium was one of the most opportunistic pathogens that caused health problems and had resistance to carbapenem antibiotics (Gorrie *et al.*, 2022; Pitout *et al.*, 2015).

The discovery of *K. pneumoniae* as a contaminant bacterium raises concerns due to its potential health implications. Therefore, it is important to remain vigilant and take necessary precautions. Efforts should be directed towards enhancing the management of the turmeric tamarind drink production process in Yogyakarta to ensure the safety of the product.

After biochemical testing, further identification was carried out for *Staphylococcus* bacteria using API Staph. In the previous test, 5 isolates were identified to the genus level, which was carried out the API STAPH test as shown in Figure 3.

Figure 3. Confirmation results of *Staphylococcus* suspect isolates using API Staph



The results of colony isolates suspected *Staphylococcus*, on biochemical tests suspected as *capitis subsp.*, *Staphylococcus aureus*, and *capitis subsp. Ureolyticus*. However, the testing with API Staph was identified as *Staphylococcus xylosus* and *Staphylococcus lentus* with the highest ID percentage of 98.4% and 88.6%, respectively. Based on the results, only 2 isolates were found, namely *Staphylococcus xylosus* and *Staphylococcus lentus*. *Staphylococcus xylosus* is a bacteria that causes health problems because it is phenotypic resistant (Leroy *et al.*, 2019; Piekarska-Radzik *et al.*, 2022). *Staphylococcus lentus* causes health problems in the urinary tract (Adil & Kundarto, 2019) and the discovery requires supervision to make traditional turmeric tamarind drink.

According to (Hiko & Muktar, 2020), contamination levels for commercial drink products that did not contain alcohol possessed an average level (Aerobic Plate Count/ALT) of 3.56 ± 0.32 log CFU/mL APC or more than 10^3 CFU/mL. From this study, the total number of bacteria also exceeded 10^3 CFU/mL. Based on the identification, no contaminant bacteria, namely *Salmonella* sp, *Escherichia coli*, and *S.aureus* were reported. The extract of rhizome turmeric contained secondary metabolite compounds such as alkaloids, flavonoids, saponins, tannins, and triterpenoids/steroids with a strong inhibitory effect on the growth of *E. coli* and *S. aureus* at 500 mg/mL (Kasta, 2020). In addition, turmeric extract also contained curcumin

Table 3

Results of identification of enteropathogenic bacteria using API staph

Isolate Code	Identified	%ID
S1P2 Yellow	<i>Staphylococcus xylosus</i>	98.4
S2P2 Yellow	<i>Staphylococcus xylosus</i>	95.1
S3P2 Yellow	<i>Staphylococcus xylosus</i>	94
S4P2 Yellow	<i>Staphylococcus xylosus</i>	90.9
S7P2 Yellow	<i>Staphylococcus lentus</i>	88.6

which inhibited the growth of gram-positive and gram-negative bacteria. Nanocurcumin extract also damaged the peptidoglycan layer in the cell wall of gram-positive and negative bacteria in tests for *S. aureus* and *E. coli* (Hettiarachchi *et al.*, 2022).

CONCLUSION

In conclusion, the traditional turmeric tamarind drink in Yogyakarta City was reported to exceed the total colony limit of SNI 10^6 CFU/mL. This was because *Salmonella* sp, *Escherichia coli*, and *S.aureus* were not detected. The traditional sour turmeric drink is still relatively safe for consumption.

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