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Occurrence of Veterinary Antibiotics in Swine Manure from Large-scale Feedlots in Zhejiang Province, China

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Abstract The occurrence and distribution of five sulfonamides and three tetracyclines in swine manure sampled from large-scale feedlots in different areas of Zhejiang Province, China were detected using solid-phase extraction and high-performance liquid chromatography. All eight test antibiotics were detected in most of the manure samples. The dominant antibiotics in swine manure were sulfadiazine, sulfamerazine, sulfadimidine, tetracycline, and chlortetracycline. The maximum concentration of residual antibiotic reached up to 57.95 mg/kg (chlortetracycline). The concentrations and distribution of both types of antibiotics in swine manure of different areas varied greatly. Relatively higher concentrations of sulfonamides were found in swine manure from the Zhejiang area in this experiment compared with previous studies. The results revealed that antibiotics were extensively used in feedlots in this district and that animal manure might act as a non-specific source of antibiotic residues in farmlands and aquatic environments.

Keywords Antibiotics · Swine manure · Feedlot · Residues · China

Antibiotics are commonly used in concentrated animal feeding operations worldwide to treat animal diseases and promote animal growth. In China, over 8000 t of antibiotics are used as feed additives each year (Ji et al. 2012). The dose of antibiotics used varies from 3.0 to 220.0 g/kg of feed, depending on the types and sizes of the animals and the type of antibiotic (Kumar et al. 2005; Venglovsky et al. 2009). However, a large percentage of these drugs are excreted in manure because they are not fully metabolized in the animal's body (Pan et al. 2011). Livestock manure means that concentrated animal feeding operations are an important source of antibiotic pollution to the surrounding environment, such as lakes, streams, ground water, and soils (Tong et al. 2009; Hu et al. 2010).

Currently, land application of manure is common practice in many parts of the world, including China. The majority of antibiotics used in medicated feeds is excreted in feces and urine and then may persist and accumulate in soils after repeated manure applications. Recently, higher levels of antibiotic residues were detected in organic vegetable fields compared with traditionally-cultivated vegetable fields (Li et al. 2009; Hu et al. 2010). Once these active ingredients reach the upper soil layer, they may accumulate, be absorbed by plants, and find their way into the food chain. This may lead to selection of antibiotic-resistant bacteria and these residues may eventually affect humans, animals, and the environment (Carballo et al. 2007).

In some 45 European countries, sulfonamides (SAs) and tetracyclines (TCs) were the most frequently detected antibiotics and exhibited a broad concentration range in animal manure (Haller et al. 2002; Karci and Balcioglu 2009). However, in China, information about the levels of residual antibiotics in swine manure is very limited, especially in Zhejiang Province, which is an important stock breeding region in China. Because swine manure is a major source of veterinary pharmaceuticals in agricultural fields, the composition of these pharmaceuticals needs to be given special attention. Thus, the objectives of this study were to determine the concentrations and the distribution of SAs and TCs in the study area in swine manures collected from

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concentrated animal feeding operations located in Zhejiang Province, China, and to compare these results with those obtained from previous studies in the region.

Materials and Methods

Sulfadiazine (SDZ, 99.0%), sulfamerazine (SM1, 99.5%), sulfadimidine (SM2, 99.6%), sulfamethoxazole (SMZ, 99.0%), Sulfamethylthiazole (SMT, 99.0%), oxytetracycline (OTC, 95.6%), tetracycline (TC, 96.4%) and chlortetracycline (CTC, 94.6%) were obtained from Dr. Ehrenstorfer (Germany). Acetonitrile and methanol were obtained from RCL Labscan (Thailand). All organic solvents used for sample preparation were of at least residue-analysis grade. High-performance liquid chromatography-grade solvents were used for the liquid chromatography analysis. The stock solution of eight antibiotics was prepared as a mixture with individual antibiotic concentrations of 100 mg/L in acetonitrile. Na_2EDTA-McIIvaine buffer was prepared by dissolving 12.90 g citric acid monohydrate, 27.50 g Na_2HPO_4, and 37.20 g Na_2EDTA in 1.00 L deionized water, with the pH adjusted to 4.00 using NaOH. This solution was prepared daily and stored in a 4°C refrigerator until use.

The manures were sampled at the time of application to agricultural soil. Manure samples were collected from six representative feed-lots in the research area: the swine breeding operation of a minimum of 10,000 pigs. Samples were collected from feeding farms in six regions including Huzhou (Hu), Hangzhou (Ha), Ningbo (N), Jinhua (J), Wenzhou (W), and Taizhou (T), from north to south of Zhejiang Province, China (Fig. 1). Manures were collected at a depth of 10 cm below the surface layer of the fresh manure heaps in doors. A stainless steel spade was used to collect small amounts of grab dung samples from different points in the swine house and transfer them into a plastic container that was immediately chilled to near freezing. Nine discrete subsamples were collected from each farm and each sample was 4 kg. All samples were transferred to the laboratory and then stored at −20°C until extraction. All samples were analyzed within a week after collection.

The antibiotics in the swine manure samples were extracted according to the modified method of selvam et al. (2013). Briefly, for analysis of antibiotics in the manure samples, 4.0 g fresh defrosted samples were weighed into 50-mL polypropylene tubes. Twenty milliliters of Na_2EDTA-McIIvaine buffer was added to each sample. After shaking for 10 min with a shaker, the samples were sonicated in a sonication bath for 10 min, and centrifuged for 10 min at 8000 rpm. The supernatants were decanted into polypropylene tubes. The pellets were redissolved in 20 mL of Na_2EDTA-McIIvaine buffer, then mixed, shaken, ultrasonically extracted, and centrifuged twice as described above. The Na_2EDTA-McIIvaine buffer extracts were homogenized for 30 s with a mini-vortex mixer and filtered through 0.45-μm glass fiber filters to remove particulates that could otherwise block the cartridge in the subsequent SPE step. The HC C18 SPE cartridges (CNW Technologies, China) were used to concentrate and purify the diluted manure extract at a flow rate of 5 mL/min. One mL solution

Fig. 1 Map of the study area and the sampling locations in Zhejiang Province, China

with a 60:40 ratio of methanol:deionized water was applied to resuspend the residue.

The target swine antibiotics in manure samples were analyzed using HPLC. An Xterra MSC18 column (Waters, MA, USA) was used to separate the analytes. For sulfonamides, the column temperature was set at 25°C, the flow rate was 0.8 mL/min, and injection volume was 20 µL. The wavelength for detection was 270 nm. The mobile phase composition was as follows: 0.01 M NaH_2PO_4:acetonitrile = 85:15. For tetracyclines, the column temperature was set at 25°C, the flow rate was 1.0 mL/min and injection volume was 20 µL. The wavelength for detection was 355 nm. The mobile phase composition was as follows: 0.01 M oxalate:acetonitrile = 85:15. The chromatograms of the antibiotics spiked into a manure matrix, and the chromatograms of the target compounds in one sample are shown in Figs. 2 and 3.

The method quantification limits (MQL) of the antibiotics were determined according to the United States Environmental Protection Agency (USEPA) method (Berthouex and Brown 2002). The MQL of sulfonamides and tetracyclines were calculated from multiple analyses of extracts from swine manure spiked with 0.5 mg/kg target antibiotics. The MQL of SDZ, SM1, SM2, SMZ, SMT, OTC, TC, and CTC were 0.03, 0.01, 0.01, 0.03, 0.01, 0.01, 0.20, and 0.28 mg/kg, respectively. The recoveries of SDZ, SM1, SM2, SMZ, SMT, OTC, TC, and CTC were 78.5%, 75.0%, 75.4%, 66.3%, 66.5%, 71.4%, 81.2%, and 72.5%, respectively.

Results and Discussion

The concentrations of nine samples of every detected antibiotic in each swine feedlot are summarized in Table 1. The results showed that all eight test antibiotics were detected in most of the manure samples. This confirms that SAs and TCs were the most frequently used antibiotics in feedlots (Zhang et al. 2008; Tai et al. 2011). Multiple SAs were detected in all of the animal swine samples. The most frequently detected analytes in swine manures were SDZ, SM1, SM2, TC, and CTC. It was observed that these analytes were detected at high concentrations in all of the swine feedlots, while SMZ, SMT, and OTC were detected at low concentrations in selected samples and below the detection limit in others. The most commonly used SAs in food-producing animals were SDZ and SM1 and they were detected at relatively higher concentration than the other three SAs; their mean concentrations were 1.79–17.50 and 3.54–11.52 mg/kg, respectively. Among the three TCs, CTC was detected at the highest concentration. Specially, the highest analyte concentrations found in swine

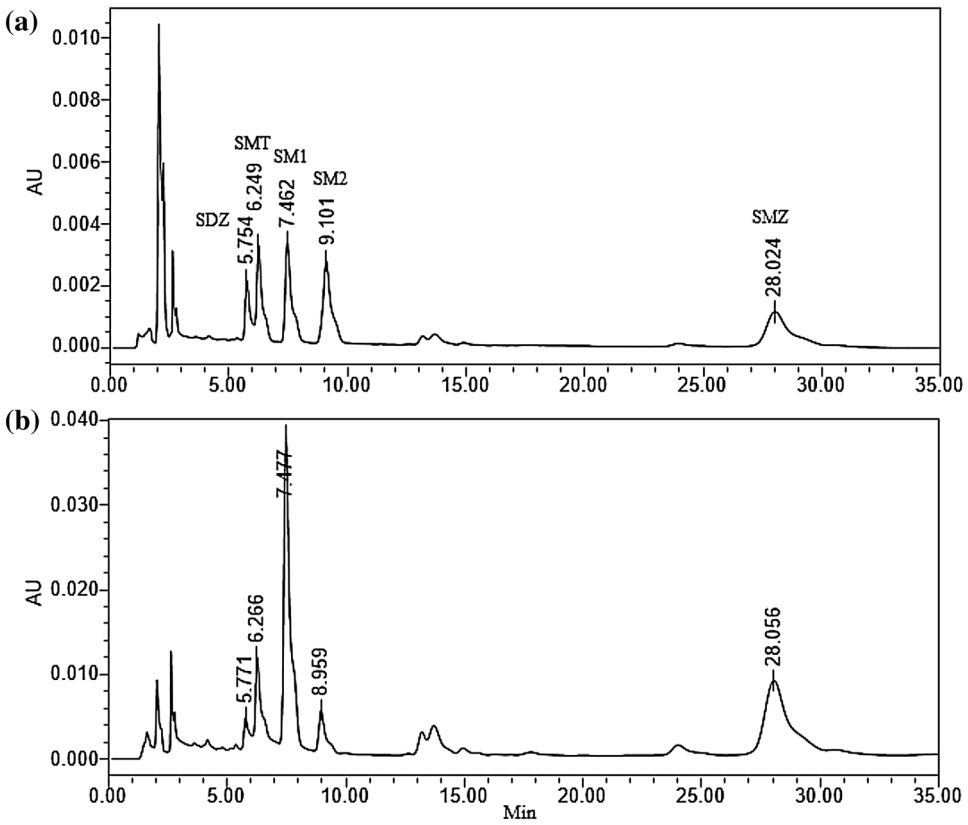

Fig. 2 The chromatograms of the sulfonamides spiked into a manure matrix (0.5 mg/kg) (**a**) and the target compounds in the sample (**b**). The number of each peak means retention time of sulfonamides. *SDZ* sulfadiazine, *SM1* sulfamerazine, *SM2* sulfadimidine, *SMZ* sulfamethoxazole, *SMT* sulfamethylthiazole, *OTC* oxytetracycline, *TC* tetracycline, *CTC* chlortetracycline

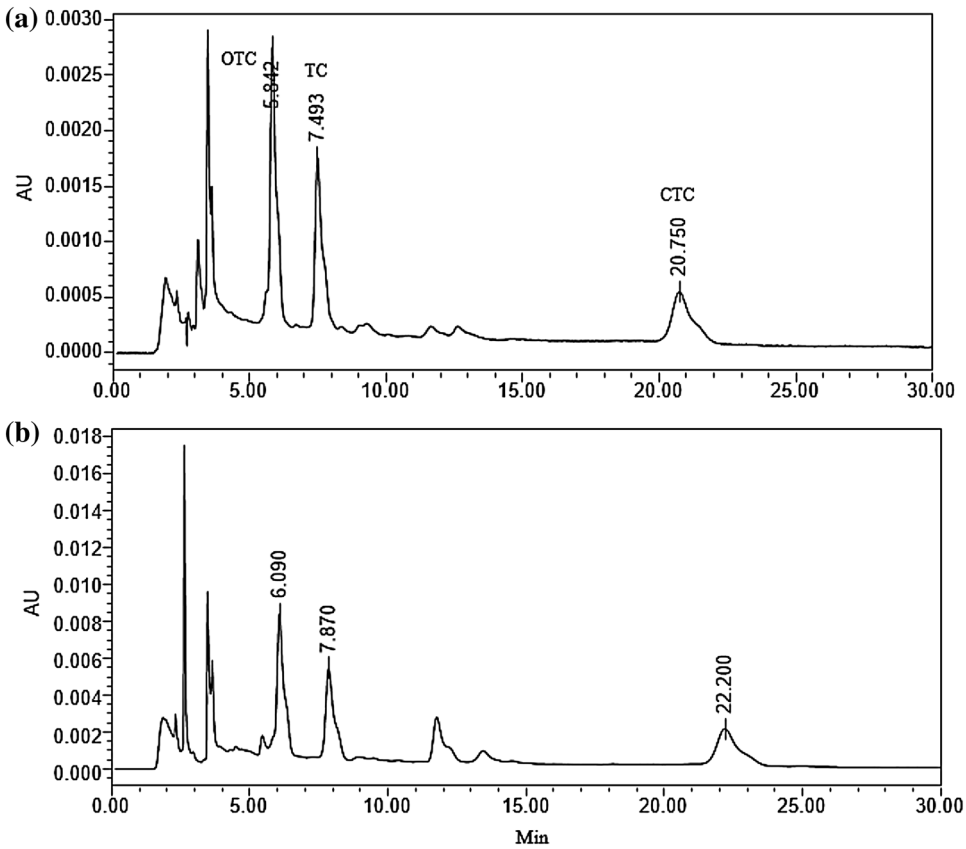

Fig. 3 The chromatograms of the tetracyclines spiked into a manure matrix (0.5 mg/kg) (**a**) and the target compounds in the sample (**b**). The number of each peak means retention time of tetracyclines

Table 1 Quantification of target antibiotics in swine manure samples (mg/kg dry weight; n = 9: 9 different manure samples from each farm)

Sample	Hu		Ha		N		J		W		T	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
SDZ	6.21–16.40	8.68	3.43–7.62	5.27	0.68–3.68	2.45	7.43–46.37	17.50	5.26–14.28	8.70	1.65–2.18	1.79
SM1	6.53–13.97	8.97	2.58–10.99	6.55	3.77–16.50	11.52	3.75–12.28	6.89	1.63–9.69	3.54	6.97–11.32	8.73
SM2	1.93–4.61	3.02	1.79–1.90	1.82	0.38–2.17	0.73	17.55–37.32	23.11	1.19–3.32	1.97	1.07–2.64	2.17
SMZ	0.06–1.15	0.66	0.08–0.51	0.13	0.16–5.22	2.71	0.13–0.76	0.42	0.46–2.76	1.39	0.05–9.35	1.84
SMT	0.36–9.41	6.42	0.03–3.31	1.86	0.01–4.30	1.17	0.35–0.89	0.66	0.02–15.05	5.69	1.70–6.24	3.15
OTC	3.16–5.51	4.10	0.32–0.50	0.43	1.19–15.68	8.38	ND-0.58	0.12	ND-1.79	0.73	1.66–3.08	2.38
TC	2.93–7.51	4.51	1.77–4.12	3.01	0.43–6.87	2.39	16.07–40.39	23.15	0.26–6.71	3.21	0.98–1.97	1.35
CTC	3.38–9.21	6.56	17.92–57.95	27.13	5.51–55.12	25.67	4.58–54.74	15.90	0.36–13.31	2.92	1.32–3.97	2.34

Hu Huzhou, *Ha* Hangzhou, *N* Ningbo, *J* Jinhua, *W* Wenzhou, *T* Taizhou, *SDZ* sulfadiazine, *SM1* sulfamerazine, *SM2* sulfadimidine, *SMZ* sulfamethoxazole, *SMT* sulfamethylthiazole, *OTC* oxytetracycline, *TC* tetracycline, *CTC* chlortetracycline; *ND* not detected

manure were CTC from Hangzhou, ranging from 17.92 to 57.95 mg/kg. The lowest analyte concentrations found in swine manure were OTC from Jinhua, ranging from under the MQL to 0.12 mg/kg.

The distributions of total concentrations of SAs and TCs in swine manure from different feedlots varied greatly (Fig. 4). The total concentrations of SAs were in the order of: Jinhua > Huzhou > Wenzhou > Ningbo > Taizhou > Hangzhou. The total concentrations of SAs were all >20 mg/kg in swine manure from Jinhua, Huzhou, and Wenzhou, while they were all <20 mg/kg in swine manure from Ningbo, Taizhou, and Hangzhou. The total concentrations of TCs were in the order of Jinhua > Ningbo > Hangzhou > Huzhou > Wenzhou > Taizhou. The total concentrations of TCs were all >30 mg/kg in swine manure from Jinhua, Ningbo, and Hangzhou, while they were all <20 mg/kg in swine manure from Huzhou, Wenzhou, and Taizhou. Thus, relatively high concentrations of the two types of antibiotics were found in swine manure from the Jinhua area;

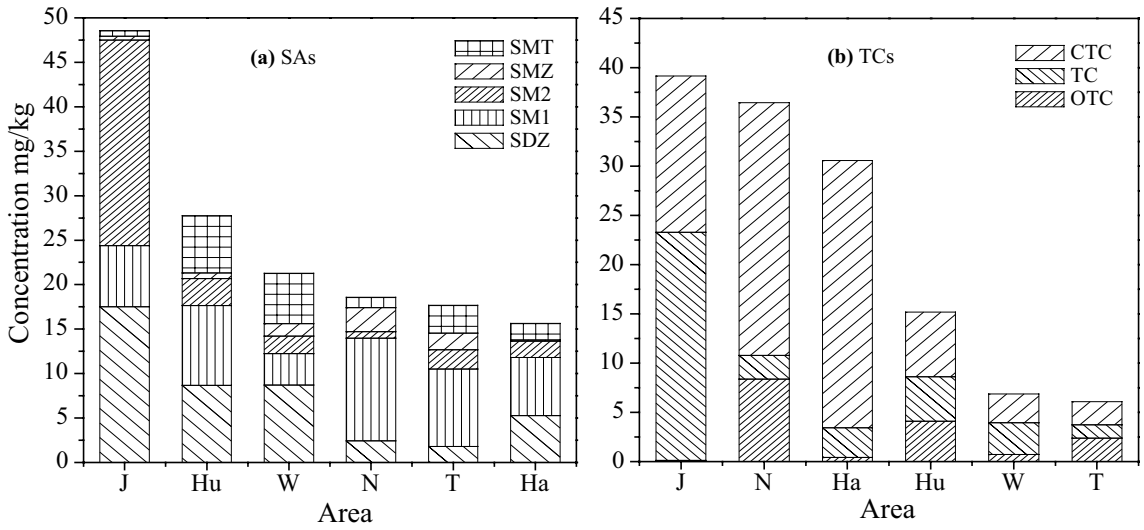

Fig. 4 Spatial distribution of antibiotics in swine manure from different areas (values presented are mean values). *Hu* Huzhou, *Ha* Hangzhou, *N* Ningbo, *J* Jinhua, *W* Wenzhou, *T* Taizhou, *SDZ* sulfadiazine, *SM1* sulfamerazine, *SM2* sulfadimidine, *SMZ* sulfamethoxazole, *SMT* sulfamethylthiazole, *OTC* oxytetracycline, *TC* tetracycline, *CTC* chlortetracycline.

however, there were no obvious trends observed in the other areas.

The concentration distribution of each SA or TC compound in swine manure from different feedlots also varied greatly (Fig. 4). For SAs, the dominant antibiotics were SDZ and SM2 in swine manure from Jinhua. The dominant antibiotic was SM1 in swine manure from Huzhou, Ningbo, Taizhou, and Hangzhou. SMZ was the least dominant in most of the areas. For TCs, the dominant antibiotic was TC in swine manure from Jinhua. The dominant antibiotic was CTC in swine manure from Ningbo and Hangzhou. The contents of three types of compounds were similar in swine manure from Huzhou, Wenzhou, and Taizhou. In general, CTC content was high in most areas.

Table 2 showed the maximum concentrations of target antibiotics in previous studies and in this experiment. As they are the most prevalent veterinary antibiotics, SAs and TCs were the most frequently detected compounds in swine manure in previous studies. In other countries, the reported maximum concentrations of SAs, such as SDZ, SM2, and SMT were 23.00, 20.00, and 12.40 mg/kg, respectively (Jacobsen and Halling-Sorensen 2006; Carballo et al. 2007; Ho et al. 2014). For TCs, the maximum detected concentrations of OTC, TC, and CTC were up to 24.40, 4.00, and 46.00 mg/kg, respectively (Hamscher et al. 2002; Jacobsen

Table 2 Maximum concentrations of target antibiotics of previous studies and this experiment (mg/kg dry weight)

Area (country)	SDZ	SM1	SM2	SMZ	SMT	OTC	TC	CTC	Instrumental technique	References
Denmark	2.00		20.00			24.40			LC-MS/MS	Jacobsen and Halling-Sorensen (2006)
Austria					12.40			46.00	LC-MS/MS	Carballo et al. (2007)
Germany							4.00		LC-MS/MS	Hamscher et al. (2002)
Malaysia	23.00								LC-MS/MS	Ho et al. (2014)
North Zhejiang, China						29.60	16.75	11.63	LC-UV	Zhang et al. (2008)
Guangzhou, China		4.69	3.70	2.74					LC-UV	Tai et al. (2011)
Tianjin, China						173.20	41.50	24.30	LC-UV	Hu et al. (2008)
Eight province, China	0.80	0.14	1.73	0.84		59.06		21.06	LC-FLD	Zhao et al., 2010
Shanghai, China	8.03			9.36			12.27	21.96	LC-MS	Ji et al., 2012
Shandong, China				0.36		16.40	136.00	172.90	LC-MS	Pan et al. (2011)
Zhejiang, China	46.37	16.50	37.32	9.35	15.05	15.68	40.39	57.95	LC-UV	This study

SDZ sulfadiazine, *SM1* sulfamerazine, *SM2* sulfadimidine, *SMZ* sulfamethoxazole, *SMT* sulfamethylthiazole, *OTC* oxytetracycline, *TC* tetracycline, *CTC* chlortetracycline

and Halling-Sorensen 2006; Carballo et al. 2007). In China, the maximum detected concentrations ranged from 0.14 to 9.36 mg/kg for SAs and from 11.63 to 173.20 mg/kg for TCs in Guangzhou, Shandong, and Shanghai Provinces and other areas (Hu et al. 2008; Zhang et al. 2008; Zhao et al. 2010; Pan et al. 2011; Tai et al. 2011; Ji et al. 2012). Thus, relatively higher concentrations of SAs (9.35–46.37 mg/kg) were measured in the present study when compared with the published literature in China, while they were comparable to the results from studies in other countries. In addition, the maximum concentration of TCs in swine manure investigated in this study ranged from 15.68 to 57.95 mg/kg, which was within the range of the previous studies. From the previous Chinese studies, the concentrations of TCs residues were significantly higher than SAs in swine manure. However, the concentrations of SAs were comparable to those of TCs in this study. Overall, the results of this study showed that SAs and TCs are popularly used antibiotics to control disease in swine feedlots in Zhejiang Province, China. The results obtained here might have been influenced by different sampling seasons, pig types, feed sources, or other factors. Future research should be conducted to investigate these factors further.

To conclude, this study investigated the levels of antibiotic pollution in swine manure samples collected from different areas located in Zhejiang Province, China. Results indicated that all eight investigated antibiotics were detected in most of the manure samples and the maximum concentration of residual antibiotic reached up to 57.95 mg/kg (CTC). The concentrations and distribution of the antibiotics in swine manure of different areas varied greatly. Moreover, relatively higher concentrations of SAs were found in this experiment compared with previous studies. The findings demonstrated that fertilization with swine manure might lead to the contamination of agricultural soil by veterinary antibiotics. The occurrence of the residues of antibiotics in the manure fertilized-soil in the studied area should be analyzed in future.

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