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Prevalence and antimicrobial resistance pattern of *Staphylococcus aureus* from frozen chicken meat

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Abstract

Staphylococcus aureus is a pathogenic bacterium known for its ability to cause infections in both humans and animals. A major concern is its rapid development of resistance to various antibiotics. Therefore, the present research aimed to screen *S. aureus* and analyze the antimicrobial resistance patterns of isolates obtained from frozen chicken meat samples collected from popular super shops in Sylhet metropolitan city, Bangladesh. *S. aureus* was identified through conventional culture and biochemical testing procedures from collected forty samples, while the cefoxitin disk diffusion technique was employed to detect methicillin-resistant *S. aureus* (MRSA). Among the samples, 65% were contaminated with *S. aureus*, with 42.31% of these isolates detected as MRSA. Notably, all MRSA isolates were found to be multidrug-resistant (MDR). Across all *S. aureus* isolates, resistance to methicillin was the highest (100%). High levels of resistance were noted against ampicillin (88.46%), nalidixic acid (84.62%), and azithromycin (65.38%). Conversely, all isolates showed 100% sensitivity to imipenem. The presence of multidrug-resistant *S. aureus* in chicken meat samples emphasizes the need of keeping good hygiene protocols by food handlers in super shops. Implementing these measures is vital to mitigating both the risk of MDR *S. aureus* contamination and spread.

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Introduction

According to the World Health Organization (WHO), food-borne diseases are typically caused by bacteria present in food or water. One of the most common causes of these illnesses is *Staphylococcus aureus* (Scallan *et al.,* 2011). It is considered as the third-leading global cause of food-related diseases and an opportunistic pathogen in both humans and animals (Aydin *et al.*, 2011). Naturally, *S. aureus* is widely distributed throughout the globe, but food is the main source of infection (Hennekinne, 2018). It grows best on a vast variety of regularly taken foods (Danbappa *et al.,* 2018), but this varies from nation to nation because of regional differences in culinary practices. Many factors, such as faulty food preparation, inadequate cooking, and tainted water or raw ingredients used in food preparation, might contribute to outbreaks (Scallan *et al.,* 2011).

Animal-derived meat serves as the main protein source, providing essential vitamins crucial for the growth, repair, and upkeep of body cells. This makes it indispensable for our daily functions in various regions across the globe (Pereira and Vicente, 2013; Olmedilla-Alonso *et al.*, 2013). Among these, chicken meat, a widely consumed food globally, is often contaminated with antibiotic-resistant strains of *S. aureus*, posing a significant risk within the food chain (WHO, 2004). *S. aureus* and other pathogens contaminated meat by poor hygiene procedures used by slaughter personnel during meat processing, as well as other flawed abattoir procedures like improper evisceration of animals, which increases the risk of gut pathogens contaminating meat (Argudín *et al.*, 2010; Leong *et al.,* 2018).

The treatment options for food-borne illness caused by *S. aureus* are becoming narrowed due to the emergence of antimicrobial resistance (AMR) in pathogens, specifically methicillin-resistant *S. aureus* (MRSA) (Sallam *et al.*, 2015). Recently, MRSA has shown multidrug-resistant (MDR) properties due to the improper use of antibiotics for treatment purposes, and as a result, infections are growing in humans (Wu *et al.*, 2018). MRSA is recognized as one of the twelve microorganism families posing the most significant threat to public health (Wu *et al.,* 2018). This threat is likely similar or higher in countries like ours. Consequently, the WHO has recently designated MRSA as "high priority 2 pathogen" (Okorie-Kanu *et al.*, 2020). Unquestionably, antibiotics are the best way for treating infection caused by *S. aureus* (Leong *et al.*, 2018). However, MRSA has developed resistance to all of the available beta-lactam antibiotics due to the presence of the *mecA* gene (Ito *et al.*, 2012).

The AMR patterns and contamination of *S. aureus* in raw chicken meat collected from live bird market in Bangladesh is reported by many earlier studies (Akhi *et al.*, 2019; Rahman *et al.*, 2018; Datta *et al.*, 2012). However, the processed and frozen meat is gaining popularities in cities like Sylhet, Bangladesh. Thus, it is imperative to assess the contamination status of processed chicken meat as well as frozen chicken, particularly with MRSA, in super shops.

Although there are few studies available in Bangladesh on MRSA presence in chicken meat from super shops (Parvin *et al.*, 2021; Islam *et al.*, 2019; Alam *et al.*, 2015), a thorough investigation is needed to ensure the safety of frozen chicken meat in such super shops. Therefore, the study aimed at figuring out the prevalence of *S. aureus* and their multidrug-resistant (MDR) patterns in frozen chicken meat from super shops within Sylhet metropolitan city, Bangladesh.

Materials and methods

Collection of sample and processing

A total of forty chicken meat samples were collected from six outlets of two available super shop brands in Sylhet, Bangladesh. Thirty samples (frozen) were collected from brand 1, while the remaining ten samples (non-frozen) were from brand 2. The samples included fifteen chicken wings, ten chicken breasts, and fifteen drumsticks (Table 1). Each sample was taken into a sterile zipper bag, kept into an ice box to avoid contamination, and transported to the laboratory.

The International Organization for Standardization's EN ISO 6888-1 standard protocol was followed for the isolation of *S. aureus* (ISO, 2003). The meat samples (25 g of each) were processed according to Parvin *et al.* (2021).

Identification of S. aureus

After incubation in buffered peptone water (BPW), 1 mL of each solution was transferred to nutrient broth (5 mL) and incubated overnight at 37°C. The next day, a loopful of each sample was streaked onto Mannitol Salt Agar (MSA) media and incubated at 37°C for 24 hours. Colonies having yellow color from each plate of MSA were identified as *S. aureus*. After that, a single colony was sub-cultured again to get pure culture. *S. aureus* isolates were confirmed by interpreting the results of a series of microbiological tests, including Gram staining, catalase, oxidase, indole, methyl red, Voges–Proskauer tests, and urease test.

Antimicrobial susceptibility testing of S. aureus

The antibiotic susceptibility profiling of each confirmed isolate of *S. aureus* against twelve antibiotic disks from Oxoid, UK belonging to nine antimicrobial classes was assessed by the disk diffusion test (DDT) method. The antibiotics tested included ampicillin (10 µg), methicillin (5 µg), cefoxitin (30 µg), azithromycin (15 µg), amoxicilin/calvulanic acid (30 µg), gentamicin (10 µg), imipenem (10 µg), cephalexin (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), doxycycline (30 µg), and oxytetracycline (30 µg). The zone of inhibitions was measured, and antimicrobial susceptibility was evaluated following the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2023) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2024). The prevalence of multidrug-resistant (MDR) *S. aureus* was counted after interpreting the susceptibility and resistant status of each isolate. Multidrug-resistant isolates were identified as those showing resistance to at least one antimicrobial agent in three or more antimicrobial classes.

Detection of MRSA

Methicillin-resistant *S. aureus* (MRSA) was phenotypically detected using the cefoxitin disk diffusion method, following the guidelines of CLSI (2023). For this purpose, a single colony from the *S. aureus* pure culture obtained after overnight incubation was transferred to nutrient broth. After that, bacterial nutrient broth culture was then spread onto Mueller-Hinton agar plates in duplicate for each sample, with both cefoxitin and methicillin disks applied; then incubated at 37°C for 18-24 hours. The isolates showing cefoxitin resistance (defined as a zone inhibition diameter of ≤21 mm) was identified as MRSA.

Results

Sample collection

We examined forty chicken meat samples (30 frozen and 10 non-frozen) collected from six outlets of two super shop brands in Sylhet metropolitan city, Bangladesh, for the presence of *S. aureus*. We found *S. aureus* in all meat samples assessed, including wings, breasts, and drumsticks (Table 1). *S. aureus* fermented MSA (Fig. 1) and showed definitive characteristics in all the biochemical tests conducted.

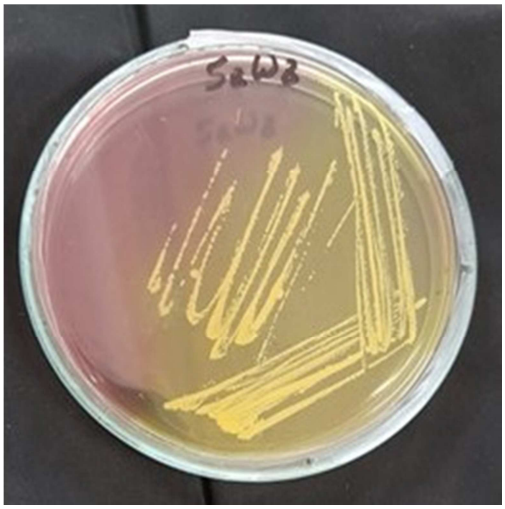

Fig. 1. Identification of *S. aureus* based on colony morphology (Yellow color) on Mannitol Salt Agar

Prevalence and distribution of S. aureus

Among the forty chicken meat samples screened, 26 were contaminated with *S. aureus*. The isolates were confirmed as *S. aureus* based on the results of culture and biochemical tests.

Table 1. Prevalence and distribution of *S. aureus* isolated from meat samples

Variables	No. of samples	No. of samples positive	Prevalence (%)	MRSA (%)	MSSA (%)
Brands					
Brand 1	30	23	76.67	11(47.83)	12(52.18)
Brand 2	10	3	30	0	3(100)
Outlets					
Outlet 1	5	4	80	0	4(100)
Outlet 2	5	4	80	2(50)	2(50)
Outlet 3	5	5	100	4(80)	1(20)
Outlet 4	5	5	100	5(100)	0(0.00)
Outlet 5	10	5	50	0	5(100)
Outlet 6	10	3	30	0	3(100)
Meat types					
Wing	15	10	66.67	2(20)	8(80)
Breast	10	8	80	4(50)	4(50)
Drumstick	15	8	53.33	5(62.5)	3(37.5)
Total	40	26	65	11(42.31)	15(57.69)

Table 2. Antibiotic susceptibility profiling of isolated *S. aureus* from meat samples

Antimicrobial agents	No. of isolates (%)		
	Sensitive	Intermediate	Resistant
Ampicillin	3(11.54)	0(0.00)	23(88.46)
Methicillin	0(0.00)	0(0.00)	26(100)
Cefoxitin	15(57.69)	0(0.00)	11(42.31)
Azithromycin	2(7.69)	7(26.92)	17(65.38)
Amoxicilin/Calvulanic acid	16(61.54)	0(0.00)	10(38.46)
Gentamicin	25(96.15)	0(0.00)	1(3.85)
Imipenem	26(100)	0(0.00)	0(0.00)
Cephalexin	5(19.23)	11(42.31)	10(38.46)
Nalidixic Acid	0(0.00)	4(15.38)	22(84.62)
Ciprofloxacin	15(57.69)	8(30.77)	3(11.54)
Doxycycline	14(53.85)	4(15.38)	8(30.77)
Oxytetracycline	14(53.85)	0(0.00)	12(46.15)

The overall prevalence of *S. aureus* among the samples was 65%. Of these, 42.31% were MRSA, while 57.69% were Methicillin-susceptible *S. aureus* (MSSA). The highest prevalence of *S. aureus* was observed in brand 1 (76.67%), whereas no MRSA was found in samples from brand 2. Moreover, in outlets 1, 5, and 6, 100% of the isolates were MSSA. In contrast, all the isolates from outlet 4 were MRSA (Table 1). In addition, the highest prevalence of *S. aureus* was observed in breasts (80%) with 50% of MRSA, and the prevalence of MSSA in wings was the highest (80%) among the meat types screened (Table 1).

Fig. 2. Antibiotic resistance profiling of *S. aureus* isolates by disk diffusion method

Antibiotic resistance profile of S. aureus

The antibiotic resistance profiling of *S. aureus* isolates was conducted using the disk diffusion test (DDT) method (Fig. 2). The overall resistance patterns for the antibiotics used are summarized in Table 2.

Among all *S. aureus* isolates, resistance to methicillin was the highest (100%). Similarly, resistance to ampicillin (88.46%), nalidixic acid (84.62%), and azithromycin (65.38%) were also significant. On the other hand, no isolates had shown resistance against

imipenem. Resistance to gentamicin was 3.85%, followed by ciprofloxacin (11.45%) and doxycycline (30.77%). Both amoxicillin/clavulanic acid and cephalexin showed equal resistance percentage of 38.46% (Table 2).

Table 3. Multidrug-resistant pattern of *S. aureus*

No. of antibiotic classes	No. of resistant isolates			
	Wing	Breast	Drumstick	Total (%)
1	0	1	0	1(3.85)
2	2	1	0	3(11.54)
3	3	2	0	5(19.23)
4	2	0	3	5(19.23)
5	2	2	3	7(26.92)
6	0	0	2	2(7.69)
7	1	2	0	3(11.54)
Total	10	8	8	26

Multidrug resistance of S. aureus

Out of the twenty-six isolates, twenty-two isolates (84.62%) were found to be resistant to antibiotics from three or more antimicrobial classes (Table 3), indicating them as multidrug-resistant (MDR). Nine antimicrobial classes were assessed, including penicillins, cephamycins, macrolides, penicillins with β-lactamase inhibitors, aminoglycosides, carbapenems, first generation cephalosporins, fluoroquinolones, and tetracyclines. The highest numbers of isolates (7) were resistant to five antimicrobial classes, while three isolates showed resistance to the maximum of seven antimicrobial classes. Equal number of isolates (5) showed resistance to three and four antibiotic classes (Table 3).

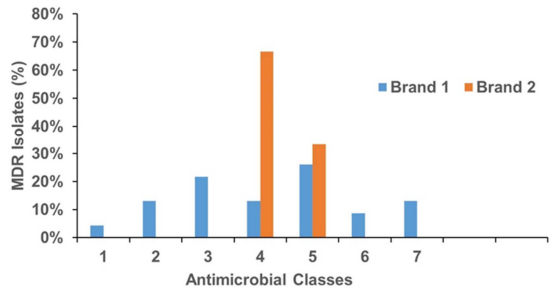

Fig. 3. Distribution of multidrug-resistant *S. aureus* among brands

In brand-wise comparisons, the highest numbers (23) of isolates from brand 1 were multidrug-resistant (MDR), with the highest percentages of isolates (26.09%) being resistant to five antimicrobial classes. On the other hand, all the isolates from brand 2 were MDR, with 66.67% of the isolates resistant to four antimicrobial classes (Fig. 3). Among meat sample types, the highest percentages of isolates (37.5%) from drumsticks were resistant to four and five classes of antibiotics. Furthermore, 80% of isolates from wings were multidrug-resistant, while 25% of isolates from breasts showed resistance to seven classes of antibiotics (Fig. 4).

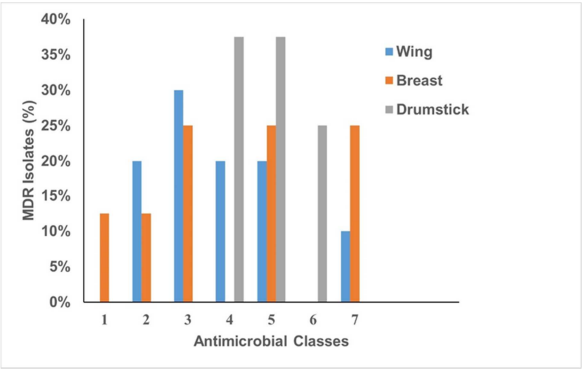

Fig. 4. Distribution of multidrug-resistant *S. aureus* by meat types

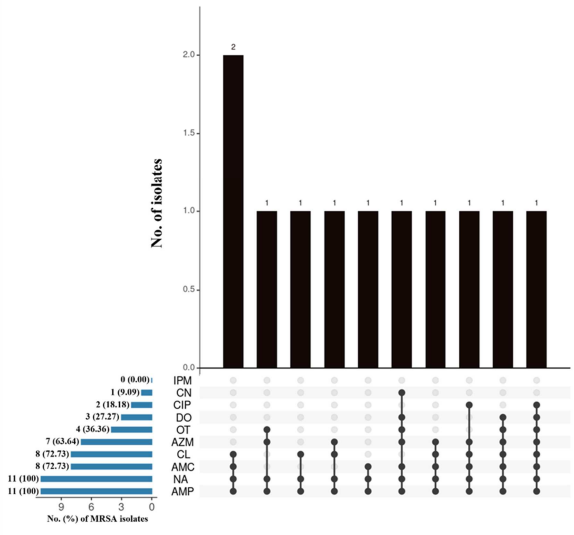

Fig. 5. An UpSet plot summarizing phenotypic resistance patterns of Methicillin-resistant *S. aureus* (MRSA)

AMP: ampicillin; NA: nalidixic acid; AMC: amoxicilin/calvulanic acid; CL: cephalexin; AZM: azithromycin; OT: oxytetracycline; DO: doxycycline; CIP: ciprofloxacin; CN: gentamicin; IPM: imipenem.

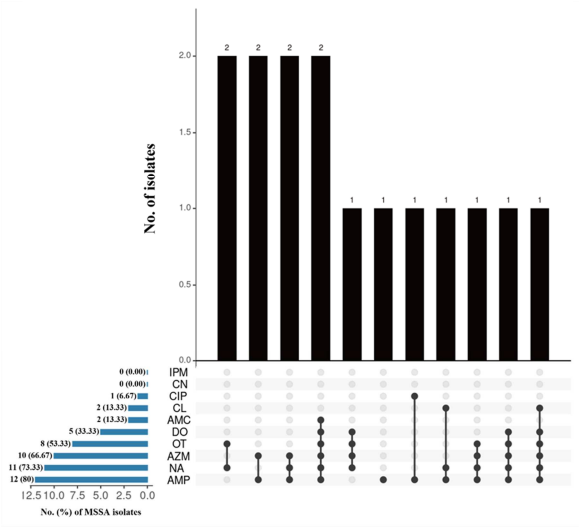

Fig. 6. The Phenotypic resistance patterns of Methicillin-susceptible *S. aureus* (MSSA) depicted in an UpSet plot

AMP: ampicillin; NA: nalidixic acid; AZM: azithromycin; OT: oxytetracycline; DO: doxycycline; AMC: amoxicilin/calvulanic acid; CL: cephalexin; CIP: ciprofloxacin; CN: gentamicin; IPM: imipenem

Phenotypic resistance pattern of MRSA and MSSA isolates

The resistance patterns shown by the isolated MRSA are summarized in Fig. 5. The most common pattern was ampicillin-nalidixic acid-amoxicillin/clavulanic acid-cephalexin, showed by two isolates. Similarly, there are resistance patterns common in different MSSA isolates (Fig. 6). For example, the pattern of six antibiotics (ampicillin-nalidixic acid-azithromycin-oxytetracycline-doxycycline-amoxicillin/clavulanic acid) was observed in two isolates (Fig. 6).

Discussion

Characterizing food-borne bacterial isolates is crucial as it offers valuable insights into their potential to cause human infections. This analysis helps to assess the risks associated with consuming contaminated food, such as meat. Particularly *S. aureus* is concerning due to its adverse effects on animals and its potential to spread between animals and humans (Peton and Le Loir, 2014). Chicken meat is recognized as a reservoir for MRSA, posing potential risks to human health. However, the extent of the risk and the effects on the health of humans associated with the contamination of food of animal origin with MRSA remain unclear. In this research, forty chicken meat samples were collected from two super shops in Sylhet metropolitan city, Bangladesh, and analyzed to find *S. aureus*. In beginning, colony morphology and growth were observed on nutrient broth and MSA to identify potential *S. aureus* isolates. After that, various biochemical tests were conducted for the final identification of the suspected bacteria.

A total of 26 *S. aureus* were found, representing a prevalence of 65%. Of them, 11 (42.31%) MRSA isolates were identified using the cefoxitin disk diffusion technique. The MRSA contamination in chicken meat observed in this study is higher than previously reported findings. For example, a study from Dhaka, Bangladesh, detected MRSA in 33.3% of isolates from packaged meat, while the overall prevalence of *S. aureus* was only 22%, which is significantly lower than the current study (Islam *et al.*, 2019). Another study from Bangladesh reported *S. aureus* and MRSA prevalence rates of 54.9% and 37.1% respectively, which also have similarities in findings with our study (Parvin *et al.*, 2021). In another research conducted in the Chittagong division of Bangladesh revealed a higher *S. aureus* prevalence of 90% but a lower MRSA prevalence of 22.2% (Ali *et al.*, 2017). Moreover, variation in occurrences of MRSA ranging from 89% to 8.1% in chicken meat has been detected in Egypt and China, respectively (Wu *et al.*, 2018; Abolghait *et al.*, 2020). Also, in Denmark, 4% MRSA prevalence was observed (Tang *et al.*, 2017). These discrepancies in MRSA prevalence could be attributed to variations in the management and handling protocols of meat samples of frozen chicken as well as geographic locations (Abolghait *et al.*, 2020).

This study revealed varying occurrences of both MRSA and MSSA across separate brands of chicken meat. The highest prevalence of *S. aureus* was found in brand 1 (76.67%), while no MRSA was detected in brand 2. Similarly, MSSA occurrence was also highest in brand 1. Significant differences were observed between outlets. Outlet 4 had the highest percentage

of MRSA, while MSSA percentage was 100% in outlet 1, 5 and 6. There was noticeable difference within meat types, as drumstick samples had the highest prevalence of MRSA. On the other hand, the highest prevalence of MSSA was observed on wing samples. Such type of MRSA contamination in chicken meat could likely be attributed to poor hygiene practices among meat handlers, as well as pathogen transfer during chicken processing and packaging procedures following slaughter (Abolghait *et al.*, 2020).

The management of infections caused by *S. aureus* heavily depends on antimicrobial treatment. However, this approach is often become ineffective due to the aggressive resistance of organism to certain antibiotics (Islam *et al.*, 2019). In this study, the antibiotic resistance profiles of *S. aureus* were assessed using 12 antimicrobial agents from 9 different classes. All *S. aureus* isolates were resistant to methicillin. High resistance levels were also observed for ampicillin (88.46%) and nalidixic acid (84.62%). *S. aureus* is recognized for its notable resistance to the penicillin-class of antimicrobials, and its resistance has been documented in Gram-positive bacteria since as early as 1940 (Guo *et al.,* 2020). On the other hand, all the isolates were sensitive against imipenem as it is not used in poultry. Resistance to gentamicin was also low (3.85%) in *S. aureus* isolates.

An important finding of this research is that all MRSA isolates showed multidrug resistance, while 22 (84.62%) *S. aureus* isolates were MDR. This indicates the serious issue of antibiotic resistance among *S. aureus* strains isolated from chicken meat in Sylhet city. Like our findings, earlier reports from Bangladesh have also noted that 100% of MRSA isolates showed multidrug resistance, (Islam *et al.*, 2019; Parvin *et al.*, 2021). Globally, multidrug-resistant MRSA isolates have been documented in various regions, with prevalence rates reported as 44% in China (Ou *et al.*, 2020), 46% in India (Zehra *et al.*, 2019), and 64% in Nigeria (Okorie-Kanu *et al.*, 2020).

The high prevalence of multidrug-resistant *S. aureus* isolates in meat samples might be related to handling, production, processing, and packaging practices, as well as with antimicrobial usage. Some Bangladeshi reports show indiscriminate use of multiple antimicrobials throughout the production of broiler chickens. This practice is believed to be a significant factor contributing to the emergence of bacterial antibiotic resistance (Masud *at al.*, 2020; Al Amin *et al.*, 2020). It would be more significant to expand the sampling to include more outlets from various retail shops. However, this research provides representative data reflecting the situation across Sylhet city.

Conclusion

Staphylococcal food poisoning is a significant foodborne illness, posing serious health risks in humans. The contamination of chicken meat with MRSA further worsens the issue by contributing to the emergence of antimicrobial resistance in humans. This research highlights a notably higher occurrence of MRSA and a concerning level of multidrug resistance among the isolates, underscoring the possibility of involvement of chicken meat in disseminating multidrug-resistant MRSA strains in Sylhet city. This represents a significant health hazard for consumers. Routine monitoring of antimicrobial resistance in animal-derived food products across various regions is therefore imperative. The elevated prevalence of multidrug-resistant MRSA in meat samples also underscores the urgency of providing enhanced training to food handlers on hygiene practices, with a focus on their role as potential sources and vectors for MRSA transmission. Furthermore, the implementation of robust practices such as good manufacturing and hygiene practices are essential to mitigate risks and protect public health.

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