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Prevalence, associated risk factors and antibiotic resistance pattern of bacterial uropathogens among pregnant women in a tertiary care hospital of Bangladesh

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ABSTRACT

Aims: The study was designed to determine the prevalence of urinary tract infection (UTI) in pregnant women depending on their various clinical and socio-demographic factors, and to assess the antibiotic susceptibility pattern of the responsible uropathogens in a tertiary care hospital of Dhaka, Bangladesh.
Methodology and results: A total of 100 midstream urine samples were collected from pregnant women and different clinical and socio-demographic variables *viz.* age, gestational weeks, living conditions, and level of education associated with UTI were determined. Bacterial isolation was carried out using blood and MacConkey agar and identified according to their phenotypic characteristics. Antibiogram profiling of the isolates was done by disc diffusion method. From 48% of positive UTI samples, the highest bacteriuria was recorded within the age group of 26-30 years (n=19; 59.38%) and in both, 1st and 3rd trimester period (50%). There was no significant association between the studied risk factors and bacteriuria, except for the age of the pregnant women. Most predominantly isolated bacteria was *Escherichia coli* (n=39; 81.25%), followed by *Klebsiella pneumoniae* (n=9; 18.75%). In *E. coli*, the highest resistance was recorded against ceftriaxone (87.18%), followed by cephalexin (84.61%) and ceftazidime (79.49%); whereas *K. pneumoniae* showed 100% resistance to ceftriaxone and cephalexin. Netilmicin was found as the only effective antibiotic against *E. coli* showing 100% sensitivity. For *K. pneumoniae*, azithromycin, imipenem, chloramphenicol, gentamicin, ciprofloxacin, amikacin and nitrofurantoin were found as the most efficacious drugs.
Conclusion, significance and impact of study: As the emergence of drug resistance is ever increasing, the study necessitates the continuous surveillance of antibiotic susceptibility of uropathogens to ensure safety and better treatment to the mother and fetus.

Keywords: Urinary tract infection, pregnancy, risk-factors, uropathogens, drug resistance

INTRODUCTION

Urinary tract infection (UTI) is the symptomatic as well as asymptomatic bacteriuria and infection with microbial invasion and inflammation of the urinary tract. UTI holds the second position among the most frequently occurring infection in pregnancy (James *et al.*, 2006). Asymptomatic or symptomatic infections may occur in 5-10% and 1-3% of pregnant women, respectively which are usually caused by vaginal, perineal and fecal microflora (Gilstrap and Ramin, 2001). Various factors are responsible for causing UTI, but some unique properties make pregnant women more prone to it such as their physiologic (compression of the urethra and enlarged uterus) and hormonal changes (elevated level of progesterone) (Macejko and Schaeffer, 2007). Besides, urethral dilatation, occurring in 90% of women during the 22nd to 24th weeks in the pregnancy, may contribute to urinary stasis and vesicoureteral reflux (Le *et al*., 2004). Amioaciduria as well as glycosuria, also encourage microbial growth (Jeyabalan and Lain, 2007). Some maternal defense mechanisms show lesser efficacies during pregnancy (McFadyen *et al.*, 1987). Besides, the previous history of UTI, number of childbirths, frequency of sexual activities per week, increased age, diabetes, sickle cell anemia, immunodeficiency and urinary tract abnormalities can increase the prevalence of UTI in pregnant women (Raza *et al*., 2011; Giraldo *et al.*, 2012). If UTI is not properly treated, the devastating effect may occur in mother and fetus. It is sometimes responsible for maternal and perinatal mortality, pre-eclamptic toxemia and anemia (Gilbert *et al.*, 2013). Women with

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complicated UTI may also give birth to low birth weight and premature newborns (Demilie *et al.*, 2014).

Evaluation of bacteriuria among infected persons presents a diagnostic challenge due to the involvement of a variety of microorganisms and requires a careful assessment to evaluate the etiologies. The bacteria responsible for UTI are mainly of the Gram-negative group with a small percentage of Gram-positive bacteria. The most frequently reported Gram-negative bacteria are *Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., and *Serratia* spp. whereas Gram-positive bacteria are *Enterococcus* spp. and *Staphylococcus* spp. (Church *et al.*, 2006; Bayram *et al.*, 2013). Among all the mentioned etiologies, *E. coli* is the most dominant contributing to UTI followed by *K. pneumoniae* (Kashef *et al.*, 2010; Theodros, 2010). Apart from urinary tract infection, both of these members of Enterobacteriaceae are associated with serious health complications such as respiratory tract infections, wound infections, septicemia and pneumonia.

However, the treatment of UTI during pregnancy is quite challenging as it is directly linked with the safety of both mother and fetus (Mitchell *et al.*, 2011; Kladensky, 2012). In the present day, ensuring proper treatment through antibiotic therapy is also very troublesome and uncertain because of high multi-drug resistance among clinically significant isolates. Multi-drug resistance in UTI during pregnancy is an emerging public health problem in developing countries. The possible reasons for the emergence of antibiotic-resistant superbug may be the irrational and widespread use of antibiotics, incomplete doses and unnecessary feeding of the livestock with antibiotics (Islam and Hasan, 2012). Prescribing antibiotics before the culture sensitivity test of the isolates is also responsible for increasing drug-resistance (Tambekar *et al.*, 2006).

As the prevalence and antibiotic susceptibility pattern vary in time and geographic location, regular monitoring of local microbiological etiology and antibiogram profiling is highly required to trace any change to provide updates on the management of UTI. Therefore, the present study was aimed at determining the prevalence and antibiotic susceptibility pattern of bacterial pathogens causing urinary tract infection in pregnant women and to correlate different clinical and socio-demographics variables with the UTI occurrence.

MATERIALS AND METHODS

Study area and population

The study was carried out in a local health-care facility of Dhaka city, Bangladesh from the period of January 2016 to July 2016. A total of 100 pregnant women were considered as study populations with or without having symptoms of UTI. Prior to urine sample collection, verbally informed consent followed by dully filled-up as well signed-up written standardized questionnaire was obtained from the patients to highlight their clinical (e.g. trimester period) and socio-demographic status (e.g. standard of living, level of education, and age) which was kept confidential during the research. Females who were under antibiotic therapy of any kind of infection and refused to give consent were excluded from the study.

Chemicals and media

Blood agar and MacConkey agar were used for the selective isolation and identification of microorganisms causing urinary tract infections, whereas Mueller-Hinton agar (MHA) was used for antibiotic susceptibility test of the isolated uropathogens. All the media and standard antibiotic discs were purchased from Oxoid Ltd., UK.

Urine sample collection

Early morning clean catch mid-stream urine samples were collected from each pregnant woman in a wide-mouthed sterile leak-proof screw cap container according to Clinical and Laboratory Standard Institute guidelines (CLSI, 2009). The containers were labeled with a unique sample number, date, time and were analyzed for the determination of uropathogens and antibiogram profiling as early as possible. In case of delay in sample analysis, buffered boric acid (20 g/L) was added for preventing false-positive culture or bacterial overgrowth (Lum and Meers, 1989).

Isolation and identification of bacterial uropathogens

A volume of 100 µL urine samples were inoculated onto sterilized blood agar and MacConkey agar plates and incubated at 37 °C for 24 h. After overnight incubation, colonies were counted to check for significant growth. Colony counts yielding 105 CFU/mL in urine indicates positive bacteriuria (Griebling, 2007). Bacterial isolates were phenotypically characterized based on their cultural, morphological and biochemical characteristics as shown in Table 1 following the taxonomic guidelines of the Cowan and Steel's Manual for the identification of Medical Bacteria (Barrow and Feltham, 1993).

Antibiotic susceptibility test of uropathogens

Kirby-Bauer disk diffusion assay was used to perform antimicrobial susceptibility testing of the selected isolates (Bauer *et al.*, 1966). Briefly, inoculum adjusted to 0.5 McFarland standard was swabbed on Mueller Hinton agar (MHA) plates by sterile cotton swab and left for 10-15 min to dry. Then, standard antibiotic discs were placed on MHA plates with sterile forceps and incubated at 37 °C for 24 h aerobically. After overnight incubation, the organisms were categorized as "resistant" or "susceptible" based on their diameter of zone of inhibition according to CLSI guidelines (CLSI, 2017). Antibiotics chosen for this study were based on the frequency of prescription and use e.g., gentamicin (30 µg), amikacin (30 µg), netilmicin (10 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), cephalexin (30 µg), ceftazidime (30 µg), azithromycin (15 µg), imipenem (10 µg), cotrimoxazole (25 µg),

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chloramphenicol (30 µg) and nitrofurantoin (30 µg). The standard strain of *E. coli* ATCC 25922 was used as a control strain for interpretations of the results for the antibiotic susceptibility test.

Statistical analysis

The data were analyzed using the Chi-square ($\chi 2$) test by GraphPad PRISM software (version 5.03). A *p* value of <0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

The prevalence and antibiogram profiles of uropathogens in each country vary because of different environmental conditions, social habits of the community, and the standard of personal hygiene and education (Alemu *et al*., 2012). Therefore, continuous surveillance and antimicrobial susceptibility pattern analysis of uropathogens is highly required.

The present study provides information regarding the prevalence of UTI of pregnant women in relation to their age, gestational period, living condition and educational status. A total of 100 midstream urine samples were collected from gravid females suspected with UTI from a local health-care facility of Dhaka city, Bangladesh. From these, 48% showed significant bacteriuria. On MacConkey agar plate, *E. coli* produced small and dark pink colonies whereas *K. pneumoniae* formed large, mucoid and light pink colonies. The cultural and biochemical characteristics of the *E. coli* and *K. pneumonia* are shown in Table 1.

Table 1: Cultural and biochemical characteristics of the isolated uropathogens.

Properties	*Escherichia coli*	*Klebsiella pneumoniae*
Colony characteristics	MacConkey agar: Small and dark pink colony Form: Circular Margin: Entire Elevation: Flat Blood agar: Non-hemolytic colony	MacConkey agar: Large, mucoid and light pink colony Form: Circular Margin: Entire Elevation: Flat Blood agar: Non-hemolytic colony
Gram staining	Gram-negative	Gram-negative
Cell shape	Short rod	Long rod
H_2S production	Negative	Negative
Motility test	Positive	Negative
Indole test	Positive	Negative
Urease test	Negative	Positive
Oxidase test	Negative	Negative
Citrate test	Negative	Positive
Catalase test	Positive	Positive

Escherichia coli was found to be the most pre-dominant bacteria among all isolated uropathogens with the incidence rate of 81.25% (n=39) as shown in Figure 1. The second most pre-dominant bacteria that was isolated was *K. pneumoniae* with the incidence rate of 18.75% (n=9). Previous reports highlighted the occurrence of enterobacteriaceae such as *E. coli* and *K. pneumoniae* as the most pre-dominant (approximately 90%) etiological agents of UTIs (Gilstrap and Ramin, 2001; Uddin and Khan, 2016). Gram-negative Enterobacteriaceae like *E. coli* and *K. pneumonia* are the most commonly isolated species from UTIs due to the fact that these bacteria can adhere to host's uroepithelium facilitated by various adhesins, pili, fimbriae, P-1 blood group phenotype receptor and poses other multi virulence factors (Annabelle *et al.*, 1999; Das *et al*., 2006).

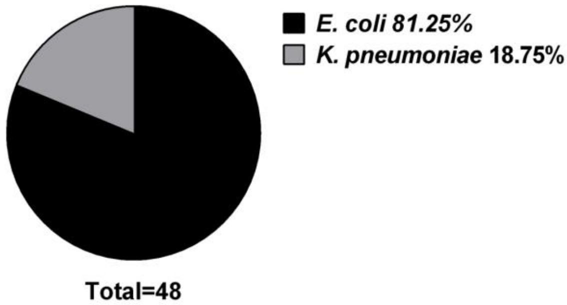

Figure 1: Frequency and distribution of bacterial uropathogens among pregnant patients.

A number of clinical and socio-demographic factors influence the prevalence of UTI during pregnancy as shown in Table 2. The risk factors which make pregnant women more vulnerable to UTI are sexual activity, diabetes, multiparity, older age, anatomical urinary tract abnormalities, changes in urine composition all of which were also evaluated by other researchers (Dwyer and O'Reilly, 2002; Thurman *et al.*, 2006; Schnarr and Smaill, 2008; Kovavisarach *et al.*, 2009). Besides, lower socioeconomic status along with poor hygienic condition may also increase the chances of infection. In this study, there was no significant association between these risk factors and bacteriuria, except for the age of the pregnant women, which may be due to the small sample size in this study.

There is a contradiction while determining the most susceptible age group in pregnancy. The pregnant women were in the age range of ≤20-35 years. Four groups were categorized to trace the prevalence of bacteriuria according to maternal age (≤20 years; 21-25 years; 26-30 years and 31-35 years). A significant correlation was found between the age groups and the occurrence of UTI during pregnancy (χ^2= 9.989; p < 0.05). Majority of the infected patients were in the group of 26-30 years (59.38%), while the least infected group was ≤20 years, which was almost similar to some previous investigations, reporting high UTI prevalence (>70%) in

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Table 2: Prevalence of UTI in relation to clinical and socio-demographics variables of the patients.

Variables	No. of sample tested	Positive bacteriuria cases, n (%)	Chi-square (χ^2) Test	Uropathogens	
				E. coli n (%)	*K. pneumoniae* n (%)
Age group (years)					
≤20	18	7 (38.89)	χ^2= 9.989 *p* =0.0187*	4 (57.14)	3 (42.86)
21-25	41	18 (43.90)		15(83.33)	3 (16.67)
26-30	32	19 (59.38)		17(89.47)	2 (10.53)
31-35	9	4 (44.44)		3 (75)	1 (25)
Gestational age (weeks)					
1st trimester (1-12)	38	19 (50%)	χ^2=0.3313 *p*= 0.8474	16 (84.21%)	3 (15.79%)
2nd trimester (13-24)	58	27 (46.55%)		22 (81.48%)	5 (18.52%)
3rd trimester (25-36)	4	2 (50%)		1 (50%)	1 (50%)
Living condition					
Residential	43	19 (44.19%)	χ^2=0.7291 *p*= 0.3932	16 (84.21%)	3 (15.79%)
Slum area	57	29 (50.88%)		23 (79.31%)	6 (20.69%)
Level of education					
Educated	49	21 (42.86%)	χ^2= 2.025 *p*= 0.1547	20 (95.23%)	1 (4.77%)
Un-educated	51	27 (52.94%)		19 (70.37%)	8 (29.63%)

* Significant at $p<0.05$.

30-34 years and 25-34 years age groups, respectively (Sibi *et al*., 2014; Derese *et al*., 2016). This highest prevalence in 26-30 years age group may be because of high sexual involvement and use of spermicide with a diaphragm or other contraceptives (Derese *et al*., 2016). However, variable findings reported elsewhere. Some reported the prevalence of UTI increases with the maternal age (Tugrul *et al.*, 2005), whereas others claimed the youngest group as the most susceptible group (Moghadas and Irajian, 2009; Parveen *et al.*, 2011).

Gestational age was not found to be a significant risk factor in the present study, probably due to the small sample size. The highest cases of bacteriuria were recorded during the1st and 3rd trimester (50%) followed by the 2nd trimester (46.55%) period. A high prevalence of bacteriuria during the gestational age is due to progressive obstruction of the urinary tract by the gravid uterus, which exerts pressure on the urethra causing urinary stasis associated with some hormonal and immunological changes (Parveen, 2011; Ranjan *et al.*, 2017). In another report, the 1st trimester period was found as the most susceptible stage of infection during pregnancy (Ayogu *et al.*, 2017). This kind of variation in results may be attributed to change in urinary stasis and vesicourethral reflux or decrease in urinary progesterone and estrogen in the different trimesters of pregnancy (Parveen *et al*., 2011).

Living conditions and levels of education are also considered risk factors associated with UTI. Findings from our study shows the higher prevalence of infection in slum areas (50.88%) rather than residential areas (44.19%) and may be attributed to the lower socioeconomic status and weakened immune system of pregnant women. Similar findings were reported in several studies (Uddin and Khan, 2016). A person having primary education may have the knowledge on the maintenance of toilet hygiene such as proper cleaning of the anus after defecating and urethral meatus, washing of genitals after urination and sexual intercourse (Parveen *et al.*, 2011; Demilie *et al.*, 2012). Lack of primary education (un-educated group) accounted for 52.94% of positive UTI cases. Nevertheless, the percentage of UTI incidences was not low among the educated group (patients having primary education level), which accounted for 42.86%. The findings show that levels of education did not have any significant variation on UTI prevalence in between the two groups *viz.* educated and un-educated.

Determination of antibiotic susceptibility patterns of uropathogens is essential to initiate prompt treatment of urinary tract infections. In the present study, the highest cause of UTI was *E. coli* which was found to be susceptible to gentamicin (94.87%), amikacin (82.05%), netilmicin (100%), ciprofloxacin (76.92%), cotrimoxazole (94.87%), chloramphenicol (94. 87%) and nitrofurantoin

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Table 3: Antibiotic susceptibility pattern of *Escherichia coli* (n= 39).

Antibiotic class	Type of antibiotic	n (% of resistance)	n (% of susceptibility)
	Gentamicin (30 µg)	2 (5.13)	37 (94.87)
Aminoglycoside	Amikacin (30 µg)	7 (17.95)	32 (82.05)
	Netilmicin (10µg)	0 (0)	39 (100)
Quinolones	Ciprofloxacin (5 µg)	9 (23.08)	30 (76.92)
	Ceftriaxone (30 µg)	34 (87.18)	5 (12.82)
Cephalosporin	Cephalexin (30 µg)	33 (84.61)	6 (15.39)
	Ceftazidime (30 µg)	31 (79.49)	8 (20.51)
Macrolide	Azithromycin (15 µg)	11 (28.21)	28 (71.79)
Carbapenem	Imipenem (10 µg)	3 (7.69)	36 (92.31)
Sulfonamides	Cotrimoxazole (25 µg)	2 (5.13)	37 (94.87)
Phenicols	Chloramphenicol (30 µg)	2 (5.13)	37 (94.87)
Nitrofuran	Nitrofurantoin (30 µg)	6 (15.39)	33 (84.61)

Table 4: Antibiotic susceptibility pattern of *Klebsiella pneumoniae* (n= 9).

Antibiotic class	Type of antibiotic	n (% of resistance)	n (% of susceptibility)
	Gentamicin (30 µg)	0 (0)	9 (100)
Aminoglycoside	Amikacin (30 µg)	0 (0)	9 (100)
	Netilmicin(10µg)	1 (11.11)	8 (88.89)
Quinolones	Ciprofloxacin (5 µg)	0 (0)	9 (100)
	Ceftriaxone (30 µg)	9 (100)	0 (0)
Cephalosporin	Cephalexin (30 µg)	9 (100)	0 (0)
	Ceftazidime (30 µg)	8 (88.89)	1 (11.11)
Macrolide	Azithromycin (15 µg)	0 (0)	9 (100)
Carbapenem	Imipenem (10 µg)	0 (0)	9 (100)
Sulfonamides	Cotrimoxazole (25 µg)	8 (88.89)	1 (11.11)
Phenicols	Chloramphenicol (30 µg)	0 (0)	9 (100)
Nitrofuran	Nitrofurantoin (30 µg)	0 (0)	9 (100)

(84.61%) as shown in the Table 3. The susceptibility patterns of *E. coli* to other include ceftriaxone (12.82%), cephalexin (15.39%), and ceftazidime (20.51%).

The second most prevalent agent of UTI in the study was *K. pneumoniae.* Here, all the *K. pneumoniae* isolates were 100% susceptible to gentamicin, amikacin, ciprofloxacin, azithromycin, imipenem, chloramphenicol and nitrofurantoin as shown in the Table 4. *Escherichia coli* showed 87.18%, 84.61% and 79.49% resistance towards ceftriaxone, cephalexin and ceftazidime respectively whereas *K. pneumoniae* were 100% resistant to ceftriaxone and cephalexin. Reckless increasing of these antibiotic resistances is very alarming. The resistance to 3rd generation cephalosporins may be caused by extended spectrum β-lactamase (ESBL) of Gram-negative uropathogens (Buscher *et al.*, 1987; Haque *et al.*, 2015).

According to the Infectious Diseases Society of America (IDSA) guidelines, no single antimicrobial agent is considered as best for the treatment of UTIs (Gupta *et al*., 2011). IDSA recommends several first-line (fosfomycin, nitrofurantoin and cotrimoxazole), second-line (ciprofloxacin, levofloxacin and ofloxacin) and third-line (amoxicillin/clavulanate, cefdinir and cefpodoxime) antimicrobial agents for the treatment of urinary tract infections (Colgan and Williams, 2011). The existing antibiotic guideline of Bangladesh, recommends nitrofurantoin, pivmecillinam and cephalexin as the drug of choice for the UTIs treatment caused by *E. coli*, *Klebsiella* sp., *Enterococcus* sp. and *Pseudomonas* sp. during pregnancy (BSMMU, 2005). However, as the antimicrobial resistance-pattern varies from time to time and according to geographical locations, the choice of antimicrobial agents should, therefore, be based on the agent's sensitivity and specificity, side-effects, resistance-pattern, cost and availability. Historically, ampicillin has been used previously for UTI treatment because of its less toxicity and high excretion in urine (Dolan *et al*.,

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1989). But owing to its increasing resistance pattern, the drug has been least likely for UTI treatment. Due to the association of ciprofloxacin with fetal arthropathy, it is also not recommended during pregnancy (Briggs *et al.*, 2001). On the other hand, aminoglycosides like gentamicin are restricted to acute pyelonephritis (Macejko and Schaeffer, 2007). Sulfonamides are the only recommended drug of choice during the 1st and 2nd trimester period, but during the 3rd trimester, the use of sulfonamides poses a risk in which the infant will develop kernicterus, especially preterm infants (Delzell and Lefevre, 2000). According to Centre for Disease Control and Prevention (CDC), the American College of Obstetricians and Gynaecologists (ACOG) recommends avoiding co-trimoxazole and nitrofurantoin in the early pregnancy, if possible, due to their links to birth defects (Ailes *et al.*, 2018). Another report says that nitrofurantoin should not be prescribed in late pregnancy as it may cause hemolytic anemia and neonatal jaundice (Nordeng *et al.*, 2013). Therefore, pregnant women should be screened for UTIs with a urine culture following standard protocols and proper treatment should be initiated on the basis of antibiotic susceptibility pattern of uropathogens considering the safety of mother and fetus. As indiscriminate use of antibiotics for medication drives the evolution of drug resistance among pathogens by selection pressure and horizontal gene transfer, it is important to manage the ever-increasing phenomena of antibiotic resistances (zur Wiesch *et al.*, 2011).

The limitations of the study were the low sample size and short duration of the study period. In order to get the broader scenario and more factual data on UTIs during pregnancy, the time period and area of this study has to be increased and the population should include patients from wider communities throughout the country. Also, further studies on the molecular level are required to understand the drug resistance mechanisms combined with various clinical and socio-demographic risk factors known to influence the frequency of occurrence of urinary tract infections during pregnancy.

CONCLUSION

From this study, it has been concluded that during pregnancy *Escherichia coli* was the most predominant uropathogen followed by *Klebsiella pneumoniae.* Among the different clinical and socio-demographic factors, age groups were found to be significantly correlated with the occurrence of UTIs. *E. coli* and *K. pneumoniae* were susceptible to cotrimoxazole, gentamicin, netilmicin, amikacin, ciprofloxacin, azithromycin, imipenem, chloramphenicol and nitrofurantoin (though *K. pneumoniae* showed the least susceptibility to cotrimoxazole). However, as the drug-resistant patterns of the microorganisms vary according to the geographical area and time, appropriate drugs for UTIs treatment during pregnancy should be recommended by considering the safety of both mother and fetus.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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