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RISK FACTORS, PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF *Staphylococcus aureus* FROM WOMEN WITH PELVIC INFLAMMATORY DISEASE IN DUTSE, JIGAWA STATE, NIGERIA

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Abstract
Pelvic inflammatory disease (PID) is an infection-driven inflammation of the upper female reproductive tract, a major cause of morbidity among women of reproductive age. The disease is traditionally attributed to *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, but increasingly linked to opportunistic pathogens such as *S. aureus*, particularly antibiotic resistant strains. This study investigated the risk factors and characterized *S. aureus* from women attending Dutse General Hospital, Jigawa State. A total of 220 consented patients completed the questionnaires, and high vaginal swabs were analyzed using standard microbiological protocols, and antibiotic susceptibility was conducted using agar well diffusion method. Significant association with PID ($p < 0.05$) were observed for educational status, nature of family, occupation, toilet facility, method of contraceptive, douching frequency, abortion history, prior PID knowledge, and previous urinary tract infections (UTIs). *S. aureus* prevalence was found to be 36%. Resistance was highest to ampiclox (95%), pefloxacin (60%), and zinnacef (52%), moderate to amoxicillin and gentamycin (42%), and lowest to azithromycin and levofloxacin (9%). This finding confirm PID as multifactorial condition shaped by socio-economic, gynecological, and sexual behavioral factors, with *S. aureus* identified as notable etiological agent. Low resistance to levofloxacin and azithromycin supports their continued therapeutic value.
Keywords: Antibiotic-resistance, Pelvic inflammatory disease, Prevalence, Risk factors, *S aureus*

INTRODUCTION

Pelvic inflammatory disease (PID) is an infectious-inflammation disorder of the upper genital tract, encompassing the endometrium, fallopian tubes, and ovaries. The condition typically results from an ascending infection originating from the lower genital tract, predominantly seen in sexually active women, arises as a result of sexually transmitted infections (Jennings & Krywko, 2018). Acute PID commonly manifest with symptoms like abdominal pain, backache and vaginal discharges (Chetana, 2018). It ranks among the most prevalent gynecological disorder associated with severe reproductive sequelae, including tubal factor infertility, endometritis, and oophoritis. These complications account for approximately 10% of infertility cases and contribute to a 0.5% mortality rate among women aged 25-34 years (Usman, 2016).

In USA, infertility that affects approximately 10-15% of all couples, attributed to tubal damage due to pelvic infections (Li *et al.*, 2024). The pathogenesis of PID is often described in two stages.

Initial acquisition of a cervical or vaginal infections (frequently sexually transmitted and potentially asymptomatic) followed by the ascent of microorganisms to the upper virginal tract culminating in infection and inflammation (Muhamed, 2024).The established risk factors include multiple sexual partners, sexually transmitted disease in past, vaginal douching, gynecological surgical procedures (Imam *et al.*, 2024).

Though, PID is traditionally linked to classic sexually transmitted pathogens like *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, there is a growing recognition of the role played by opportunistic pathogens. Notably, *S. aureus* particularly antibiotic resistant strains like MRSA are increasingly implicated in genital tract infections including PID. This trend poses a critical threat due to propensity of *S. aureus* for developing multidrug resistance.

Therefore, this study was conducted to evaluate the risk factors associated with PID and to isolates, identify, and determine the antibiotic susceptibility profiles of *S. aureus* isolates from PID patients in Dutse, Jigawa state.

MATERIALS AND METHODS

Study Area and Design

This cross sectional hospital based study was carried out at Dutse General Hospital (11.76^{0}N and 9.34^{0}E). Dutse is a capital city of Jigawa State with an estimated population of about 251,135 as at 2006 population census (National Population Commission, 2006).

Ethical Clearance
The approval to conduct this research was secured from Jigawa State Ministry of Health (Ethical Committee).

Sample Collection
The Cohran's formula for determining minimum sample size for prevalence studies ($n = Z^2pq/d^2$) was used for calculating the sample size. A total of 220 structured questionnaires were distributed to consented patient prior to collection of their sample. The collection of the sample was carried out by gently rolling a sterile swab stick at the site below cervix to collect as much pus as possible.
The collected samples were transported immediately to the Microbiology and Biotechnology department laboratory of Federal University Dutse, in a cold box for further analysis.

Isolation of *S. aureus*
The High Vaginal Swab samples collected were inoculated onto Mannitol Salt Agar (MSA) and incubated at 37 °C for 24 hours. The plates were examined for growth, and the colonies that appeared yellow and mucoid were selected for identification (Cheesbrough, 2018).

Biochemical Test
All the recovering isolates showing golden yellow colonies on MSA plates were confirmed by a biochemical test using standard bacteriological procedure according to Cheesbrough (2018), which includes catalase and coagulase tests.

Antibiotic Sensitivity Test
Antimicrobial susceptibility test was carried out on the confirmed *S. aureus* using Kirby-Bauer diffusion method. Briefly, a bacterial suspension adjusted to 0.5 McFarland standard was inoculated onto Mueller-Hinton agar (Oxoid, UK). The commercially prepared antibiotic discs (Oxoid, UK) containing ampiclox (30 µg), pefloxacin (10 µg), gentamicin (10 µg), zinnacef (20µg), amoxicillin (30 µg), rocephin (25 µg), ciprofloxacin (10 µg), azithromycin (15µg), levofloxacin (15µg) and erythromycin (15µg), were gently applied onto the agar plates, and incubated at 37 °C for 24 hours. To measure the zones of inhibition, the plates were held a few inches above a black, non-reflective surface illuminated with reflected light. A ruler was used with the unaided eye, while viewing the back of the Petri dish (Clinical and Laboratory Standards Institute (CLSI), 2024). The results were recorded and compared with the zone of inhibition diameter interpretive standard of CLSI (2024).

RESULTS
Socio-Demographic Factors Associated with PID
Two hundred and twenty (220) questionnaires were distributed to the consented patients and the information regarding the risk factors associated with PID among women in Jigawa state were generated. The socio-demographic factors indicated statistical significant (x^2 =12.376 p = 0.0062) association between prevalence of PID and different ages. The age brackets of 18-25 and 26-32 had the highest prevalence of 33 (47%) and 29 (39.2%) respectively, compare to the other age groups ranging from 33-39 and 40-47 with 12(30%) and 6(16%) respectively. The prevalence of PID in relation to marital status showed no significant (x^2 = 0.9736, p = 0.3238) association, with married women 54(38.3%) had the highest rate of occurrence, than single women 25(31.6%). The association of PID in relation to educational status have shown statistical significant difference (x^2 = 28.0982, p = 0.0001), with primary certificate and non-formal education having the highest percentage of occurrence of 15(31.6%) and 42(55.4.3%) respectively. Meanwhile, those with secondary/diploma, degree and other have 15(26.3%), 4(12.5%) and 4(16%) respectively (Table 1).
The occurrence of PID in relation to settlement showed no significant (x^2 = 2.6419, p = 0.1041) association, with those that are living in rural settlement had the highest prevalence of 46(41.1%), than those of urban settlement 33(31%). The prevalence of PID in relation to nature of family showed significant (x^2 = 9.3414, p = 0.0022) association, with polygamous families have the highest prevalence of 46(47%) than monogamous families 33(27%). The prevalence of PID in relation to occupation revealed significant (x^2 = 14.5399, p = 0.0001) association, with unemployed 64(45.3%) had the highest occurrence than employed 16(20%). The prevalence of PID in relation to types of toilet showed significant (x^2 =26.8775, p=0.0001) association, with open air had the highest occurrence of 44(59.5%), followed by pit latrine 17 (24.3%) and water closet 18(23.7%) as presented in Table 1.

Table 1: Socio-Demographic Factors Associated with PID

Parameter	Total	Positive (%)	Negative (%)	X^2
Age				
18-25	70	33(47.00)	37(53.00)	12.376*
26-32	74	29(39.20)	45(61.80)	
33-39	40	12(30.00)	28(70.00)	
40-47	36	5(13.00)	31(87.00)	
Marital Status				
Single	79	25(31.60)	54(68.40)	0.9736
Married	141	54(38.30)	87(61.70)	
Educational Status				
Primary	32	15(46.9)	17(53.10)	28.0982*
Secondary/ Diploma	57	15(26.30)	42(73.70)	
Degree	32	4(12.50)	28(87.50)	
Other	25	4(16.00)	21(84.00)	
Non-formal	74	41(55.40)	33(44.60)	
Settlement				
Urban	108	33(31.00)	75(69.00)	2.6419
Rural	112	46(41.10)	66(58.90)	
Nature of Family				
Monogamy	122	33(27.00)	89(730)	9.3414*
Polygamy	98	46(47.00)	52(53.00)	
Occupation				
Employed	81	16(20.00)	65(80.00)	14.5399*
Unemployed	139	63(45.30)	76(54.70)	
Types of Toilet				
Water Closet	76	18(23.70)	58(76.30)	26.8775*
Pit Latrine	70	17(24.30)	53(75.70)	
Open Air	74	44 (59.50)	30(40.50)	

Key: * = Statistically significant

Gynecological Factors Associated with PID

The gynecological factors associated with PID are presented in Table 2. The prevalence of PID in relation to practice of birth control indicated significant ($x^2 = 6.2008$, $p = 0.0128$) association, with those practice birth control had the highest percentage of occurrence 48(44.9%), than those that did not 31(27%). The prevalence of PID in relation to types of birth control showed no significant ($x^2 = 0.609$, $p = 0.7258$) association, with those using intra uterine device had the highest percentage of 23(48%) compare to those using pills and condoms with 15(42.9%) and 10(38.5%) respectively. The prevalence of PID in relation to douching indicated no significant (($x^2 = 0.88727$, $p = 0.350$) association, with 58(38%) had the infection while 21(31.1%) had no infection. The prevalence of PID in relation to frequency of douching revealed significant ($x^2 = 21.5501$, $p = 0.0001$) association, with those douche daily had the high occurrence 31 (64.6%). Whereas those douche weekly and monthly have 8(22%) and 19(27.9%) respectively.

Table 2: Gynecological Factors Associated with PID

Parameter	Total	Positive (%)	Negative (%)	X^2
Practice of Birth control				
Yes	109	48 (44.90)	61 (55.10)	6.2008*
No	111	31 (27.90)	80 (72.10)	
Types of Birth Control				
Intra uterine Device	48	23 (48.00)	25 (52.00)	0.6409
Pills	35	15 (42.90)	20 (51.10)	
Condom	26	10 (38.50)	16 (61.50)	
Douching				
Yes	153	58 (38.00)	95 (62.00)	0.8727
No	67	21 (31.3)	46 (68.70)	
Douching Frequency				
Daily	48	31 (64.60)	17 (35.40)	
Weekly	37	8 (22.00)	29 (78.00)	21.5501*
Monthly	68	19 (27.90)	49 (63.10)	

Key: * = Statistically significant

Sexual Behavioral Factors Associated with PID

The prevalence of PID in relation to sexual behavioral factors was presented in Table 3. The prevalence of PID in relation to history of abortion revealed significant (x^2 = 30.6205, p = 0.0001) association, with those that have gone abortion had the highest occurrence 54(55.2%) compare to those that have never gone abortion 24(20.1%). The relationship between PID and previous knowledge of the disease showed significant ((x^2 = 3.9020, p = 0.0482) association, with those with previous knowledge of PID had the high occurrence 44(43%) than those who have no knowledge of the disease 35(30%). The prevalence of PID in relation to previous exposure indicated significant (x^2 = 50.9848, p = 0.0001) association, with those that have previously infected had the highest occurrence 65(72.2%) than those that have never infected 14(11%). The prevalence of PID in relation to previous exposure to UTIs showed significant (x^2 = 23.6284, p = 0.0001) association with those that exposed to UTIs before had the highest occurrence of 61(50%) compare to those that have not exposed before (18.4%).

Table 3: Sexual Behavioral Factors Associated with PID

Parameter	Total	Positive (%)	Negative (%)	X^2
History of Abortion				
Yes	96	54 (55.20)	42 (44.80)	20.6205 *
No	124	25 (20.10)	99 (79.9 0)	
Previous Knowledge on PID				
Yes	103	44 (43.00)	59 (57.00)	3.9020*
No	117	35 (30.00)	82 (70.00)	
Previous Exposure to PID				
Yes	90	65 (72.20)	25 (27.8)	50.9848*
No	130	14 (11.00)	66 (82.5)	
Previous Exposure to UTIs				
Yes	122	61 (50.00)	61(50)	23.6284*
No	98	18 (18.40)	80 (81.6)	

Key: *= Statistically significant

Morphological and Biochemical characteristics of Presumptive *S. aureus*

From the two hundred and twenty (220) patients presented with PID, 79 were positive for *S. aureus* as presented in Table 4.

Table 4: Morphological and Biochemical characteristics of Presumptive *S. aureus*

Isolate	Colonial morphology on MSA	Gram Reaction	Biochemical test		Organism
			Cat	Coa	
HVS 002, 004, 005, 007, 009, 010	Creamy yellow	Gram-positive cocci (cluster)	+	+	*S. aureus*
011, 012, 015, 017, 020, 025, 026	Creamy yellow	Gram-positive cocci (cluster)	+	+	*S. aureus*
027, 028, 032, 037 038, 041, 045	Creamy yellow	Gram-positive cocci (cluster)	+	+	*S. aureus*
050, 052, 058, 060 to HVS 220	Creamy yellow	Gram-positive cocci (cluster)	+	+	*S. aureus*

Key: Cat = Catalase, Coa = Coagulase

Prevalence and Antibiotic susceptibility pattern of *S. aureus* from women with PID

The prevalence of *S. aureus* in this research among women suspected to have PID was found to be 36% (79/220) as presented in Table 5. Antimicrobial susceptibility test showed that 75(95%) of the *S. aureus* isolate were resistant to Ampiclox, 48(60%) were resistant to Pefloxacin, 41(52%) were resistant to zinnacef, 33(42%) were resistant to amoxicillin and gentamycin, while 15(19%) were resistant to rocephin, ciprofloxacin and erythromycin. The least resistant was observed among azithromycin and levofloxacin with only 7(09%) isolates were resistant as presented in Table 6.

Table 5: Prevalence of *S. aureus* among PID Patient in Dutse General Hospital

Number of sample (n)	Positive (%)	Negative (%)
220	79(36)	141(64)

Table 6: Antibiotic susceptibility pattern of *S. aureus* from women with PID

Antibiotic	R (%)	I (%)	S (%)
APX	75(95.00)	4(5.00)	0(0.00)
PEF	48(60.00)	17(22.00)	14(18.00)
CN	33(42.00)	7(9.00)	39(49.00)
Z	41(52.00)	36(45.50)	2(2.50)
AM	33(42.00)	33(41.80)	13(16.20)
R	15(19.00)	49(60.10)	15(20.9)
CPX	15(19.00)	29(37.00)	35(44.00)
AZ	7(9.00)	16(20.10)	56(70.90)
LEV	7(9.00)	7(9.00)	65(82.00)
E	15(19.00)	44(55.70)	20(25.30)

Key: R = resistant, I = intermediate, S = sensitive, APX = ampiclox, PEF = pefloxacin, CN = gentamycin, Z = zinnacef, AM = amoxicillin, R = rocephin, CPX= ciprofloxacin, AZ = azithromycin, LEV = levofloxacin, E = erythromycin

DISCUSSION

Pelvic inflammatory disease (PID) is a condition caused by various microorganisms and present with severe clinical implication including chronic pelvic pain, ectopic pregnancy, infertility etc. (Spencer *et al*., 2014). The study showed high occurrence of PID in the age group 18-25 (47%) and 26-32 (38.5%) compared to other age groups. This may likely be due to these demographics being more sexually active, and may have multiple partners increasing their risk of sexually transmitted diseases that predispose them to PID. This result is consistent with the works of Ahmed *et al*. (2017); Shinde *et al*. (2018) and Oyedum *et al*. (2021). However, it contrasts with Dayal *et al*. (2016) who found the highest prevalence of PID in women aged 31-40 years. The study revealed that married women had the higher percentage (38.3%) of the disease compare to single women (31.6%). This may be attributed to the fact that the disease can easily be transferred among the couple. The finding is in agreement with Ahmed *et al*. (2017) who reported that 90% of women with PID are married. The result also agrees with finding of Baruti *et al*. (2022) who reported 73.5% cases of PID to be married women. However, the result is in disparity with Nkwabong and Dingom (2015) who found that 58.6% of women with PID are single.

The study further revealed that those with primary school qualification and non-formal education have the highest percentage of PID with 46.9% and 55.4% respectively.

This high prevalence of PID among these categories is due the fact that lower education level limits their knowledge on STIs including PID which increase the risk of infection. Another reason is because most of these people are living in rural areas, where education is not encouraged more especially among female, so they are zero oriented about the disease and preventive measures. Naaz *et al*. (2016) reported the high percentage of PID among illiterate and primary with 30% and 25% respectively. The finding is also in concordance with Oyedum *et al*. (2021) who found 83.5% and 65.5% were uneducated and primary education respectively.

Geographically, rural resident had the higher prevalence (41%) compare to urban dwellers (31%), which is link to a lack of awareness, lack of standard health care facilities and poor personal hygiene. This finding is supported by Dayal *et al*. (2016) and Oyedum *et al*. (2021), while in contrast with Usman (2016). Women in polygamous families had a higher percentage (47%) than those in monogamous families (27%), potentially due to multiple sexual partnerships increasing STIs risk, and are associated with economic instability limiting healthcare access. This is supported by Usman (2016) and Semwal *et al*. (2022). Unemployed women showed a high PID prevalence (45.3%), this is linked to financial constraints, limiting access to healthcare services, as well autonomy, which limits them to make decision about their own medical attention for PID among the others. Ahmed *et al*. (2017) reported the prevalence of 90% of PID. The use of open-air toilets was associated with a highest prevalence (59.5%), likely due to poor sanitation, limited clean water, and inadequate waste management fostering bacterial growth. This aligns with Oyedum *et al*. (2021).

The study revealed a higher occurrence of PID (44.9%) among women that practice birth control, this may be due to the fact that many women in developing countries like Nigeria accepted the practice of birth control due to the economic instability. With practice of intrauterine device, it is very easy to introduce the pathogen in to the upper genital track which in turn causes PID. The result is in disagreement with finding of Oyedum *et al*. (2021) who reported that 68.4% had not practice birth control compare with 53% who practice it. Among those who practice birth control, intrauterine device users had the high prevalence of PID (77%). This finding is supported by Oyedum *et al*. (2021) who reported 53.6% PID cases among women using intrauterine device.

Vaginal douching was linked to a high prevalence (38%), potentially due to disruption of protective vaginal flora allowing the opportunistic pathogen. The result agrees with finding of Short *et al*. (2015). According to this research, women who had abortion has the high prevalence of PID (55.2%), this is due to the fact that some of abortions are carried out under unsafe condition that at times lead to the introduction of pathogenic microorganisms to the upper genital track leading to acquisition of PID. Ahmed *et al*. (2017) asserted that 50(33.3%) out of 150 patients sampled practice unsafe abortion. However, Oyedum *et al*. (2021) reported low prevalence of PID among women who practiced abortion.

The present study reveals a significant association between prior UTIs and the development of PID (50%). The findings indicates that individual with a history of UTIs are more likely to develops PID, suggesting a potential link between two conditions. Several mechanisms may contribute to the development of PID in women with prior UTIs. One possible factor is the ascending infection hypothesis, where bacteria from the urinary tract migrate to the upper genital tract causing inflammation and infection. Additionally, shared risk factors such as sexual behavior and bacterial colonization may also play role in development of PID. This association is consistent with previous study of Ahmed *et al*. (2017). However, this finding disagrees with finding of Oyedum *et al*. (2021).

Phenotypic identification confirmed *S. aureus* in 36% (79/220) of the samples. Isolates exhibited characteristics creamy yellow colonies on MSA, which is a classic indicator of *S. aureus* due to its ability to ferment mannitol. This fermentation leads to acid production, causing a noticeable color change in the medium (Moraes *et al*., 2021). The organisms are Gram-positive cocci clustered together, which is a t typical feature of the *Staphylococcus* genus when viewed using 100x objective lenses. This is supported by (Tong *et al*., 2015) who reported similar cellular appearance of *S. aureus*. Biochemically, all isolates tested positive for catalase and coagulase, both of which are essential diagnostic markers for the identification of *S. aureus*. The presence of catalase helps differentiate *Staphylococci* from *Streptococci*, while coagulase production is a significant virulence factor and a definitive marker for pathogenic *S. aureus* (Ochei and Kolhatkar, 2010).

The study reveals a significant prevalence of *S. aureus* 36% (79/220) of the samples collected from women with PID at Dutse General Hospital. This finding highlights the significant prevalence of *S. aureus* as a potential etiological agent in PID in the study area. *S. aureus* is a well-known opportunistic pathogen that colonizes various body sites including the female genital tract and has been implicated in many gynecological and obstetric infections (Eze *et al*., 2008). The finding of this research is in alignment with other studies in Nigeria (Eze *et al*., 2008; Nanjebe *et al*., 2023). The finding is in contrast with (Akelere *et al*., 2013; Adirimo *et al*., 2023). These variations in prevalence rates of *S aureus* among women with PID may be attributed to many factors, including the study population that may comprise the socio-economic status, age, educational level, access to health care facilities and geographical location etc. Other factors that may contribute to these variations are sampling method, sample size, diagnostic criteria, and study design among many more.

The antibiogram analysis revealed high resistance observed in beta-lactams: ampiclox (95%) and amoxicillin (42%), this is in consistent with global pattern of *mecA*-mediated methicillin resistance (Olowe *et al*., 2020; Adesida *et al*., 2021). Finding from this study is similar to that of Ogunbanwo *et al*. (2018) who reported that ampiclox is 100% resistant by *S. aureus.* Moderate resistance was noted to gentamicin (42%), potentially due to enzymatic modification or efflux pumps. This is aligning with (Opintan *et al*., 2019). However, Olowe *et al*. (2012) and Bobai *et al*. (2022) reported that *S. aureus* was 90% and 91.82% sensitive to gentamycin respectively.

Resistance to cephalosporin was observed, with 52% resistance to cefuroxime (zinnacef), and 60.1% isolates showing intermediate resistance to ceftriaxone. This can be linked to the mechanisms such as beta-lactamase production, target modification and efflux pumps (Reygaert, 2018). This aligns Ojo *et al*. (2013). Variable susceptibility pattern among fluoroquinolones was observed. Levofloxacin was most effective (81% sensitive), followed by ciprofloxacin (44% sensitive) and pefloxacin (18% sensitive). This stepwise resistance is due to mutations in *gyrA* and *parC* genes, with newer agents like levofloxacin requiring multiple mutations for a high level resistance (Sartory *et al*., 2025). The low ciprofloxacin sensitivity supports CLSI, (2023) guidelines against its empirical use for *S. aureus* infections.

A stark disparity was noted between azithromycin (70.9%) versus erythromycin (25.3%). This is likely due to erythromycin susceptibility to *erm* gene-mediated ribosomal methylation and efflux pumps, from which azithromycin's structural stability offers protection (Luo *et al*., 2024). The low erythromycin sensitivity aligns with (Umar *et al*., 2023; Bawonda *et al*., 2024).

CONCLUSION

Pelvic inflammatory disease can be concluded as a multifactorial condition influenced by socioeconomic, gynecological and sexual behavioral factors in Dutse, Jigawa State. It has been observed in this research that there is significant association between the prevalence of PID and some risk factors ($p < 0.05$); these include educational status, nature of family, occupation, types of toilet, practice of birth control, douching frequency, history of abortion, previous knowledge of PID, and previous exposure to UTIs. This finding highlights a significant prevalence of *S. aureus* as a potential etiological agent in PID in the study area; and it signify the importance of considering other opportunistic pathogens apart from *Neisseria gonorrhea* and *Chlamydia trachomatis* that usually implicated in the disease. The isolates continue to exhibit high resistance to beta-lactam antibiotics, while showing moderate susceptibility to fluoroquinolones and macrolides. These findings emphasize the importance of molecular studies to identify resistance genes such as *mecA*, *femA*, and *femB*, which could further explain the resistance mechanisms observed. Future research should focus on alternative therapies, including combination treatments and novel antimicrobial agents, to combat antibiotic-resistant *S. aureus* infections effect.

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CONFLICTS OF INTEREST

All the authors declared that there are no conflicts of interest.

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