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Original article

Assessment of bacterial etiologic agents, antimicrobial susceptibility status and associated factors of isolates among hospitalized patients suspected for blood stream infection at Dessie comprehensive specialized hospital, Northeast Ethiopia

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Abstract

Background: The world's biggest hazard to public health is the rise in antimicrobial resistance bacterial infections, particularly in underdeveloped nations. These multidrug-resistant bacterial infections increase hospital stays, raise mortality rates, and have impact on both the national and individual economies. This study aimed to assess the bacterial etiologic agents, antimicrobial susceptibility status, and associated factors of isolates among hospitalized patients. **Methods:** A hospital-based cross-sectional study was conducted using a consecutive sampling technique. All bacterial isolates were identified based on standard bacteriological techniques. Antibiotic susceptibility testing was performed on muller-hinton agar and interpreted according to Clinical Laboratory Standard Institute guidelines. Data were collected and entered and analyzed using SPSS version 25. Descriptive statistics and logistic regression analysis were employed. Variables with P-value < 0.05 with a 95% confidence interval was considered statistically significant. **Results:** Out of 294 clinical samples processed 96 (32.7%) were culture positive. Overall, *S. aureus* was the predominant isolate 25 (26%) followed by *E. coli* 14 (14.6%). The levels of drug resistance of gram-negative isolates were higher for ampicillin 89.6% and tetracycline 75.0%. The overall multi-drug resistances were 84.4%. Having a history of invasive procedures, chronic underlying diseases, history of previous hospitalization, and having urinary and intravenous catheterization were statistically significant variables for the acquisition of bacterial infection. **Conclusion:** *E. coli* and *S. aureus* were the most common isolates. Most of the isolates were resistant to commonly prescribed antibiotics. Therefore, routine antimicrobial susceptibility testing and wise use of antibiotics are recommended.

Introduction

Bloodstream infection (BSI) caused by bacteria is among the main causes of mortality and morbidity across the globe, particularly, the mortality ranges

from 4 to 41.5% [1]. Bloodstream infections (BSIs) range from benign infections to potentially fatal sepsis and are a leading cause of sepsis-related morbidity and mortality. The spectrum of bacteria

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has broadened and is subject to geographical variation, making hospitals still burdened with this problem [2, 3].

Bacterial bloodstream infections (BSIs), which are common around the world, can result in both direct and indirect social and economic issues. It is estimated that 30 million individuals are considered to be impacted by BSI, which causes 6 million deaths globally each year [4]. Microbes that invade the bloodstream can cause very serious acute adverse effects such as multiple organ failure, shock, and disseminated intravascular coagulopathies [5]. The primary factors contributing to the increase in BSI-related morbidity and mortality are changing epidemiological trends, insufficient local antimicrobial guidelines, the emergence of antibiotic resistance, and a lack of excellent diagnostic facilities [6]. In sub-Saharan countries, fatality owing to BSI in hospitals is very high, i.e., 39% [7]. There have been reports indicating that between 11 and 28% of people in Eastern African countries have BSI [8].

Antimicrobial resistance (AMR) is posing a danger to global health, especially in sub-Saharan Africa where the greatest rates of infectious diseases are found [9]. Additionally, underlying illnesses such as HIV promote the growth of resistant germs, which has boosted the use of antibiotics to treat opportunistic infections [10].

Despite the paucity of information addressing BSIs in low- and middle-income nations, it is thought that they may be quite widespread in sub-Saharan Africa [11]. The absence of staff, equipment, and diagnostic kits required for conducting antibiotic susceptibility testing in these locations leads to a deficiency of data. Therefore, the purpose of this study was to determine the bacterial profile, patterns of antimicrobial susceptibility, and related variables of isolates among hospitalized patients who were admitted for less than 48 hours to Dessie comprehensive specialized hospital in Northeast Ethiopia.

Methods

Study design, period, and setting

A hospital-based cross-sectional study was conducted among hospitalized patients at Dessie comprehensive specialized hospital, North-east Ethiopia from February to April 2021. Dessie comprehensive specialized hospital is among the biggest hospitals in the country and provides a

healthcare service for more than 4 million people around the area.

Study population

Patients admitted to different wards for less than 48 hours

Sample size and sampling technique

A single population proportion formula was used and taking a 25.78% prevalence of bloodstream bacterial infection [12]. The final sample size was determined to be 294 and study participants were selected consecutively.

Data collection and sample collection

Information on demographic variables and clinical data was collected from each participant through face-to-face interviews using a structured questionnaire.

Blood sample: 10 ml blood from adults, 2-5 ml from children, and 2 ml from neonate was collected aseptically and added to tryptic soya broth (Oxoid, LTD) in two bottles. The sample was transported to Wollo University Laboratory and incubated at 35–37°C [13].

Culture, isolation and identification

The blood culture was inspected daily for the signs of bacterial growth for 7 days. After 7 days of incubation for those which do not show growth, were reported as negative. However those which show bacterial growth, were subcultured on blood agar, chocolate agar, and macConkey agar. Identifications of isolates were made by the conformation of relevant biochemical tests [14]. Since there is no availability of anaerobic media, anaerobic bacteria was not isolated.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using mueller–hinton agar by Kirby Bauer disk diffusion technique as modified by the 2020 Clinical and Laboratory Standard Institute (CLSI) [15]. Different antimicrobial disks were used for susceptibility testing. The inoculated plates with antimicrobial disk were incubated at 35–37°C for 16 to 18 hours, and the diameter of the zones of inhibition was measured and interpreted according to the standards of CLSI [15].

Data and laboratory quality control

The questionnaires were checked for completeness. All laboratory assays were done by maintaining quality control procedures. The sterility of the media was checked by incubating 5% of the batch at 35–37°C overnight. For antimicrobial sensitivity testing

disks were checked using standardized reference strains of *S. aureus* ATCC25923, *E. coli* ATCC25922, and *P. aeruginosa* ATCC27853 [15].

Data analysis

The data was entered and analyzed by using Statistical Package for Social Sciences (SPSS) version 25. The descriptive statistics were calculated and presented with tables. Binary logistic regression was used to assess the association between explanatory and dependent variables. The odds ratio was calculated and variables with $P \leq 0.2$ were entered into multivariable logistic regression. P-value ≤ 0.05 was considered to be significant.

Results

Socio-demographic and clinical characteristics

Among study participants enrolled, 174 (59.2%) were males. The age of the study participants ranged from 4 days to 85 years, with a median age of 36 years. The majority 174 (59.2%) of the participants were male and 125 (42.5) were rural residents. In this study, 73(24.8%) of the study participants had known comorbidity (Table 1).

Bacterial isolation rate

A single specimen was collected from each participant and the overall culture positivity rate was (32.7%). Among the isolates gram negatives were predominant 50/96 (52.1%) and from all gram-negative bacteria *E. coli* 14 (28%) was predominant where as among gram positives *S. aureus* was the most prevalent 25(54.3%) (Table 2).

Antimicrobial resistance profiles of gram positive bacterial isolates

The overall resistance rate of gram-positive bacteria to ampicillin and tetracycline was 76.1%, 73.9% respectively. *Staphylococcus aureus* showed higher resistance to ampicillin (76%) and tetracycline (72%) (Table3).

Antimicrobial resistance profile of Gram-negative bacterial isolates

The overall resistance rate of Gram-negative bacteria to ampicillin, tetracycline, and trimethoprim-sulphamethoxazole was highest at 89.6%, 75.0%, and 75.0%, respectively. However, these isolates showed the lowest overall resistance for ceftriaxone, gentamycin, and chloramphenicol with 42%, 44.0%, and 44.7%. From the isolates, *E. coli* showed the lowest resistance for ceftriaxone, chloramphenicol, and ciprofloxacin 42.8% but highest for ampicillin (92.9%) and trimethoprim-sulphamethoxazole (85.7%)(Table 4).

Multidrug resistance profiles of isolates

Of all bacterial isolates, 90.6% of the isolates were resistant to at least one of the selected antibiotics. The overall multi-drug resistance rate of isolated bacteria was 81(84.4%). Among Gram-negative bacteria the MDR rate of *E. coli*, *K. pneumoniae*, and *Pseudomonas* spp is 100% and 90%, and 80% respectively. Whereas from Gram-positive bacteria the MDR rate of *S. aureus* was 68% (Table 5).

Associated factors of bacterial infection

Bivariable logistic regression analysis was done for socio-demographic and clinical variables. Those with $p \leq 0.2$ were further analyzed using multivariable logistic regression. In the multivariable analysis, study participants who had urinary catheterization were 2.22 times more likely to develop bacterial infection [95% C.I, 1.19-4.11]) as compared with those who had not, and study participants who had chronic underlying disease were 5.36 times more likely to develop bacterial infection [95% C.I, 3.04-9.44]) as compared with those who had not such disease. Moreover, a history of having an invasive procedure had 5.86 times the odds of acquiring bacterial infection than the counterparts [95% C.I, 2.87-11.97]) (Table 6).

Table 1. Socio-demographic and clinical characteristics of the study participants (N=294) at Dessie comprehensive specialised hospital, Northeast Ethiopia.

Demographic Characteristics	Category	Frequency, n (%)
Age in years	≤14	32(10.9)
	15-29	50(17.0)
	30-44	121(41.2)
	45-59	65(22.1)
	≥60	26(8.8)
Sex	Male	174(59.2)
	Female	120 (40.8)
Residence	Urban	125 (42.5)
	Rural	169 (57.5)
Educational status	Can't read and write	75(25.5)
	read and write	61(20.7)
	primary school	45(15.3)
	Secondary school	32(10.9)
	Diploma or certificate	53(18.0)
	Degree and above	28(9.8)
	No	212(72.1)
Chronic underlying disease	Yes	73(24.8)
	No	221(75.8)

Table 2. Proportion of bacterial isolates (n=96) among patients admitted for less than 48 hours at Dessie comprehensive specialized hospital, Northeast Ethiopia.

	Frequency (%)	Species	Frequency (%)
Gram-negative	50	<i>Acinetobacter spp</i>	2(4.0)
		<i>C. diversus</i>	3(6.0)
		<i>C. freudii</i>	5(10.0)
		<i>E. auregenosa</i>	3(6.0)
		<i>E. coli</i>	14 (28.0)
		<i>K. ozenae</i>	4 (8.0)
		<i>K. pneumonia</i>	5 (10.0)
		<i>K. oxytoca</i>	3 (6.0)
		<i>P. vulgaris</i>	1 (2.0)
		<i>pseudomonas spp</i>	10 (20.0)
Gram-positive	46	<i>S. agalactia</i>	1 (2.2)
		<i>S. epidermidis</i>	7 (15.2)
		<i>s. saprophyticus</i>	13 (28.3)
		<i>S. aureus</i>	25 (54.3)

Table 3. Antimicrobial resistance profile of Gram-positive bacteria among study participants admitted for less than 48 hours Dessie comprehensive specialized hospital, Northeast Ethiopia February – April 2021

Isolated spp(n=46)	Antibacterial agents N (%)										
	FOX	CL	E	P	CIP	CHF	CN	SXT	AMP	CEF	TET
<i>S.aureus</i>	10 (40)	13 (52)	10 (40)	17(68)	7 (28)	8 (32)	6 (24)	16 (64)	19 (76)	10(40)	18 (72)
<i>s. saprophyticus</i>	–	8(61.5)	6 (46.2)	9 (69.2)	3(23.1)	5(38.5)	6 (46 .1)	10 (76.9)	11(84.6)	4 (30.8)	10(76.9)
<i>S. epidermidis</i>	–	3(42.8)	4 (57.1)	5 (71.4)	3 (42.8)	3 (42.8)	3 (42.8)	5 (71.4)	4 (57.1)	4 (57.1)	5 (71.4)
<i>S. agalactia</i>	–	0(0)	1 (100)	1(100)	0(0)	0(0)	–	–	1 (100)	1 (100)	1(100)
Overall resistance	10 (40)	24 (52.2)	21 (45.7)	32 (69.6)	13(28.3)	16(34.8)	15 (33.3)	31 (68.9)	35(76.1)	19 (41.3)	34(73.9)

FOX; Cefoxitin, CL; Clindamycin, CIP: Ciprofloxacin, CN: Gentamicin; E: Erythromycin, SXT: Trimethoprim-Sulphamethoxazole; P: Penicillin; AMP: Ampicillin, CHF; Chloramphenicol, CEF; Cefotaxime, TET; tetracycline

Table 4. Antimicrobial resistance rate result of Gram-negative bacteria among study participants admitted for less than 48 hour at Dessie comprehensive specialized hospital Northeast, Ethiopia

Isolated spp (n=50)	Antibacterial agents									
	AMC	CIP	CHF	CN	SXT	CRO	AMP	CEF	TET	
<i>Acinetobactersp p</i>	–	2(100)	–	1(50)	2(100)	1(50)	–	1(50)	2(100)	
<i>C. diversus</i>	2(66.7)	0(0)	2(66.7)	0(0)	2(66.7)	1(33.3)	3(100)	1(33.3)	3(100)	
<i>Citrobacter.spp</i>	2(40)	1(20)	3(60)	1(20)	3(60)	2(40)	4(80)	2(40)	3(60)	
<i>E. auregenosa</i>	2(66.7)	2(66.7)	1(33.3)	1(33.3)	2(66.7)	1(33.3)	2(66.7)	1(33.3)	2(66.7)	
<i>E.coli</i>	7(50)	6(42.8)	6(42.8)	7(50)	12(85.7)	6(42.8)	13(92.9)	6(42.8)	11(78.6)	
<i>K. ozenae</i>	2(50)	3(75)	1(25)	3(75)	3(75)	1(25)	4(100)	2(50)	2(50)	
<i>K. pneumoniae</i>	3(60)	2(40)	2(40)	3(60)	3(60)	2(40)	4(80)	2(40)	4(80)	
<i>K.oxytoca</i>	2(66.7)	1(33.3)	1(33.3)	1(33.3)	2(66.7)	1(33.3)	3(100)	2(66.7)	2(66.7)	
<i>P.vulgaris</i>	1(100)	0(0)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)	1(100)	
<i>Pseudomonsspp</i>	–	7(70)	–	5(50)	–	6(60)	9(90)	5(50)	–	
Overall resistance	21(55.3)	24(48)	17(44.7)	22(44.0)	30(75.0)	21(42)	43(89.6)	23(46)	30(75)	

CIP: Ciprofloxacin, AMC: Amoxicillin- Clavulanic acid CRO: Ceftriaxone, CN: Gentamicin, SXT: Trimethoprim-Sulphamethoxazole, AMP: Ampicillin, CEF; Cefotaxime, CHF; Chloramphenicol, TET; Tetracycline

Table 5. Multidrug resistance profile of bacteria isolated among patients admitted for less than 48 hours at Dessie comprehensive specialized hospital, Northeast Ethiopia.

Antibiogram	Isolated spp (n=96)													
	<i>Acinetobacter</i> spp	<i>C. diversus</i>	<i>Citrobacter</i> spp	<i>E. aerogens</i>	<i>E. coli</i>	<i>K. ozaenae</i>	<i>K. pneumoniae</i>	<i>K. oxytoca</i>	<i>P. vulgaris</i>	<i>Pseudomonas</i>	<i>S. agalactiae</i>	<i>S. epidermidis</i>	<i>Saprophyticus</i>	<i>S. aureus</i>
R 0(%)	0(0)	0(0)	1(20)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(4.0)
R 1 (%)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(10)	0(0)	0(0)	2(20)	0(0)	0(0)	2(15.4)	4(16.0)
R 2(%)	0(0)	0(0)	0(0)	1(33.3)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	3(42.9)	1(7.7)	3(12.0)
R 3(%)	0(0)	1(33.3)	0(0)	0(0)	0(0)	1(25)	0(0)	0(0)	0(0)	3(30.0)	0(0)	1(14.3)	0(0)	1(4.0)
R 4(%)	1(50)	0(0)	2(40)	1(33.3)	2(14.3)	0(0)	0(0)	2(66.7)	0(0)	4(40.0)	1(100)	0(0)	3(23.1)	4(16.0)
R 5(%)	1(50)	1(33.3)	0(0)	0(0)	6(42.9)	0(0)	1(20)	0(0)	0(0)	1(10)	0(0)	0(0)	0(0)	1(4.0)
R 6(%)	0(0)	1(33.3)	1(20)	0(0)	6(42.9)	3(75)	2(40)	0(0)	1(100)	0(0)	0(0)	0(0)	2(15.4)	6(24.0)
R 7(%)	0(0)	0(0)	1(20)	1(33.3)	0(0)	0(0)	1(20)	1(33.3)	0(0)	0(0)	0(0)	3(42.9)	5(38.5)	5(20.0)
MDR	100%	100%	80%	66.7%	100%	100%	90%	100%	100%	80%	100%	62.5%	76.9%	68%
Over all MDR	81(84.4%)													

R0: susceptible for all tested antibiotics, R1-6: resistance to 1, 2, 3, 4, 5, and 6 antibiotics; R7 for ≥ 7 antibiotics. $\geq R3$: resistance to 3 or more antibiotics from different classes

Table 6. Bivariable and Multivariable analysis of associated factors for acquiring bacterial infection among study participants attending (N=294) at Dessie comprehensive specialized hospital

Characteristics (Total No.)		Bacterial infection		COR (CI 95%)	P-value	AOR	P-value
		Positive N, %	Negative N, %				
Urinary catheterization	Yes (91)	43(47.3)	48(52.7)	2.54 (1.51-4.25)	0.001	2.22(1.19-4.11)	0.012*
	No (203)	53(26.1)	150(73.9)	Ref			
Previous hospitalization	Yes (53)	26(49.1)	27(50.9)	2.35(1.28-4.31)	0.006	2.31(1.11-4.80)	0.026*
	No (241)	70(29.0)	171(71.0)	Ref			
IV catheterization	Yes(82)	45(54.9)	37(45.1)	2.61(1.51-4.51)	0.001	2.13(1.08-4.19)	0.029*
	No (212)	51(24.1)	161(75.9)	Ref			
invasive procedure	Yes (41)	28(68.3)	13(31.7)	5.86(2.87-11.97)		5.31(2.32-12.17)	0.00*
	No (253)	68(26.9)	185(73.1)	Ref			
chronic disease	Yes (73)	45(61.6)	28(38.4)	5.36(3.04-9.44)	0.001	5.94(3.12-11.33)	0.00*
	No (221)	51(23.1%)	170(76.9%)	Ref			

Note: COR: crude odds ratio, AOR: adjusted odds ratio, CI: confidence interval, Ref: Reference. *: Those have an association with bacterial infection

Discussion

The growing number of bacterial isolates and their resistance makes it more and more important to generate up-to-date data on antibiotic resistance patterns and bacterial isolates among hospitalized patients. The bloodstream profile of bacterial pathogens, risk factors for bacterial infection, and

the emergence of antibiotic resistance in hospitalized patients were all assessed in this study. In this study, the prevalence of bacterial bloodstream infection was 32.7%. This was comparable to findings from Hawassa, 35.9% [16], Addis Ababa, (32.8%) [17], Mekelle 28% [18], and Pakistan 36% [19]. However the finding was lower

compared to another study from Gondar (46.6%) [20], Bahir Dar 39.2% [21], India 47% [22], and Egypt 45.9% [23]. Furthermore, this result was higher as compared with another previous study in Gondar 19.7% and 25% [24], Jimma 22.8% [25], Ghana 13.1% [26] Italy 16.0% [27] and India 14.8% [28]. The variation might be due to differences in geography, study population, study design, and lifestyle of the population.

Gram-negative bacteria were dominant isolates which account for 52.1%. This was similar to a study conducted in Jimma [25], Rwanda Kigali [29] and Italy [30]. However, gram positives are dominant study from Arbaminch [31], Mekelle [18], and India [28]. The observed disparity may stem from the epidemiological variability of the bacteria that causes bloodstream infections, given the historical fluctuations in the incidence and etiology of bloodstream infections [32].

From gram-positive isolates *S. aureus* was the most dominant isolate. Even though there was a prevalence difference, another study also showed *S. aureus* as a predominant blood isolates: like in Addis Ababa [17] Arbaminch [31] Jimma [25], and Rwanda [29]. But this was different from other studies in which *CoNS* were their predominant blood isolates such as in Jimma [33], Hawassa [16], and Brazil [34]. The reason for the high prevalence of *CoNS* could be that they are found as the most common skin commensal that may get access to blood during medical procedures and increase the infection rate since most of our respondents were admitted patients. Whereas from gram-negative bacteria *E. coli* is the predominant isolate. This was supported by studies from Jimma [25], Arbaminch [31], and India [28]. The reason for the predominance of *E. coli* may be its relationship with the high risk of surgical procedures.

Antibiotic resistance presents another challenge for poor countries like Ethiopia. In this study, the level of antimicrobial resistance among Gram-negatives was highest for ampicillin, tetracycline, and trimethoprim-sulphamethoxazole with overall resistance of 89.6%, 75%, and 75% respectively. This was supported study conducted by Addis Ababa, Jimma, and Gondar [17, 25, 35]. The reason might be due to overuse of antibiotics and self-medication resulting in antibiotic resistance. In addition, the use of broad-spectrum agents, and underuse of medications as a result of insufficient dose or incomplete treatment courses may also play

a significant role in the emergence of antimicrobial resistance [36].

The selection of an antibiotic treatment becomes increasingly complicated as MDR diseases spread. Antibiotic overuse and abuse by patients is a major factor in the development and spread of multidrug-resistant bacteria in Ethiopia. In this study, the overall multi-drug resistance profile of isolates was 84.4% CI (77.1-91.6). This was a comparable finding from Jimma [37]. However, This was higher than other studies from Jimma and Arbaminch [25, 31]. The reason for this might be due to pharmacists carry the majority of the antibiotics that are routinely prescribed. Consequently, it is typical to purchase and use medications from independent pharmacies without a prescription [36, 38].

In this study, socio-demographic and clinical factors were analyzed as independent risk factors for bacterial infection. According to this study, patients with chronic underlying disease had 5.94 odds of increased acquisition of bacteria than patients who did not have such disease at 95% CI (3.12-11.33). This is supported by other studies done in Ethiopia [25] and Brazil [39]. This might be due to participants with underlying chronic diseases being exposed to many drugs, having frequent interaction with health professionals, and having poor immune status which can contribute to bacterial infection [40].

The other independent factor associated with bacterial infection was a history of previous hospitalization. Having a history of previous hospitalization had 2.31 odds of increased acquisition of bacteria than patients with no history of previous hospitalization. This is also supported by studies in Ethiopia and Brazil [31, 39, 40]. History of invasive procedures. In these studies participants who had a history of invasive procedures had 5.31 times the odds of acquiring bacterial infection than those who didn't have a history. This is also supported by other studies conducted in Brazil [39].

Conclusion

E. coli and *S. aureus* were the most common isolates. Most of the isolates were resistant to commonly prescribed antibiotics.

Recommendation:

Routine antimicrobial susceptibility testing should be before prescribing any drug and there should be wise use of antibiotics for any patients.

List of abbreviations and acronyms

AMR Anti-Microbial Resistance

BSI	Blood Stream Infection
CoNS	<i>Coagulase Negative Staphylococcus</i>
HIV	Human Immune Deficiency Virus
MDR	Multi-Drug Resistant
NICU	Neonatal Intensive Care Unit
TSI	Triple Sugar Iron
WHO	World Health Organization

Ethical approval and informed consent

The study protocol was reviewed and approved by the Research and Ethics Review Committee of Wollo University. Written permission letters were also obtained from Dessie comprehensive specialized hospital. The purpose and procedures of the study were explained to the study participants.

Consent for publication

Not applicable.

Data availability

The finding of this study is generated from the data collected and analyzed based on the stated methods and materials. All the data are already found in the manuscript and there are no supplementary files. The original data supporting this finding will be available at any time upon request.

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Competing interests

The authors declare that they have no competing interests

Authors' contributions

AsS: Conceptualized the study, collected the data, performed the microbiological investigation, analyzed the data, and critically edited the manuscript. AbA, GK, CM: and MA Involved interpretation of the data and critically edited the manuscript,

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