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Antibacterial resistance patterns of bacteria isolated from clinical specimens at Uttara IbnSina Diagnostic Centre, Dhaka

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Nowadays, antibiotic resistance is a global public health threat. Bangladesh is accelerated to this owing to its sub-standards healthcare along with the self-medication and overuse of antibiotics. The study aimed to assess patterns of antibacterial resistance in the clinical samples. The study was carried out at Ibn Sina Diagnostic and Consultation Center Uttara, Dhaka, from January to December 2019. All cultures and antimicrobial susceptibility test results of patients were extracted from laboratory records, using a semi-structured checklist. Data were analyzed using Microsoft Excel and SPSS version 20.0. To ensure confidentiality coding was used instead of the patient's identity. A total of 925 culture-positive results were analyzed, of which blood 620(65.0%) and urine 297(32.1%) samples were commonly diagnosed. The most frequently isolated bacterial were *Salmonella* spp. [601(65%)], *Escherichia coli* [244(26.4%)] and *Klebsiella spp.* [57(6.16%)]. The majority of the patients were females [540(58.4%)]. *E. coli* was found to be highly sensitive (>80%) to nitrofurantoin, meropenem, amikacin, amoxiclav, and imipenem; simultaneously, resistant (>45%) to cefixime, cephalexin, piperacillin, aztreonam, ampicillin, cefuroxime, and ciprofloxacin. *S. typhi* and *S. paratyphi* were sensitive (>80%) for cefepime, ceftriaxone, imipenem, tetracycline, cefixime, ceftazidime, cephalexin, cotrimoxazole, aztreonam, cefuroxime, and amoxiclav; concurrently, above 80% resistance for ciprofloxacin, azithromycin, gentamycin, and ampicillin. Overall, most of the isolates showed a significant rising rate of microbial resistance to ciprofloxacin, azithromycin, piperacillin, cephalexin, gentamycin, and ampicillin. The study findings revealed gradually rising rates of antibiotic resistance to commonly prescribed antibiotics. The study suggested the prescribers should be avoided overuse and irrational use of drugs to reduce antimicrobial resistance.

Key words: Bacteriology, antibiotic susceptibility, clinical samples, Dhaka.

INTRODUCTION

Antibiotic resistance is a well-known public health concern at the community, national and global levels (Nordberg et al., 2004). Decreasing the effectiveness of antibiotics in treating bacterial common infections and a decline in the new drug development rate is a concerning issue (Kandelaki et al., 2015; Luepke and Mohr, 2017; Spellberg et al., 2004). Antibiotic resistance poses a significant risk of mortality and economic burden

worldwide (Ahmed et al., 2019). The causes of antibiotic resistance are complex which include enzymatic degradation of antibacterial drugs, alteration of bacterial proteins that are antimicrobial targets, and changes in membrane permeability to antibiotics (Kandelaki et al., 2015). The low- and middle-income countries are more affected because of extensive misuse of antibiotics, non-human antibiotic use, poor quality of drugs, insufficient surveillance, and other factors associated with poor healthcare standards, malnutrition, chronic and repeated infection, unaffordability of more effective and costly drugs (Ayukekbong et al., 2017; Sosa et al., 2010). In 2014, the World Health Organization (WHO) reported on global surveillance of antimicrobial resistance, significant gaps prevail in surveillance, absence of standards methodology, data sharing and coordination. WHO identified the major gaps in the South-East Asia Region, the African Region, and the Eastern Mediterranean region (WHO, 2014).

Bangladesh is one of the South-East Asian developing countries and has a high rate of antibiotic resistance which poses a regional and global concern (Rahman and Huda, 2014). Enteric fever caused by salmonella spp. has been detected among children aged <5 years of age than the age group ≥5 years in the South-East Asian especially in India and Bangladesh. Though, there are no valid data regarding paratyphoid fever in Bangladesh (Naheed et al., 2010). Therapeutic failures in Bangladesh are not uncommon. Multiple studies have demonstrated irrational antibiotic prescribing by physicians, self-medication habits of patients, and indiscriminate use of antibiotics in agriculture and farming in different segments of the country (Biswas et al., 2014a; Biswas et al., 2014b; Sutradhar et al., 2014). Therefore, the prevalence of antibiotic resistance in Bangladesh is high, but no attempts have been undertaken to alleviate it. This study aims to serve as a reference for future works and to guide policymakers and prescribers to adopt the best strategy to lower the extent of antibiotic resistance as well as combat the problems following the expanding resistance.

METHODOLOGY

Study design and setting

This retrospective cross-sectional study was executed from January to December 2019 at IbnSina Diagnostic and Consultation Center Uttara, Dhaka. The sample was collected by using a sterile ascetic technique. A total of 925 culture-positive test result samples were analyzed. All cultures and antimicrobial susceptibility test results of patients were extracted from laboratory records notebook by using a semi-structured checklist. The sample-set included blood, urine, stool and sputum samples as well as wound swabs.

Bacterial isolates and identification

All of the received clinical specimens were initially cultured and subcultures into brain heart infusion, blood agar, Salmonella-Shigella agar, Chocolate agar and Mac-Conkey agar as per need, and after overnight incubation at 37°C, the bacteria identification was completed by gram staining as well as standard biochemical tests (catalase, coagulase, oxidase). This was done by sub-culturing on mediums such as triple sugar iron agar (TSI), SIM medium, and Simmons' citrate agar.

Antimicrobial susceptibility tests

The antimicrobial sensitivity tests of the isolated bacteria were performed by using the Kibry Bauer disk diffusion test on Mueller-Hinton agar (Bauer et al., 1966). The antibiotics agents used were: tetracycline (30 µg), nitrofurantoin (300 µg), azithromycin (15 µg), gentamicin (10 µg), ciprofloxacin (5 µg), doxycycline (30 µg), cotrimoxazole (25 µg), imipenem (10 µg), ceftriaxone (30 µg), ceftazidime (30µg), cefepime (30µg), meropenem (10µg), ampicillin (10 µg), penicillin (g) (10 µg). cefixime (5µg), cephalexin (30µg), piperacillin (75 µg), aztreonam (30 µg), cefuroxime (30 µg) amikacin (30µg), amoxiclav (30µg) vancomycin (30µg) fusidic acid (10µg) and cloxacillin (30 µg). The pattern of sensitivity and resistance was interpreted according to the guideline of the National Committee for Clinical Laboratory Standards (NCCLS) (National Committee for Clinical Laboratory Standards and Barry, 1999).

Statistical analysis

The data were entered into Microsoft Excel and analyzed by SPSS version 20. The results were presented as descriptive statistics in terms of relative frequency, percentage, mean ± standard deviation (SD) and to summarize patients' attributes and other related information.

Ethical considerations

Ethical approval was obtained from the Institutional Review Board. Administrative authorization for this study was obtained from the Branch Manager of the Diagnostic Center. The researchers highly consider the human right of the participants. To ensure the confidentiality coding method was used instead of other identifiers of the patients.

RESULTS

A total of 925 samples were analyzed; 32.1% presented urine, followed by stool (0.5%), blood (67%), sputum (0.2%) and wound swab (0.1%) (Figure 1). Among them males were 385 (41.6%) and females were 540 (58.4%). The ages of the patients ranged from 3 months to 90 years

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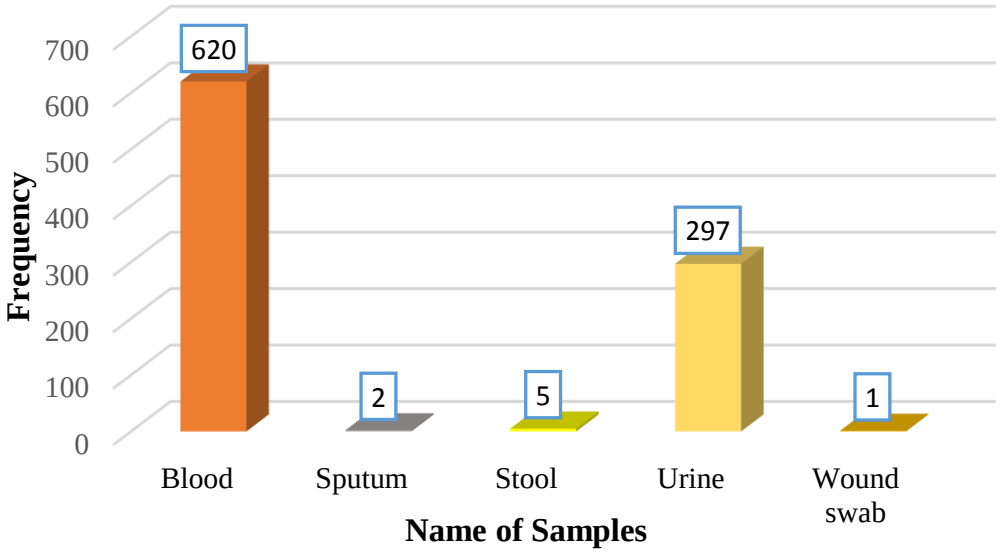

Figure 1. Types of samples.

Table 1. Age and sex status of patients.

Age and sex status of patients	Frequency	Percentage
Age of the patients (years)		
<10	193	20.9
10-19	201	21.7
20-29	209	22.6
30-39	115	12.4
>40	207	22.4
Sex of the patients		
Male	385	41.6
Female	540	58.4
Total	925	100.0

with a mean age of 27 years. The infection was most common among age groups of above >40 years (22.4%) followed by age groups of 20-29 years (22.6%) (Table 1). The set of most frequently isolated organisms included Salmonella spp., 601(65.0%); *Escherichia coli* were,244(26.4%) and *Klebsiella spp*. and *Staphylococcus aureus*, 57(6.16%) and 15(1.62%) respectively (Figure 2 and Table 2).

The sensitivity pattern of *E. coli* shows that the microbes were highly (>80%) sensitive for imipenem, nitrofurantoin, gentamycin, meropenem, amikacin, and amoxiclav. *E. coli* is booming developed resistance (>45%) for some antibiotics such as cefixime, cephalexin, piperacillin, aztreonam, ampicillin, cefuroxime, and ciprofloxacin. The microbes *Salmonella* spp. (*S. typhi* and *S. paratyphi)* were more than 80% sensitive tocefepime, ceftriaxone, imipenem, tetracycline, cefixime, ceftazidime, cephalexin, gentamycin, cotrimoxazole, aztreonam, ampicillin, cefuroxime, amikacin, and amoxiclav. At the same time above 80% had developed resistance to ciprofloxacin and azithromycin. *S. typhi* has developed (>20%) resistant to several antibiotics like ampicillin, piperacillin, and cotrimoxazole.

Klebsiella spp. was above 80% sensitive tocefepime, ceftriaxone, imipenem, gentamycin, meropenem, amikacin, and amoxiclav. This bacteriumhas developed significant resistance (>40%) to some antibiotics such as ciprofloxacin, piperacillin, cephalexin, and cefepime. *Staphylococcus aureus* was highly (>80%) sensitive tocotrimoxazole, gentamycin, amikacin, doxycycline, vancomycin, fusidic acid, cloxacillin. *S. aureus* developed resistance for ciprofloxacin, penicillin, and azithromycin. *Pseudomonas* spp. was substantially sensitive to cefepime, imipenem, ceftazidime, piperacillin,

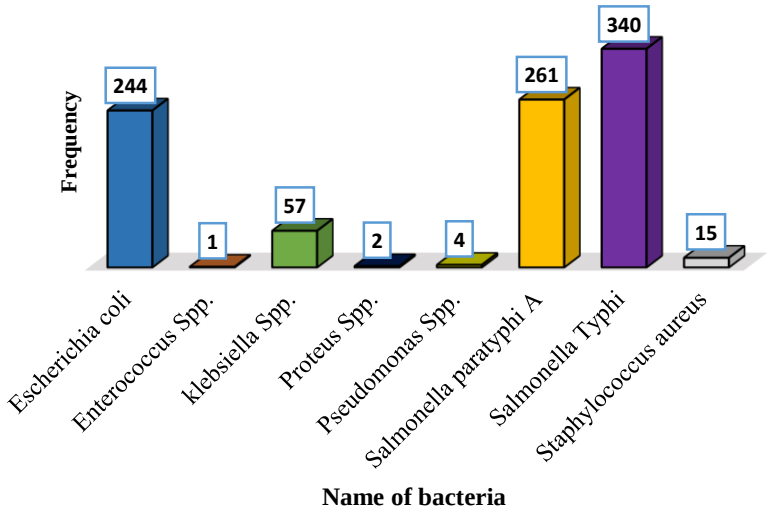

Figure 2. Name of isolated bacteria.

Table 2. Distribution of bacteria among sex of patients.

Name of bacteria	Sex	
	Male	Female
E. coli	40	204
Enterococcus spp.	0	1
Klebsiella Pneumoniae	1	0
Klebsiella spp.	6	51
Proteus spp.	1	1
Pseudomonas spp.	2	2
Salmonella paratyphi A	145	116
Salmonella typhi	184	156
Staphylococcus aureus	6	9
Total	385	540

gentamycin, and amikacin. These bacteria developed resistance to aztreonam, cefepime, and tetracycline. *Proteus* spp. was 100% sensitive tocefepime, imipenem, cotrimoxazole, piperacillin, amikacin, gentamycin, meropenem, amikacin, and amoxiclav. Of the resistance to ceftazidime and cefixime, throughout the study period, only two samples were positive *Proteus* spp. *Enterococcus* spp. was 100% resistance tocefepime, ceftriaxone, imipenem, cotrimoxazole, tetracycline, cefixime, ceftazidime, gentamycin, amikacin and meropenem and resistive for piperacillin, ampicillin, azithromycin. Though, throughout the study period, we have found a single culture positive of *Enterococcus* spp. (Table 3).

DISCUSSION

Bacterial infections are the predominant problem in developing countries like Bangladesh where water, sanitation, and hygiene (WASH) continue to be below international standard. The shortage of reliable microbial and antimicrobial data is also a problem in managing the physicians treating patients with a bacterial infection before the appropriate treatment is applied to get the best outcome (Tjaniadi et al., 2003). The major cause behind antibiotic resistance makes the bacteria to be smart. However, in Bangladesh, prescribers usually diagnose microbial infection based on clinical finding and choose antimicrobial drugs on an experiential basis (Faiz and Rahman, 2004), which critically distresses the sensitivity pattern of microorganisms. Besides, the unwillingness of the policymakers and officials to sanction law to overcome insufficient guidelines and instruction to control antimicrobial prescription and administration leads to the deteriorating of the circumstance.

In the present study, female patients were found to be higher than the males as found in other studies (Derbie et

Table 3. Pattern of antibiotic resistance among isolated bacteria.

Antimicrobials		Bacterial isolates							
		E. coli **(n-244)**	***Salmonella Typhi*** **(n=340)**	***Salmonella paratyphi A*** **(261)**	***Staphylococcus aureus*** **(n=15)**	***Klebsiella*** **spp. (n=57)**	***Pseudomonas*** **spp. (n=4)**	***Proteus*** **spp. (n=2)**	***Enterococcus*** **spp. (n=1)**
Cefepime	S	129(73.30)	285(99.65)	217(83.1)	ND	38 (82.60)	3 (75.0)	1 (100)	0
	R	47(26.70)	1(0.35)	44(16.9)	ND	8 (17.40)	1 (25.0)	0	1 (100)
Ceftriaxone	S	156(63.93)	339(99.71)	259(99.2)	0	38 (82.60)	ND	1 (50)	0
	R	88(36.07)	1(0.29)	2(0.8)	1 (100)	8 (17.40)	ND	1 (50)	1 (100)
Imipenem	S	225(94.94)	331(97.35)	251(96.2)	1(100)	51 (89.47)	3 (100)	2 (100)	0
	R	12(5.06)	9(2.65)	10(3.8)	0	6 (10.53)	0	0	1 (100)
Nitrofurantoin	S	203(88.26)	ND	ND	1 (100)	44(78.57)	ND	2 (100)	1 (100)
	R	27(11.74)	ND	ND	0	12(21.43)	ND	0	0
Tetracycline	S	156(65.27)	332(96.51)	244(93.5)	0	39 (68.42)	ND	1 (50)	0
	R	83(34.73)	13(3.78	17(6.5)	1 (100)	18 (31.58)	ND	1 (50)	1 (100)
Cefixime	S	132(54.32)	338(99.41)	258(98.9)	0	33(57.89)	ND	0	0
	R	111(45.68)	2(0.59)	3(1.1)	1 (100)	24(42.11)	ND	2 (100)	1 (100)
Ceftazidine	S	175(73.53)	338(99.71)	258(98.9)	1 (100)	33(63.46)	3 (75)	0	0
	R	63(26.47)	1(0.29)	2(1.1)	0	19(36.54)	1 (25)	2 (100)	1 (100)
Cephalexin	S	127(53.59)	331(98.51)	250(97.7)	0	32(56.14)	ND	1 (50)	0
	R	110(46.41)	5(1.29)	6(2.3)	1(100)	25(43.86)	ND	1 (50)	1 (100)
Cotrimoxazole	S	149(61.07)	263(77.35)	227(87.3)	9 (90)	42(73.68)	ND	2 (100)	0
	R	95(38.93)	77(22.65)	33(12.7)	1 (10)	15(26.32)	ND	0	1 (100)
Piperacillin	S	117(49.37)	262(78.68)	216(86.4)	1 (100)	33(57.89)	3 (100)	2 (100)	1 (100)
	R	120(50.63)	71(21.32)	34(13.6)	0	24(42.11)	0	0	0
Aztreonam	S	126(54.31)	324(97.30)	251(96.5)	0	35(62.5)	2 (50)	0	0
	R	106(45.69)	9(2.70)	9(3.5)	1 (100)	21(37.5)	2 (50)	2 (100)	1 (100)
Ampicilin	S	97(40.76)	272(80.47)	239(91.9)	1 (100)	10(17.5)	ND	2 (100)	1 (100)
	R	141(59.24)	66(19.53)	21(8.1)	0	47(82.45)	ND	0	0
Cefuroxime	S	135(56.72)	333(98.23)	251(96.2)	1 (100)	37(64.91)	ND	1 (50)	0
	R	103(43.28)	6(1.77)	10(3.8)	0	20(35.8)	ND	1 (50)	1 (100)
Ciprofloxacin	S	132(55.0)	49(14.50)	30(11.5)	1 (7.70)	32(57.14)	3 (100)	1 (50)	0
	R	108(45.0)	289(85.50)	231(88.5)	12 (92.30)	24(42.85)	0	1 (50)	1 (100)
Gentamycin	S	205(85.42)	337(99.12)	260(99.1)	13 (100)	51(91.07)	4 (100)	2 (100)	0
	R	35(14.58)	3(0.88)	1(0.9)	0	5(8.92)	0	0	1 (100)
Meropenem	S	230(96.23)	ND	ND	1 (100)	49(87.5)	ND	2 (100)	0
	R	9(3.77)	ND	ND	0	7(12.5)	ND	0	1 (100)
Amikacin	S	215(92.27)	336(99.41)	257(98.5)	13 (92.85)	46(93.87)	4 (100)	2 (100)	0

Table 3. Contd

Amoxyclav	R	18(7.73)	2(0.59)	4(1.5)	1 (7.15)	3(6.13)	0	0	1 (100)
	S	183(78.54)	325(97.31)	255(98.1)	1 (100)	42 (85.71)	ND	2 (100)	1 (100)
Azithromycin	R	50(21.46)	9(2.69)	5(1.9)	0	7 (14.29)	ND	0	0
	S	1(50.0)	118(34.71)	222(85.5)	4(30.76)	0	ND	ND	1 (100)
Doxycycline	R	1(50.0)	222(65.29)	39(14.9)	9(69.24)	2 (100)	ND	ND	0
	S	ND	ND	ND	12(85.71)	ND	ND	ND	ND
Vancomycin	R	ND	ND	ND	2(14.29)	ND	ND	ND	ND
	S	ND	ND	ND	12(100)	ND	ND	ND	ND
Fusidic acid	R	ND	ND	ND	0	ND	ND	ND	ND
	S	ND	ND	ND	11(84.61)	ND	ND	ND	ND
Cloxacillin	R	ND	ND	ND	2 (15.39)	ND	ND	ND	ND
	S	ND	ND	ND	10(100)	ND	ND	ND	ND
Penicillin	R	ND	ND	ND	0	ND	ND	ND	ND
	S	ND	ND	ND	7(53.84)	ND	ND	ND	ND
	R	ND	ND	ND	6(46.16)	ND	ND	ND	ND

S- Sensitive, R- Resistant , ND - Not Done.

al., 2017; Tahira and Singh, 2017; Kolawole et al., 2009). The females were more infected than males due to their physiological and anatomical differences (Kibret and Abera, 2014). The current study revealed that *Escherichia coli* was highly sensitive (>80%) to imipenem, nitrofurantoin, gentamycin, meropenem, amikacin, and amoxiclav. The susceptibility pattern of *E. coli for* nitrofurantoin was (89.7%), which compares to a study done in London (Bean et al., 2008). *E. coli* is developing resistance (>45%) to some antibiotics such as cefixime, cephalexin, piperacillin, aztreonam, ampicillin, cefuroxime, and ciprofloxacin. Ahmed et al. (2019) showed that *E. coli* was highly resistance to commonly used antibiotics like ampicillin (94.6-100%), amoxiclav (67.1-85.5%), ciprofloxacin (65.2-80.5%) and cotrimoxazole (72-82.2%). Patil and Mule described the isolates of *E. coli* were resistant to ampicillin (96.6%), tetracycline (79%), ceftriaxone (62%) and gentamicin (51.7%) (Patil and Mule, 2019).

Salmonella spp. showed (>80%) sensitive to cefepime, ceftriaxone, imipenem, tetracycline, cefixime, ceftazidime, cephalexin, gentamycin, cotrimoxazole, aztreonam, ampicillin, cefuroxime, amikacin, and amoxiclav. This result is comparable with Ahmed et al. (2019) who demonstrated *Salmonella spp.* was highly sensitive to cefixime and ceftriaxone. *Salmonella typhi* developed resistance to ciprofloxacin and azithromycin. Ebrahim et al. (2016) showed that *Salmonella typhi* has developed resistance from 2003 to 2014 from 0% to 14% in Canada. The previous literature showed Salmonella isolates had 100% sensitivity to ceftriaxone and cefixime (Bhan et al., 2005; Bhetwal et al., 2017; and Mule, 2019).

Klebsiella spp. was above 80% sensitive to cefepime, ceftriaxone, imipenem, gentamycin, meropenem, amikacin, and amoxiclav. These bacteria developed significant resistance (>40%) to some antibiotics like ciprofloxacin, piperacillin, cephalexin, and cefepime. *Klebsiellaspp*.

Sensitive to Meropenem (100%), Nitrofurantoin (83.3%), whereas it is resistant to Penicillin (100%), Ampicillin (93.61%) and intermediate to Gentamicin (18.5%), Augmentin (17.4%) (Ahmed et al., 2019). Antimicrobial sensitivity pattern reveals the development of resistance to common antibiotics, which is comparable with that of previous studies (Akond et al., 2009; Rogers et al., 2011). However, antibiotics like ciprofloxacin, ceftazidime, ampicillin, piperacillin, cotrimoxazole, and ceftriaxone have become ineffective because of the beginning of the resistance. The microbes are still sensitive to imipenem, azithromycin, vancomycin and amikacin, nitrofurantoin, gentamycin, meropenem, and amoxiclav. This outline of sensitivity does not compare with

previous findings (Hasan et al., 2011). Prescribers cited diagnostic insecurity and advent of resistance as pediment reasons for prescribing antimicrobials; nevertheless some of them revealed the possibility of losing patients as one reason (Rahman and Huda, 2014).In this study, we found a very small proportion of *S. aureus* 15(1.7%), *Pseudomonas spp*. 4(0.43%), *Proteus* spp. 2(0.21%), *Enterococcus* spp. 1(0.10%). Therefore, we did not compare it with other studies.

Conclusion

The present study revealed that *E. coli, salmonella typhi,* and *salmonella paratypi* were the most frequently isolated bacterial in the clinical samples. The majority of the isolated bacteria showed certain levels of antimicrobial resistance to commonly recommended drugs like ampicillin, norfloxacin, ciprofloxacin, azithromycin, cephalexin, piperacillin and cotrimoxazole. However, strict policy and appropriate use of antibiotics can assuage the burden of antimicrobial resistance. It is highly suggested to perform antimicrobial susceptibility testing before the administration of antibiotics and ensure the rational use of drugs to reduce antibiotic resistance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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