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POTENTIALLY PATHOGENIC MULTIDRUG-RESISTANT *VIBRIO* SP. ISOLATED FROM DUMPSITES IN RIVERS STATE, NIGERIA

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ABSTRACT

Vibrio sp. is associated with several disease conditions. An assessment of the pathogenic potential of such strains is key in assessing the risk these organisms could pose to human health. This study therefore was aimed at analyzing the pathogenic potential of MDR Vibrio sp. isolated from select dumpsites in Port Harcourt, Rivers State. Two dumpsites in Port Harcourt, Rivers State were assessed for the presence of Vibrio sp. using standard microbiological techniques involving a pre-enrichment using alkaline peptone water, culture on Thiosulfate-citrate-bile salts-sucrose and biochemical characterisation. Following antimicrobial susceptibility testing using the standard Kirby Bauer disc diffusion method, pathogenic potential was assessed based on haemolytic activity and biofilm forming potential. Results showed that the highest rates of resistance were against cefuroxime (68.8%) while the lowest resistance rates (3.2%) were against the quinolone antibiotics (ofloxacin and ciprofloxacin). In total, 38 antibiograms were observed among the 93 isolates with AUG-CAZ-CRX-CXM noted as the most commonly occurring antibiogram. Only 31.2% (29/93) of isolates were multidrug resistant. A total of 47.3% of the test isolates exhibited pathogenic potential (either haemolytic ability or biofilm forming potential), with more (37.6%) exhibiting biofilm forming potential. A co-occurrence of both pathogenic characteristics was observed in only 6 (13.6%) isolates. Of the 93 isolates, a co-occurrence of MDR and pathogenic potential was observed in 16.1% of isolates (15/93). This study shows a moderate association between potentially pathogenic multidrug resistance Vibrio sp. and the sampled dumpsites indicating a low potential public health risk because of the non-pathogenic nature of strains isolated.

Keywords: *Vibrio* sp., dumpsites, pathogenic potential, MDR, Nigeria

INTRODUCTION

Over the years, the phenomenon of multidrug resistance has not abated but rather has grown in scope and magnitude. *Vibrio* sp. is a diverse group of organisms made up of over 100 species, of which only about 12 cause infections in man (Baker-Austin et al., 2018). There are however only three key players involved in human infection. Specific strains of *Vibrio cholerae* (the O1 and O139 serotypes) are associated with cholera which is a severe secretory diarrheal illness transmitted by the faecal-oral route via contaminated food and water and characterized by vomiting and the typical rice-water stool. Non O1 and O139 and *Vibrio parahaemolyticus* and *Vibrio*

vulnificus have been associated with gastrointestinal tract disorders ranging from mild to severe cases (Baker-Austin et al., 2018)

Dumpsites are designated sites for the disposal of wastes; unwanted products, are no longer fit for purpose. Considering the role of dumpsites as receptors of different types of wastes especially household and human waste, there is a danger of these sites harbouring multidrug-resistant (MDR) *Vibrio* sp. Subsequently, these could be transported via runoffs into local untreated drinking water systems (Turner et al., 2021). This phenomenon of the presence of MDR in *Vibrio* sp from dumpsites has recently been analyzed by Omoruyi and Ojubiaja (2022) in Edo State. This study described a 90% occurrence of MDR in the isolated *Vibrio* sp. Most studies simply carry out a general bacteriological assessment without a pre-enrichment step and hence no detection of *Vibrio* sp. (Oshoma et al., 2017; Ogunleye & Akinneye 2019; Odum et al., 2020). No study focused specifically on *Vibrio* sp. has however been carried out in Port Harcourt, Rivers State.

Taking into cognizance variations between pathogenic and commensal/environmental strains and the impact that this has on human health, an assessment of the pathogenic potential of strains is often key. This could provide a more complete understanding of the risk such non-clinical strains may pose to human health. This study therefore was aimed at analyzing the pathogenic potential of MDR *Vibrio* sp. isolated from select dumpsites in Port Harcourt, Rivers State, Nigeria.

MATERIALS AND METHODS

Sampling Procedure and Processing

Two dumpsites in Port Harcourt, Rivers State, served as the sampling area for this study. These dumpsites known as the Rivers State Waste Management Authority (RIWAMA) dumpsite and the Alakahia dumpsite were found at GPS locations 4˚ 54'12''N 6˚ 57'52''E and 4˚ 53'14.3''N 6˚ 55'19.9''E respectively. Soil samples were randomly collected from twenty-five points within each dumpsite at two unique sampling depths per point. Topsoil (TS) samples were obtained from the surface while subsurface (SS) samples were collected at a 15 cm depth.

Soil samples collected were immediately transferred to the laboratory and processed for the presence of *Vibrio* sp. as previously described (Otokunefor et al., 2023). In brief, a pre-enrichment was carried out using Alkaline Peptone Water (APW) and culture was subsequently done on the Thiosulfate-citrate-bile salts-sucrose (TCBS) agar using the streak method.

Isolation and Identification

Characteristic *Vibrio* colonies showing as yellow or green colonies were then purified by sub-culturing on nutrient agar plates. The identities of the organisms were then determined using standard previously described biochemical tests such as citrate test, motility test, TSIA test, indole test, urease test, catalase test, sugar fermentation tests, methyl red/Voges Proskauer test, oxidase test and sodium chloride tolerance (Cowan & Steel, 1985; Cheesbrough 2006).

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of isolates was then determined using the Kirby-Bauer disc diffusion test (Bauer et al., 1966). This method involves the creation of a bacterial lawn on a Mueller Hinton agar plate from a bacterial cell suspension corresponding to 0.5 McFarland using a sterile swab followed by the application of the susceptibility discs. Incubation was then done at 37°C for 24 hours after a five-minute pre-incubation step. Antibiotics tested include; Nitrofurantoin (300µg), Cefuroxime (300µg), Ceftriaxone (45µg), Ampiclox (10µg), Cefixime (5µg), Levofloxacin (5µg), Amoxicillin/Clavulanic acid (50µg), Cefotaxime (20µg), Imipenem/ Cilistatin (10µg), Ofloxacin (5µg), Gentamicin (10µg), Nalidixic acid (30µg), Ceftazidime (30µg), Cefuroxime (30µg),

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Augmentin (30µg), Ciprofloxacin (5µg) and tetracycline (30µg).

Assessment of Pathogenic Potential

Hemolytic Activity

This test was used to determine isolates that can produce hemolysin which is one of the major factors that is responsible for pathogenicity among *Vibrio* species. To determine haemolytic activity, freshly prepared blood agar was prepared and aseptically inoculated with the test isolates (Arunagiri and Sivakumar 2021). The inoculated plates were properly labelled and incubated at 37°C for 24 hours, after which organisms were classed as either alpha haemolytic (complete clear zones), beta haemolytic (partial clear zones) or gamma haemolytic (non-haemolytic).

Biofilm Formation Assay

The biofilm forming potential of isolates was assessed as previously described (Otokunefor et al., 2020). In brief, freshly prepared plates of Congo Red Agar were aseptically inoculated with the test isolates, properly labelled and incubated at 37°C for 24 hours. After the incubation period, the plates were observed for biofilm-forming ability as indicated by the production of a black pigment (Otokunefor et al., 2020).

RESULTS

Results of the isolation and identification of *Vibrio* sp. from the dumpsite samples showed a higher occurrence of *Vibrio* sp. in the topsoil as opposed to the subsoil samples (Figure 1). In general, more *Vibrio* sp. were isolated from the Airport Road dumpsite (Figure 2).

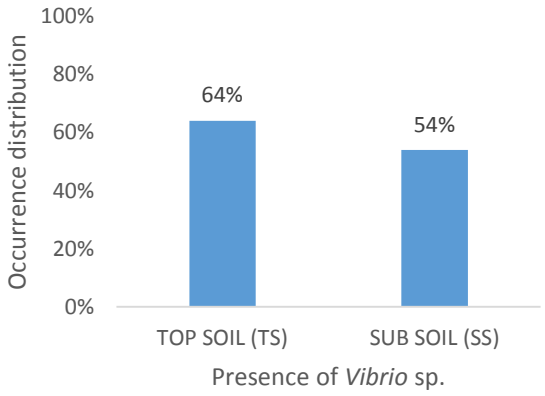

Figure 1: Occurrence distribution of *Vibrio* Species isolated from 2 points at dumpsites.

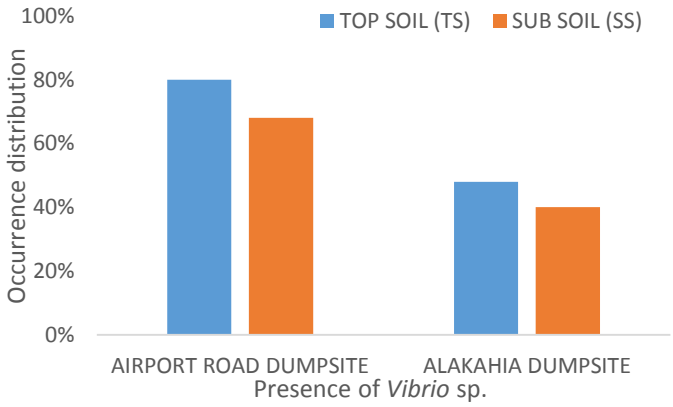

Figure 2.: Diversity of occurrence of *Vibrio* spp based on dumpsite

Antimicrobial Susceptibility Rates of Test Isolates

An analysis of the results of the susceptibility testing of the isolates (Figure 3) showed that resistance rates less than 50% were observed for most antibiotics (6/9, 66.7%). The highest rates of resistance were against Cefuroxime (68.8%) and Augmentin (67.7%), while the lowest resistance rates (3.2%) were noted against the quinolone antibiotics (ofloxacin and ciprofloxacin).

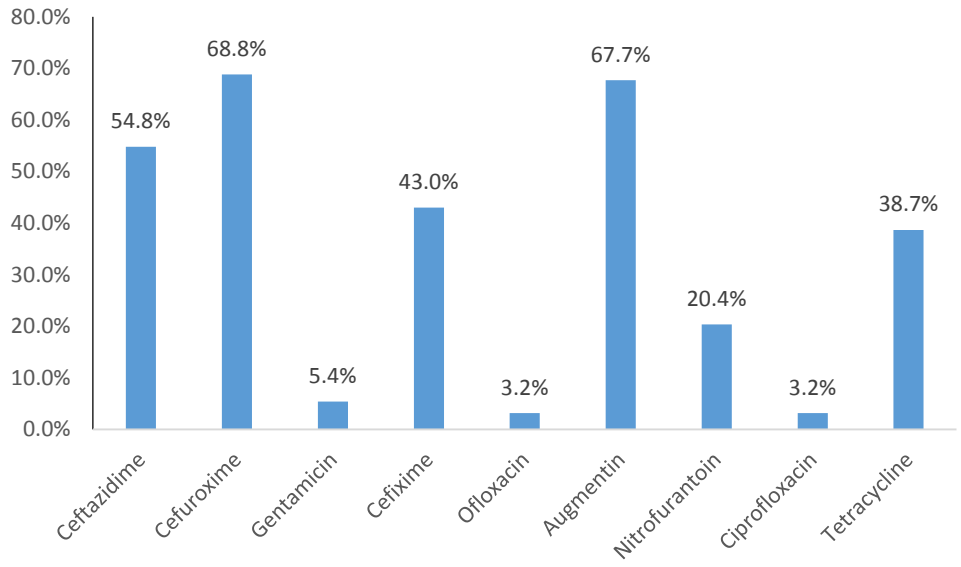

Figure 3: Occurrence distribution of resistance rates among test isolates

Antibiotic Resistance Pattern

The generation of unique antibiotic resistance patterns (antibiograms) for individual isolates from the results of the susceptibility testing showed a total of 38 antibiograms (Table 1). The majority of these (23/38, 60.5%) however were singletons, containing only a single organism. Four isolates were sensitive to all antibiotics, while one isolate was resistant to all antibiotics. TET and AUG-CAZ-CRX-CXM were the most commonly occurring antibiograms with 10 and 12 isolates expressing this antibiogram respectively.

Table 1: Antibiotic resistance patterns and distribution among test isolates

S/N	ANTIBIOTIC RESISTANCE PATTERN	NUMBER of ISOLATES
1.	-	4
2.	AUG	4
3.	CRX	1
4.	TET	10
5.	AUG- NIT	2
6.	AUG-CAZ	1
7.	AUG-CRX	7
8.	AUG-TET	2
9.	CAZ- CRX	1
10.	CAZ-CXM	2
11.	CAZ-TET	1
12.	CRX-CXM	1
13.	CRX-GEN	1
14.	CRX-TET	3
15.	AUG-CAZ-CRX	7
16.	AUG-CAZ-CXM	1
17.	AUG-CRX-TET	2
18.	AUG-NIT-TET	1
19.	CAZ-CRX-CXM	1
20.	CAZ-CRX-TET	1

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21.	CRX-CXM-TET	1
22.	AUG-CAZ-CRX-CXM	12
23.	AUG-CAZ-CRX-NIT	1
24.	AUG-CAZ-CRX-TET	1
25.	AUG-CRX-CXM-NIT	1
26.	CAZ-CRX-CXM-TET	1
27.	AUG-CAZ-CPR-CRX-CXM	1
28.	AUG-CAZ-CRX-CXM-GEN	1
29.	AUG-CAZ-CRX-CXM-NIT	6
30.	AUG-CAZ-CRX-CXM-OFL	1
31.	AUG-CAZ-CRX-CXM-TET	5
32.	AUG-CAZ-CRX-NIT-TET	2
33.	AUG-CRX-CXM-NIT-TET	2
34.	CAZ-CRX-CXM-NIT-TET	1
35.	AUG-CAZ-CRX-CXM-NIT-TET	1
36.	AUG-CAZ-CRX-GEN-NIT-TET	1
37.	AUG-CAZ-CPR-CRX-CXM-GEN-OFL	1
38.	AUG-CAZ-CPR-CRX-CXM-GEN-NIT-OFL-TET	1

Code: AUG: ; CAZ-CPR-CRX-CXM-GEN-NIT-OFL-TET

Multiple Antibiotic Resistant (MAR) Index

MAR index represents a ratio of the number of antibiotics to which an organism is resistant in relation to the total number of antibiotics assayed for. The MAR index is calculated using the formula MAR index=a/b.,

Where:

a= how many antibiotics the isolate was resistant to
b= represents the total amount of antibiotics the isolate was exposed to.

An estimation of the MAR index in this study revealed that quite a high proportion (43%) of isolates were found at MAR index values of 0.22 and less (Figure 4), where this range is indicative of "low antibiotic exposure risk source".

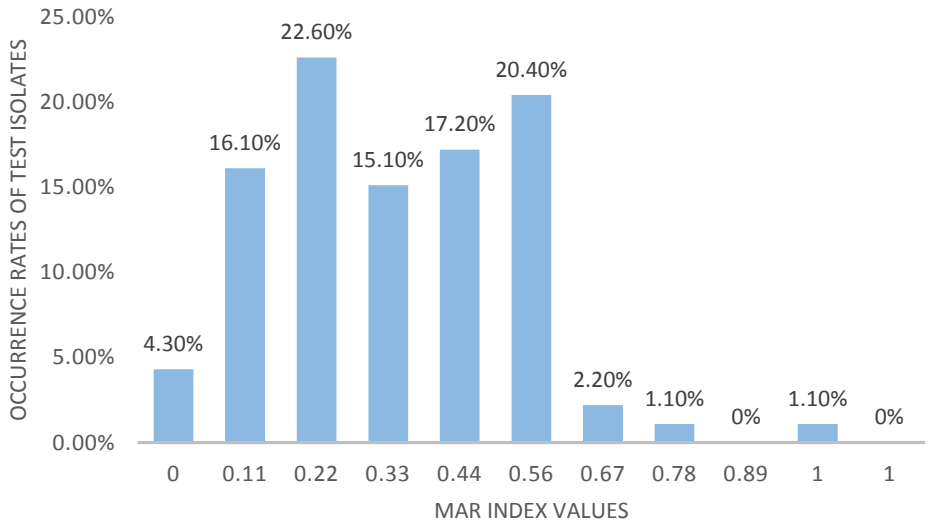

Figure 4: Occurrence distribution of test isolates among the different MAR indices

Occurrence of Multidrug resistance

An assessment of multidrug resistance (MDR) defined by resistance to three or more classes of antibiotics revealed that 31.2% (29/93) of isolates were multidrug resistant. There was however a preponderance (72.4%) of isolates resistant to only 3 classes of antibiotics (Figure 5), with very few organisms (6.4%) resistant to more than 4 drug classes.

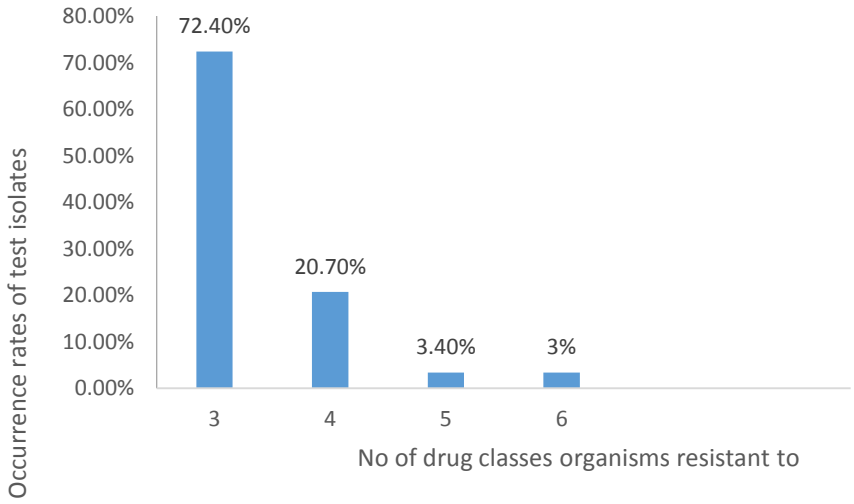

Figure 5: Occurrence distribution of multidrug-resistant isolates

Pathogenic Potential of test Isolates

A total of 47.3% of the test isolates exhibited pathogenic potential as evidenced by either haemolytic ability or biofilm forming potential. More organisms however exhibited biofilm forming potential (37.6%) than haemolytic ability (16.1%). A co-occurrence of both pathogenic characteristics was observed in 13.6% (6) of isolates (SS31B, SS32A, SS33B, TS11B, TS13B, TS31A).

Co-occurrence of MDR and Pathogenic Potential

Of the 93 isolates, a co-occurrence of MDR and pathogenic potential was observed in 16.1% of isolates (15/93).

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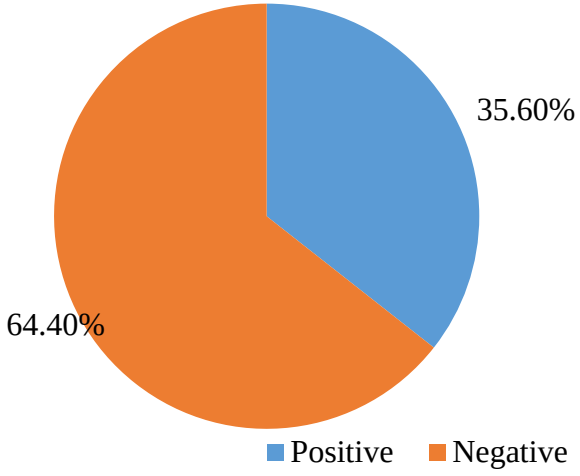

Figure 6: Occurrence of biofilm-forming ability in test isolates

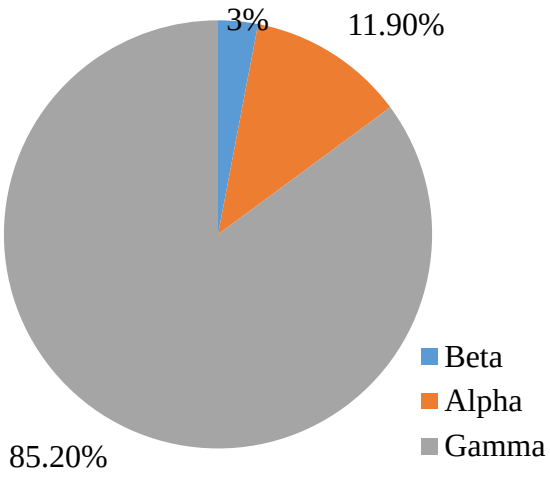

Figure 7: Hemolytic activity of *Vibrio* isolates.

DISCUSSION

Vibrio species which are more commonly reported to be isolated from aquatic and marine environments where they occur naturally (Baker-Austin et al., 2018; Ebob et al., 2022), have also been isolated from dumpsites particularly in Nigeria (Idahosa et al., 2017; Nyandjou et al., 2018; Omoruyi & Ojubiaja 2022). While there is a tendency to consider these isolates to all be pathogenic, few studies assess the pathogenic potential of such isolates especially in relationship to the drug-resistant status of such isolates.

This study focused specifically on isolating *Vibrio* sp., found a much higher occurrence (61%) of *Vibrio* than other studies focusing specifically on dumpsites. Nyandjou et al (2018), reported only 26 *Vibrio* sp. from 520 samples obtained from dumpsites, while Ajuzie et al, (2010) and Obire et al, (2002) failed to detect *Vibrio* sp. from their sampled dumpsites. This is probably a result of the sampling protocol. The pre-enrichment step recommended for the isolation of *Vibrio* sp. was employed in this present study.

The highest rates of resistance in this study were observed against two beta-lactam antibiotics, augmentin (67.7%) and cefuroxime (68.8%). This rate was slightly higher than observed in a 2021 study (Adekanmbi et al., 2021) which reported a 44.3% resistance. Other studies simply tested against ampicillin and reported rates ranging from 76.92% to 94.1% (Islam et al., 2021; Gxalo et al., 2021). A 2022 study however had a much lower rate of amoxicillin resistance (45%) reported (Adesiyan et al., 2022). Low rates of resistance were noted against gentamicin, ofloxacin and ciprofloxacin. This trend is similar to previous reports from both a study on *Vibrio* sp from dumpsites in Nigeria (Omoruyi & Ojubiaja 2022), as well as other studies exploring *Vibrio* sp from various sources (Abioye et al., 2023; Odjadjare & Igbinosa 2016). In all of these cases, resistance rates were less than 20%. Gxalo and colleagues (2021) however reported higher rates of ofloxacin resistance (59.5% to 66.9%). These isolates were however from freshwater environments rather than dumpsites. These variations could be a result of differences in strain source and antibiotic exposure to the environment.

Unlike the 31.2% MDR rate observed in this study, some other studies had recently reported 100% MDR rates in *Vibrio* isolates (Gxalo et al., 2021; Omoruyi & Ojubiaja 2022). These studies also differed from this

present study in having high levels of organisms resistant to more than 4 drug classes. Several studies however reported only resistance rates to different individual antibiotics rather and failed to report specifically on multidrug resistance (Odjadjare & Igbinosa, 2017; Adesiyan et al., 2022; Oko et al., 2022).

Haemolysis has been described as a crucial marker of virulence among *Vibrio* sp. Biofilms have also been thought to play a crucial part (Silva & Benitez 2016). Reports on haemolysis in *Vibrio* sp., in Nigeria are few, but reports from around the globe show that haemolytic activity within our strains was relatively low with studies reporting rates ranging from 20.5% to 36% (Costa et al., 2013; Hikmawati et al., 2019; Arunagiri & Sivakumar 2021). The much higher rates of biofilm-forming ability of isolates in this study were similar to reports by a previous study which noted biofilm-forming potential in 56.14% of their test isolates (Santajit et al., 2022). This same study reported a co-occurrence of haemolysis and biofilm-forming ability in 14 out of 35 (40%) *Vibrio* sp., detected. A rate much higher than that reported in this present study. Additionally, only a single isolate in the Santajit study showed co-occurrence of multidrug resistance and pathogenicity.

CONCLUSION

This study shows a moderate association between potentially pathogenic multidrug resistance *Vibrio* sp. and the sampled dumpsites. This is encouraging as it indicates that while these dumpsites showed a high occurrence of *Vibrio* sp., there is a low potential public health risk because of the non-pathogenic nature of the strains isolated.

Statements and Declarations

Competing Interests

All authors declare that they have no conflict of interest.

Ethical Statement

This article does not involve human participants or animals.

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