Original Article

Detection of *bla*$_{OXA-10}$ and *bla*$_{OXA-488}$ Genes in Multidrug-Resistant *Pseudomonas aeruginosa* from Clinical Isolates

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Abstract

Background: *Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen. In order to halt the development of extended spectrum lactamases (ESBLs), which are produced by multidrug-resistant *P. aeruginosa*, effective infection management measures are required. The frequency of clinical isolation fluctuates greatly globally and rapidly over time. **Objective:** The goal of this study was to ascertain the distribution of ESBLs (oxacillin-hydrolyzing class D β-lactamase 10 [*bla*$_{OXA-10}$] and oxacillin-hydrolyzing class D β-lactamase 488 [*bla*$_{OXA-488}$]) genes among *P. aeruginosa* isolates and to determine the susceptibility of the antibiotic of these isolates in order to reflect the frequency and severity of these bacteria's pathogenicity in Babylon province. **Materials and Methods:** A total of 194 clinical specimens from patients hospitalized in Babylon, Kirkuk, and Baghdad hospitals were taken, including wound, burn, urine, lung fluid, gunfire, bomb, and diabetic foot swabs. Biochemical tests, molecular study of *P. aeruginosa*-specific gene, and antibiotic resistance testing were used to identify isolates, along with morphological features (culturally and microscopically). **Results:** Thirty-six *P. aeruginosa* isolates were found and identified; all the isolates were resistant to piperacillin (100%) as well as to ticarcillin (100%) and ceftazidime (89%). *Pseudomonas aeruginosa* isolates had the lowest resistance to piperacillin-tazobactam (42%), colistin (8.3%), and polymyxin B (8.3%). *Pseudomonas aeruginosa* was found to carry *bla*$_{OXA-10}$ 30/36 (83%) and *bla*$_{OXA-488}$ 24/36 (67%) according to polymerase chain reaction (PCR) tests. **Conclusion:** Antimicrobial resistance in *P. aeruginosa* is a real problem that affects individuals all over the world and is an unintended consequence of excessive use of antibiotics.

Keywords: ESBLs *bla*$_{OXA-10}$ and *bla*$_{OXA-488}$ genes, MDR, *P. aeruginosa*

Introduction

Owing to their link with numerous community and healthcare-associated illnesses (HAIs), gram-negative bacteria (G-ve) present a real problem for health systems.[1,2] Given the few suitable treatment choices, increased morbidity and mortality, and high cost of medical care, multidrug-resistant (MDR) *Pseudomonas aeruginosa* is a dangerous source of HAIs.[3,4] The cornerstone of antimicrobial resistance, notably in (G-ve), has been identified as β-lactamase genes, despite the fact that infections have a variety of methods of resistance.[5,6]

The gram-negative bacteria *P. aeruginosa* infections are highly resistant to many microbial antibiotics, making it difficult to manage and treat infections. The public's health is in danger because *P. aeruginosa* bacteria are showing positive extended spectrum lactamases (ESBLs) at an alarming rate.[1]

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Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_307_23

Gram-negative bacteria have high rates of antibiotic resistance, which is associated with increased disease and death, according to a World Health Organization (WHO) report. Clinical isolates of *P. aeruginosa* quickly acquire antibiotic resistance and spread across hospitals, putting them on the list of MDR bacteria by WHO.[7,8]

Risk factors for *P. aeruginosa* infection in soft tissues include burns, open wounds, and the postoperative state; urinary catheters; immune compromise; bloodstream infections; and chronic obstructive pulmonary disease,

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Submission: 14-Mar-2023 **Accepted:** 13-Apr-2023 **Published:** 23-Jan-2026

How to cite this article: Hussien MKJ, Jarallah EM, Al-Taee ZM. Detection of *bla*$_{OXA-10}$ and *bla*$_{OXA-488}$ genes in multidrug-resistant *Pseudomonas aeruginosa* from clinical isolates. Med J Babylon 2025;22:1002-8.

advanced age, cystic fibrosis, and mechanical ventilation in the treatment of respiratory infections.[9] In terms of respiratory infections, tracheostomy, prior colonization or infection with *P. aeruginosa*, bronchiectasis, severe chronic obstructive pulmonary disease, and prior invasive respiratory or vasopressor support were factors related to *P. aeruginosa* community-acquired pneumonia, whereas tracheostomy, before colonization or infection with *P. aeruginosa*, and respiratory or vasopressor support were factors related to multidrug resistance *P. aeruginosa* with community-acquired pneumonia (MDR-PA CAP).[10] ventilator-associated pneumonia (VAP) brought on by *P. aeruginosa* was linked to prolonged hospital stays and intensive care units stays.[11,12]

Pseudomonas aeruginosa demonstrates its resistance by the ability to produce biofilm,[13] the ability to rapidly develop resistance to current therapies, and innate one-to-many drug classes.[14,15] The permeability of outer membrane, excessive expression of efflux systems, and antibiotic-inactivating enzymes are a few of the known mechanisms of *P. aeruginosa* resistance. Acquired resistance is caused by mutations in the genes encoding efflux pumps, porins, penicillin-binding proteins, and enzymes and horizontal gene transfer. Adaptive resistance is brought on by repeated antibiotic exposure and excessive environmental stress.[16] Classic antipseudomonal antibiotics have a variety of mechanisms of action, including inhibition of bacterial cell walls for β-lactam agents like ceftazidime/cefepime, piperacillin-tazobactam, imepenem-cilastatin, meropenem, dorepenem, and aztreonam, which block the synthesis of DNA for fluoroquinolones, and inhibition the synthesis of protein for aminoglycoside.

Resistance to β-lactamases in *P. aeruginosa* has increased due transmissible antibiotic resistance genes that play a role in promoting resistance. A, B, D, and C are β-lactamases. Class B mechanism is a MBLs (metallo-lactamases), which opposed to A, C, and D.[17] Some microorganisms have the ability to produce enzymes that enhance resistance to beta-blockers. Most of these organisms are *P. aeruginosa* bacteria.[18,19]

The presence of such a many different groups of β-lactamase genes was noticed in MDR-*P. aeruginosa* isolated form clinical samples that were phenotypically resistant to ceftazidime-avibactam and/or ceftolozane-tazobactam. Resistance to ceftazidime-avibactam and ciftolozane-tazobactam was linked to the class B β-lactamases genes (e.g., bla_{VIM-2}) and class D β-lactamases genes (e.g., oxacillin-hydrolyzing class D β-lactamase 10 [bla_{OXA-10}]), whereas ciftolozane-tazobactam resistance was linked to the class A β-lactamases (e.g., bla_{VEB-9}), class (e.g., oxacillin-hydrolyzing class D β-lactamase 488 [$bla_{OXA-488}$]). Resistance to these novel therapeutic agents was also associated with the presence of other β-lactamase genes such as bla_{PDC-35} and bla_{OXA-10}.[6]

Materials and Methods

Collection of samples

A total of 194 clinical samples (including urine, burns, diabetic foot, gun fire bombs, and bombs) were collected from both genders and different age groups from July to October 2022. These specimens were collected from several hospitals in the Hilla city and (Burns Specialized, AL-Shaheed Ghazi Hariri, Baghdad Teaching, Ibn AL Bitar Hospital and National Center for Educational Laboratories) in Baghdad and (Kirkuk General Hospital, Azadi Teaching Hospital) in Kirkuk.

Bacterial isolation and identification

All samples were grown using the streaking method on blood agar, MacConkey agar, and cetrimide agar (incubation at 37ºC for 24 h).[20] The bacterial isolates were maintained in nutrient broth that contains 15% glycerol at –20ºC. MacFaddin's description, morphological characteristics, and biochemical tests were used to identify the isolates (for cells and colonies).

DNA extraction

Whole genomic DNA was extracted for *P. aeruginosa* molecular identification in accordance with the manufacturer's standard Kit (Favorgen, Taiwan).

PCR primers and conditions

The bla_{OXA-10} and $bla_{OXA-488}$ genes, which are unique to *P. aeruginosa* were detected in this reaction using polymerase chain reaction (PCR) cycling thermal program settings. The PCR primers produced by Humanizing Genomics Macrogen (Macrogen, Inc., Seoul, Korea) that were used in this study are displayed in Table 2.

Preparation of PCR reaction mixture

Table 3 provides an illustration of the reaction mixture. On a 1.5% agarose gel, the PCR products were run for 60 min at 80 V and stained with 5 mL of GoldView (biosharp India). The gel well received 1 µL of loading dye and 5 µL of amplification products. The 100 bp DNA ladder was used to scale the amplicon of PCR product (solGent100bp plus DNA Ladder Korea). The PCR products were photographed by the Gel Documentation System (Biometra, Germany).[21]

Antibiotics susceptibility

According to CLSI 2022[22] recommendations, the disk diffusion of susceptibility tests was accomplished. The antibiotics disc potency given included ticarcillin (75 mg), piperacillin (100 mg), and piperacillin-tazobactam (110 mg), ceftazidime (30 mg), cefepime (30 mg), aztreonam (30 mg), ciprofloxacin (30 mg), levofloxacin (15 mg), norfloxacin (10 mg), tobramycin (30 mg), gentamycin (10 mg), amikacin (30 mg), colstin (40 mg), polymyxin B (300 mg), dorpenem (10 mg), and ipenem (10 mg). Muller Hinton agar was the platform for all tests (Merck, Germany).

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 7/17/1336 (including the number and the date in 21/2/2022) to get this approval

Results

Isolation and identification

One hundred ninety-four clinical samples, including wound, urine, burns, diabetic patients' feet, gun fire, bomb, and pulmonary fluid, were used to obtain a total of 36 isolates of *P. aeruginosa* (from 1 to 36). Out of these isolates, 18 (50%) were isolated from wounds, 12 (33.33%) from burns, three (8.33%) from gun fire, bomb, and three (8.33%) from diabetic patients' feet [Table 1].

The process of identifying these isolates involved the use of culture, microscopic examination, biochemical assays, and confirmation by PCR detection for the 16S rRNA gene. Gram stain experiments revealed a single or bi-arrangement in these isolating cultures on MacConky agar, which were pale in color because of their inability to ferment the lactose contained in this culture medium and had a smell reminiscent of fermented grapes.

Antibiotic susceptibility testing

Testing for antibiotic resistance to 17 common antibiotics was done on 36 identified *P. aeruginosa* isolates. Antibiotics, especially those with β-lactamase activity, were resistant to the majority of isolates. Antibiotics such as piperacillin and ticarcillin exhibited 100% resistance, whereas piperacillin-tazobactam (42%), ceftazidime (89%), tobramycin (83%), gentamycin (67%), cefepime (50%), imipenem (50%), levofloxacin (36%), amikacin (83%), meropenem (42%), ciprofloxacin (42%), doripenem (36%), colistin (8.3%), polymyxin B (8.3%), aztreonam (58%), and norfloxacin (39%) [Figure 1].

PCR assay results

The β-lactamase genes under investigation (bla_{OXA-10} and $bla_{OXA-488}$) varied in their abundance in *P. aeruginosa* bacterial isolates, the results of PCR utilize products of

Table 1: Distribution of distinct bacterial strains and their percentages

Sample type	*Pseudomonas aeruginosa*		Others		Total
	Number	Percentage (%)	Number	Percentage (%)	
Wound	17	17.7	79	82.3	96
Diabetic foot	3	23	10	77	13
Burns	12	16.4	61	83.6	73
Urine	0	0	5	100	5
Gun fire (bomb)	3	60	2	40	5
Pulmonary fluid	1	50	1	50	2
Total	36	18.5	158	81.5	194

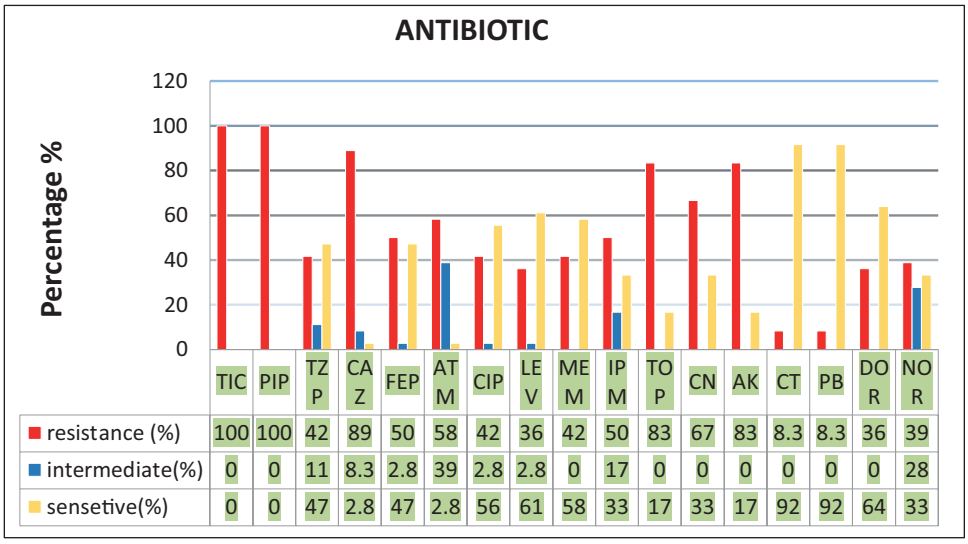

Figure 1: *Pseudomonas aeruginosa* pattern of antibiotics susceptibility to various antibiotics used in current study

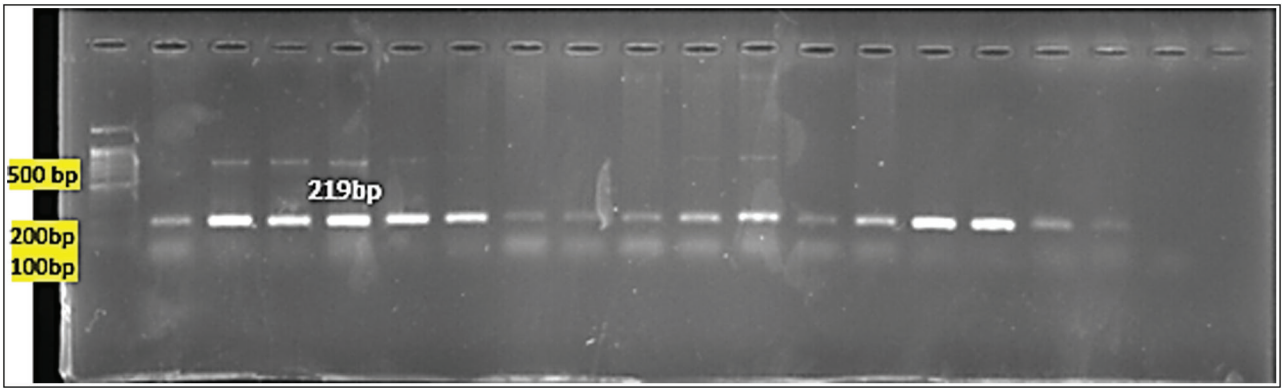

Figure 2: Agarose gel electrophoresis of bla_{OXA-10} polymerase chain reaction product (219bp), 1.5% agarose gel with safe red stain, at 80 V Cm, it took 60 min. M: marker DNA ladder

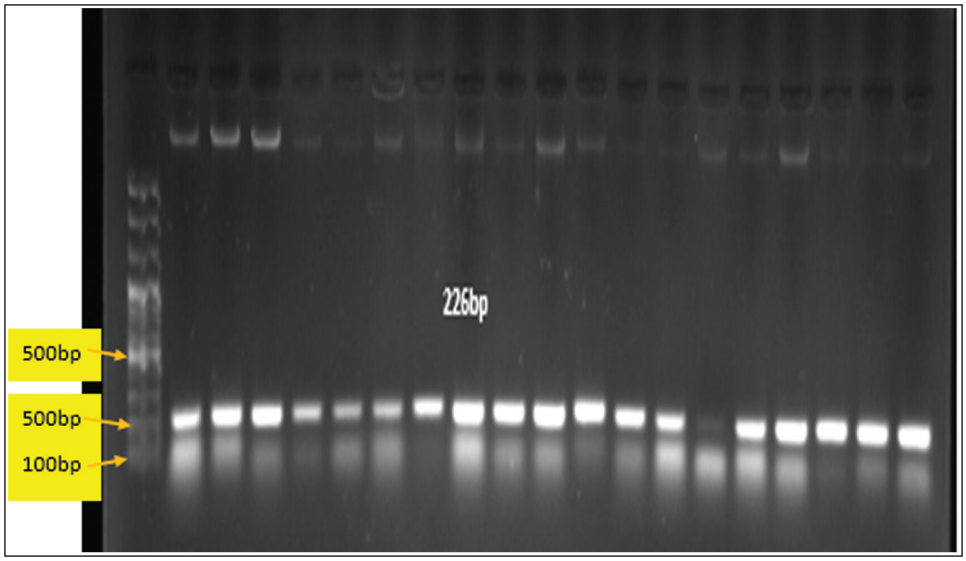

Figure 3: Agarose gel electrophoresis of $bla_{OXA-488}$ polymerase chain reaction product (226 bp), 1.5% agarose gel with safe red stain, at 80 V Cm, it took 60 min. M: marker DNA ladder

Table 2: Polymerase chain reaction primers and their conditions used in this study (designed by this study)

Primer		Sequence (5′ → 3′)	Product (bp)	Condition		Cycle No.
				Temp (°C)	Time (s)	
bla_{OXA-10}	F	GGTAGTGCGGATCAACCTGT	219	Denaturation 95	30	30
	R	TCATATCGTCGAGTGGTGGG		Annealing 60		
				Extension 72		
$bla_{OXA-488}$	F	GTGGGGTTGTTTGGCATGAT	226	Denaturation 95	30	30
	R	ACTGGTAATGCCGACCAGAC		Annealing 62		
				Extension 72		

bla_{OXA-10}: oxacillin-hydrolyzing class D β-lactamase 10, $bla_{OXA-488}$: oxacillin-hydrolyzing class D β-lactamase 488

β-lactamases genes showed that 30/36 (83.33%) isolates carried bla_{OXA-10} at 219 bp [Figure 2] and 24/36 (66.66%) isolates at 226 bp [Figure 3] had $bla_{OXA-488}$.

There were 19 isolates (52.78%) which possessed bla_{OXA-10} and $bla_{OXA-488}$ genes. The bla_{OXA-10} genes were identified in 11 (30.55%) isolates, and $bla_{OXA-488}$ genes were identified in five isolates (13.88%) [Table 4].

Positive molecular detections for the bla_{OXA-10} and $bla_{OXA-488}$ genes and *P. aeruginosa* isolates selected were based on the positive ESBLs test for β-lactamase and were produced using antibiotic resistance; this was shown to be the case in the present study. The most antibiotic-resistant *P. aeruginosa* strains were listed in [Table 5].

Discussion

Isolation and identification

In blood agar, the colony of Gram-negative bacteria is dark in color, and the majority of them have haloes around them. All *P. aeruginosa* isolates tested (for oxidase and catalase) were positive, and it can also pass the urease test and make the pigments (yellow-green pyoverdine and blue water-soluble pigment pyocyanin).[23,24] Isolates were tested positive for the citrate test, despite IMVIC tests being negative for the indol, methyl red, and Voges-Proskauer (VP) tests (C). H_2S is positive (Kligler iron agar has supplied an alkaline slant) and gas production because Kligler iron agar is strictly aerobic, Gram's stain negative, and has no effect on the bottom.[25]

Table 3: Polymerase chain reaction mixture

Contents	Volume
Master mix	12.5 µL
Target DNA	3 µL
F primer (10 pmol/µL)	1.5 µL
R primer (10 pmol/µL)	1.5 µL
Nuclease free water	4 µL
Stain	2.5 µL
Total volume	25µL

Table 4: Distribution of bla_{OXA-10} and $bla_{OXA-488}$ genes of *P. aeruginosa*

Gene	Isolate No.	%
Bla_{OXA-10}	11	30.55
$Bla_{OXA-488}$	5	13.88
Bla_{OXA-10} and $Bla_{OXA-488}$	19	52.77
None	1	2.77

bla_{OXA-10}: oxacillin-hydrolyzing class D β-lactamase 10, $bla_{OXA-488}$: oxacillin-hydrolyzing class D β-lactamase 488

Antibiotic susceptibility testing

The findings of Sedighi demonstrated that MBL-producing *P. aeruginosa* populations pose a serious therapeutic threat. The rate of imipenem resistance caused by MBL has dramatically increased. The best antimicrobial strategies for this organism are early detection and infection control practices.[26]

Baban's research focuses on the development of antimicrobial stewardship to prevent the indiscriminate use of carbapenem antibiotics, and the early detection of carbapenem-resistant isolates is critical to avoiding the risk of cross-transmission of these isolates among critically ill patients. As a result of Baban's work, active surveillance and strict adherence to infection prevention and control measures may be effective strategies for slowing the rise of carbapenemase resistance.[27]

Gram-negative bacilli are one of the most frequent causes of serious nosocomial and community-onset bacterial infections in people, and antimicrobial resistance has become a major challenge to the delivery of healthcare globally.[28]

Table 5: Relationship between the resistance of *P. aeruginosa* isolates to antibiotics and the presence of genes responsible for resistance to β-lactam antibiotics

Gene antibiotic	Bla_{OXA-10}		$Bla_{OXA-488}$		Bla_{OXA-10} and $Bla_{OXA-488}$		None	
	No	%	No	%	No	%	No	%
Ticarcillin	11	100	5	100	19	100	1	100
Piperacillin	11	100	5	100	19	100	1	100
Ceftazidime	9	81.8	5	100	17	89.4	1	100
Piper./Tazo.	3	27.2	3	60	9	47.3	0	0
Cefepime	2	18.18	1	20	14	73.6	1	100
Aztreonam	7	63.63	3	60	11	57.89	0	0
Ciprofloxacin	0	0	1	20	14	73.4	0	0
Levofloxacin	0	0	1	20	12	63.15	0	0
Meropenem	1	9	1	20	13	68.42	4	50
Imipenem	4	36.36	2	40	11	57.89	0	0
Tobramycin	8	72.72	4	80	17	89.4	1	100
Gentamicin	3	27.2	4	80	16	84.2	1	100
Amikacin	9	81.8	4	80	16	84.2	1	100
Colstin	2	18.18	0	0	0	0	1	100
Polymyxin B	2	18.18	0	0	0	0	1	100
Doripenem	1	9	2	40	9	47.36	1	100
Norfloxacin	4	36.36	1	20	8	42.1	1	100

bla_{OXA-10}: oxacillin-hydrolyzing class D β-lactamase 10, $bla_{OXA-488}$: oxacillin-hydrolyzing class D β-lactamase 488

In most hospitals and community, *P. aeruginosa* is a frequent nosocomial infection that can cause serious illnesses. *P. aeruginosa* isolates that produce ESBLs have been associated with high rates of morbidity, mortality, and medical expenses. It is a public health emergency that these strains are rapidly developing antibiotic resistance.[29]

Following identification verification testing, the bacterial resistance profiles of the samples were compared with CLSI report of 2023. *P. aeruginosa* isolates had increased levels of resistance to many studied antibiotics, particularly β-lactams. The majority of the isolates identified were resistant to at least six different antibiotics.

Conclusion

Carbapenems such as imipenem and meropenem are the most important drugs for treating *P. aeruginosa* infections. Unfortunately, this study found a high level of imipenem resistance. Many ESBL genes, including bla_{OXA-10} and $bla_{OXA-488}$, were also found to be expressed in clinical isolates of *P. aeruginosa*. To prevent the development of resistant *P. aeruginosa* strains, it is best to avoid prescribing and using antibiotics when they are not absolutely necessary.

Acknowledgments

The authors thank the laboratory staff at Al-Hillah City Hospitals (Al-Hillah Teaching, Mirgian, and Imam AL-Sadiq Teaching), Kirkuk Hospitals (Kirkuk General Hospital, Azadi Teaching Hospital), and Medical City Hospitals in Baghdad (Burns Specialized, AL-Sheed Ghazi Hariri, Baghdad Teaching, and National Center for Educational Laboratories) in Iraq for processing clinical specimens to facilitate research requirements.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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