Antibiotic resistance profile of *Pseudomonas aeruginosa* isolated from aquaculture and abattoir environments in urban communities

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**ABSTRACT**

**Objective:** To characterize multiple antibiotic resistance profile of *Pseudomonas aeruginosa* from aquaculture and abattoir environments.  

**Methods:** Wastewater samples were obtained from the abattoir and aquaculture environments between May 2016 and July 2016 and analysed using standard phenotypic, biochemical and PCR-based methods.  

**Results:** The mean pseudomonads count ranged from \((4 \times 10^2 \pm 1.01)\) to \((2 \times 10^4 \pm 0.10)\) colony-forming unit/mL in the aquaculture environment and \((3 \times 10^3 \pm 0.00)\) to \((1 \times 10^5 \pm 1.00)\) colony-forming unit/mL in the abattoir environment. A total of 96 isolates of *Pseudomonas aeruginosa* confirmed by PCR were thereafter selected from both aquaculture and abattoir environments and further characterized for their antimicrobial susceptibility profile by adopting the disc diffusion method. High level of resistance was observed against the aminoglycosides [gentamycin 64/96 (66.67%) and kanamycin 52/96 (54.17%)], monobactams [aztreonam 76/96 (79.17%)], carbapenems [meropenem 52/96 (54.17%)], tetracyclines [tetracycline 72/96 (75.00%)] and cephems [ceftazidime 72/96 (75.00%) and cefuroxime 48/96 (50.00%)]. Multiple antibiotic resistant index of the respective isolates ranged from 0.4 to 0.8 while multidrug resistant profile of the isolates revealed that 28 of the respective isolates were resistant to ceftazidime, cefuroxime, gentamycin, kanamycin, aztreonam which belongs to cephs, aminoglycosides and monobactam class of antimicrobials.  

**Conclusions:** Findings from the present study therefore underscores the need for effective monitoring of the abattoir and aquaculture environments as they could be the significant source for spreading antibiotic resistant bacteria within the environment.

### 1. Introduction

*Pseudomonas aeruginosa* (*P. aeruginosa*) is a Gram-negative, oxidase positive organism found in various ecological niches including soil, water, rhizosphere, plant, animal and sewage environment[1,2]. It comprises strains with economic, ecological and health-related significance and has also been categorized as an emerging waterborne pathogen[3,4]. Species of *Pseudomonas* have been implicated in infections of public health importance among which include cystic fibrosis, urinary tract infections, bone and joint, gastrointestinal infection, bacteremia, soft tissue and respiratory system. They are also of concern in immune-compromised patients such as AIDS and cancer infected patients[5]. *Pseudomonas* as an emerging pathogen is centred principally within their capability to exist in biofilms (a combined population of bacterial that adheres to specialized surfaces within the aquatic ecosystem) that frequently serves as shielding asylum for the bacteria against physical, biological, environmental and chemical stress conditions. Proliferation inside biofilms enhances genetic diversity which, in turn, heightens the potential for susceptibility against antimicrobials, disinfectants, environmental stress as well as increases the virulence capacity of the pathogen[6].  

Wastewater effluents from abattoir environments are usually...
characterized by the presence of pathogenic microorganisms which include the genus *Pseudomonas*, *Aeromonas* and *Enterococcus* species which are of significance to public health[3,7]. The presence of these organisms coupled with their inherent capacity to resist multiple antibiotics is an enigmatic problem of public health importance[5]. These multidrug resistant pathogenic bacterial isolates which might have emanated from the carcasses as a consequence of the continuous use and misuse of antimicrobials as therapeutic/prophylactic agent as well as growth promoters can be transferred via the food chain to humans[8,9]. Resistance to antimicrobials by pathogenic bacterial isolates can also be a consequence of the ability of the organisms to harbour resistant genes and transmit these genes within bacterial populations via horizontal gene transfer and mobile genetic elements (transposons, plasmids, etc.)[10,11]. This is a crucial issue as these organisms might migrate into the food chain resulting in food infection or directly to humans resulting in difficulties to treat infections[12].

Microbial drug resistance is a significant public health concern. There has been less attention paid to the potential for antibiotic use in the aquaculture and abattoir environments, which compromises environmental and human health. In developed countries, such as members of the European Union, Great Britain, United States of America, Canada and Norway, the use of antibiotics (as growth promoters, prophylactic/therapeutic agents) is declining because of the progresses in management and advances of effective vaccines[13]. However, this does not seem to be followed suit in Nigeria where monitoring controls are fragile and compromised, and the administration of antibiotics seems to be abused.

Previous studies have isolated and characterized *Pseudomonas* species from poultry[14], abattoir environments[1,3,4,7,15], effluent water from a brewery[16] and fresh and dry fish species[12,17]. Abattoirs, aquaculture environment and associated wastewaters are potential sources of pathogenic bacteria that could possess, act as reservoirs and spread antibiotic resistance genes within bacterial population to food of animals as well as humans. Thus, the present investigation aimed to detect and characterize the multiple antimicrobial susceptibility profile of *P. aeruginosa* from aquaculture and abattoir environments.

### 2. Materials and methods

#### 2.1. Study area

The site of the sampling fish ponds (aquaculture) was located at Ikpoba-Oka and Egor Local Government Areas (LGA) of Benin Metropolis, Edo State, Nigeria. The pond at Ikpoba-Oka LGA was a trampoline pond. Respective ponds examined were periodically drained on a daily basis. Fishes were fed in the morning and evening with commercially available feed. No antibiotic was given to the fish or placed in the water except in cases where there were challenges like spotting on the fish in the ponds. More so, fowl droppings were placed in the pond at Ikpoba-Oka LGA for a period of 3 days before the fishes were placed in the pond at the fingerling stage. The pond at Egor LGA was a concrete pond. Artificial fertilizer was also used to facilitate the growth of green algae in the fish pond. No animal waste was used in fish ponds from Egor LGA.

Slaughter houses (abattoir) investigated were located in Ikpoba-Okha LGA. Cattles were slaughtered on a daily basis, chopped to sizes and transported to different open markets and cold rooms where they were sold out to consumers. The abattoir in this LGA was located close to the Ikpoba River. As such, all wastewaters from the abattoir found their way directly to the river or indirectly to the river through leaching or erosion.

#### 2.2. Sample collection

Wastewater samples were collected on a bimonthly basis at margins and in the middle of the pond in 50 mL sterile universal containers. Wastewater samples were collected from different points at the abattoir such as the drainage, stagnant water around the abattoir environment and washed meat slaughtered at the abattoir using 50 mL sterile universal containers and were labelled appropriately. Samples were collected between May and July, 2016. All samples were transported in cold ice pack to the Applied Microbial Processes & Environmental Health Research Group Laboratory, Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, where they were analysed within 4 h after collection.

#### 2.3. Enumeration of *Pseudomonas*

All samples were serially diluted to obtain dilution factors (10⁻¹–10⁻³). An aliquot of 100 µL was then transferred from each of the diluents to glutamate starch phenol red agar (Merck, Germany) and cetrimide agar (Merck, Germany) in triplicates with the aid of sterile pipette and spread on the solidified agar surface by spreading the aliquot with a sterile glass rod. The plates were allowed to stand for 10–20 min before they were incubated at (37 ± 2 °C) for 18 h. After incubation, discrete colonies (pink colonies) and fluorescent, green colonies were counted and expressed as colony-forming unit (CFU)/mL.

#### 2.4. Enrichment and isolation method

An aliquot of 100 µL from each of the samples obtained was dispensed into a conical flask containing 5 mL of tryptone soy broth (Merck, Germany) and incubated at room temperature of (37 ± 2) °C for 24 h. Thereafter, streak plate method was employed in streaking the 18–24 h old broth medium on Glutamate Starch Phenol Red Agar which was selective for the isolation of *Pseudomonas* and *Aeromonas* species and incubated for 18 h at (37 ± 2) °C. After incubation, discrete colonies (pink colonies) were sub-cultured on cetrimide agar for another 18 h to obtain pure *Pseudomonas* isolates. Gram staining was carried out on isolates. All *Pseudomonas* isolates were stored at −20 °C in brain heart infusion broth with 30% glycerol.

#### 2.5. Biochemical characterization of the isolates

Gram-negative rod shapes and polar flagellated oxidase positive isolates were biochemically characterized using the Analytical
Profile Index 20NE according to the manufacturer’s instructions (BioMerieux, Marcy-l’Étoile, France) as previously described by Igbinosa et al.[3]. Positive control used included P. aeruginosa (ATCC 27853). The strips were studied and final identity of the isolates was revealed adopting Analytical Profile Index lab plus software (BioMerieux, Marcy l’Étoile, France).

2.6. Extraction of genomic DNA

Discrete colonies of P. aeruginosa cultivated at 37 °C on tryptone soy agar plates were selected and re-suspended on 100 μL of sterilized distilled water. The presumptive cells were lysed with the heat block (MK200-2, Shanghai, China) at 100 °C for 15 min. The preparation was thereafter centrifuged at 11 000 r/min for 2 min using a MiniSpin microcentrifuge to remove the cell debris. The supernatant was then stored at –20 °C and used as DNA template.

2.7. PCR amplification assay

Presumptive P. aeruginosa isolates previously characterized using phenotypic and biochemical methods were further confirmed using PCR. All PCR amplification procedures were accomplished in 22.5 μL volume of reaction buffer which contained 2.5 μL of DNA template and 0.05 IU/μL Taq polymerase recommended by the manufacturer (Fermentas Life Sciences, USA). DNA templates of P. aeruginosa ATCC 27853 was used as a positive control while sterilized distilled H2O was added as a negative control into each PCR assay. All PCR assay was carried out using a Peltier-based thermal cycler (Bio-Separation System, Shanxi, China) at the succeeding thermal cycler conditions: 95 °C for 1 min; 40 rounds of thermal denaturation at 95 °C for 15 s; primer annealing at 58 °C for 20 s; concluding extension at 68 °C for 40 s and holding temperature of 4 °C. P. aeruginosa primers used were pa722F (5’-GGCGTGTTGTGGAAGTC-3’) and pa899R (5’-TGTTGCGATCT TGAAC TCTT-3’) ampiclon size of 199 bp[18]. Amplicons were electrophoresed with 1% agarose gel (Hispanagar, Spain) encompassing ethidium bromide 0.5 mg/L (Merck, Germany) at 100 V for 1 h in 0.5 × tris-acetate-ethylene diamine tetracetic acid buffer (40 mmol/L tris-HCl, 20 mmol/L Na-acetate, 1 mmol/L ethylene diamine tetracetic acid, pH 8.5) and imagined under a UV transilluminator (EBOX VX5, Vilber Lourmat, France).

2.8. Antimicrobial susceptibility testing

Susceptibility profiles of the P. aeruginosa isolates were determined by the disc diffusion technique of Kirby Bauer on Mueller-Hinton agar (Merck, Germany) plates. For the disc diffusion assay, bacteria were cultivated between 18 and 24 h on Mueller-Hinton agar, reaped and then re-suspended in 0.85% sterilized physiological saline solution that was adjusted to a 0.5 McFarland turbidity standard, conforming to 104 CFU/mL. The inoculum was thereafter streaked onto plates of Mueller-Hinton agar with the aid of a sterilized cotton swab and aseptically impregnated with commercially available antibiotics discs acquired from Mast Diagnostics, Merseyside, United Kingdom. The P. aeruginosa isolates were then subjected to 10 antibiotics belonging to 7 groups of antimicrobials. The antibiotics used were penicillins [piperacillin (100 μg)], cephems [cefazidime (3 μg), cefuroxime (30 μg)], monobactams [aztreonam (30 μg)], carbapenems [ertapenem (10 μg), meropenem (10 μg)], aminoglycosides [gentamicin (10 μg), kanamycin (30 μg)], tetracyclines [tetracycline (30 μg)] and fluoroquinolones [ciprofloxacin (5 μg)]. After incubated for 24 h at room temperature, organisms were classified as sensitive (S) or resistant (R) on the basis of the clear zone of inhibition in accordance to the guidelines of the Clinical and Laboratory Standard Institute[19].

2.9. Statistical analysis

All data were analyzed using the statistical package IBM SPSS version 21.0. Descriptive statistics, one-sample t-test and One-way ANOVA were adopted. P < 0.05 was considered statistically significant.

3. Results

The mean P. aeruginosa counts from aquacultures and abattoir were presented. During the month of May, June and July, the mean Pseudomonas counts from aquacultures in Ikpoba-Okha were (2 × 109 ± 0.96), (2 × 109 ± 0.07) and (2 × 107 ± 0.10) CFU/mL, respectively, while those of Egor were (4 × 107 ± 1.01), (5 × 107 ± 0.11) and (3 × 107 ± 0.01) CFU/mL, respectively (P < 0.05). During the month of May, the mean P. aeruginosa counts from drains, environment and wash water in the abattoir were (4 × 102 ± 0.10), (1 × 102 ± 1.00) and (2 × 104 ± 0.01) CFU/mL, respectively (P < 0.05). In the month of June, the mean counts were (3 × 103 ± 0.00), (9 × 104 ± 0.00) and (1.2 × 107 ± 0.00) CFU/mL, respectively, while in July, the mean counts were (1 × 103 ± 0.01), (7 × 102 ± 0.00) and (7 × 103 ± 0.10) CFU/mL, respectively (P < 0.05).

The resistance profile of the 96 P. aeruginosa detected from abattoir and fish pond water was further phenotypically characterized using ten antibiotics which were representatives of seven groups of antimicrobial agents. The resistance profile of the isolates revealed that 12/96 (12.50%) of the isolates were resistant to piperacillin, 72/96 (75.00%) were resistant to ceftazidime and tetracycline, 48/96 (50.00%) were resistant to cefuroxime, 64/96 (66.67%) were resistant to ceftazidime and tetracycline, 72/96 (75.00%) were resistant to gentamycin, 52/96 (54.17%) were resistant to kanamycin, 8/96 (8.33%) were resistant to ertapenem, 16/96 (16.67%) were resistant to ciprofloxacin, 52/96 (54.17%) were resistant to meropenem and 76/96 (79.17%) of the isolates were resistant to aztreonam (Table 1).

The antimicrobial susceptibility profile of the P. aeruginosa isolates from aquacultures showed that none of the isolates were resistant to cefuroxime, ertapenem and ciprofloxacin in Ikpoba-Okha and Egor LGAs while they revealed different levels of resistance to other antibiotics assayed. More so, all isolates from abattoir were sensitive to piperacillin and resistant to other antibiotics used in variable percentages of resistance (Table 2). A clear pattern of multiple antibiotic resistance (MAR) along the lines of some P. aeruginosa isolates origin was observed in the present study. The MAR index ranged from 0.4 to 0.8. The modal value of MAR index
for the isolates was 0.6. The least pattern of MAR was resistant to 4/10 antibiotics while the highest level of MAR was resistant to 8/10 antibiotics (Table 3). Extensive drug resistance profile in the present study showed that 36 P. aeruginosa were resistant to ceftazidime, cefuroxime, gentamycin and kanamycin while multidrug resistant (MDR) profile in the present study showed that 28 P. aeruginosa were resistant to ceftazidime, cefuroxime, gentamycin, kanamycin and aztreonam (Table 4).

Table 2
Distribution of the resistant profile of P. aeruginosa from abattoir and aquaculture.

<table>
<thead>
<tr>
<th>Antibiotic Class</th>
<th>Abattoir [n (%)]</th>
<th>Aquaculture [n(%)]</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudoplatinum</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>23 (100.00)</td>
<td>16 (100.00)</td>
<td>1</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>23 (100.00)</td>
<td>16 (100.00)</td>
<td>1</td>
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<tr>
<td>Gentamycin</td>
<td>23 (100.00)</td>
<td>16 (100.00)</td>
<td>1</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>23 (100.00)</td>
<td>16 (100.00)</td>
<td>1</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>23 (100.00)</td>
<td>8 (50.00)</td>
<td>0</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>0 (0.00)</td>
<td>9 (56.25)</td>
<td>0</td>
</tr>
<tr>
<td>Meropenem</td>
<td>12 (50.00)</td>
<td>10 (62.50)</td>
<td>1</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>23 (100.00)</td>
<td>16 (100.00)</td>
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</tr>
</tbody>
</table>

Table 3
MAR index of P. aeruginosa isolates from abattoir and aquaculture.

<table>
<thead>
<tr>
<th>Isolate code</th>
<th>Antibiotics</th>
<th>MAR index</th>
</tr>
</thead>
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<tr>
<td>PDR1</td>
<td>PTZ, CAZ, GEN, KAN, ATM, MEM, TET</td>
<td>0.7</td>
</tr>
<tr>
<td>PDR2</td>
<td>CAZ, CXM, KAN, ATM, ETP, MEM, TET</td>
<td>0.7</td>
</tr>
<tr>
<td>PDR3</td>
<td>CAZ, CXM, GEN, KAN, ATM, MEM, CIP, TET</td>
<td>0.8</td>
</tr>
<tr>
<td>PDR4</td>
<td>CAZ, CXM, GEN, KAN, ETP, MEM, CIP, TET</td>
<td>0.8</td>
</tr>
<tr>
<td>PEN1</td>
<td>PTZ, CAZ, GEN, KAN, ATM, MEM</td>
<td>0.6</td>
</tr>
<tr>
<td>PEN2</td>
<td>CAZ, CXM, GEN, KAN, ATM, CIP, TET</td>
<td>0.7</td>
</tr>
<tr>
<td>PEN3</td>
<td>CAZ, CXM, GEN, KAN, ATM, TET</td>
<td>0.6</td>
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<td>PEN4</td>
<td>CAZ, CXM, GEN, KAN, ATM, TET</td>
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<tr>
<td>PEG4</td>
<td>CAZ, CXM, GEN, KAN, ATM, TET</td>
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</tr>
</tbody>
</table>

4. Discussion

The poor management practices adopted in abattoirs can affect the safety of meat in various ways, such as diseases in animals (zoonotic diseases), chemical (pesticides and antibiotics) residues deposition, most importantly by microbial contamination with pathogenic microbes and toxins [20]. Microbial populace are always in constant evolution, with unlimited adaptive capacities towards conditions that are used to inhibit/kill them, all making food safety a long-lasting issue. Ignorance concerning food safety measures provides potentials for increased food borne diseases in man [21]. Due to the diversity of P. aeruginosa, they are found in diverse ecological niches. Under stressful environmental conditions, these opportunistic microbial populations may become pathogenic, invade the body tissue and initiate infections. It is thus imperative to regulate and monitor the bacterial parameters in the current aquaculture and abattoir system where lots of profit maximization is done to enhance production.

Significant differences were observed in the mean pseudomonads count from Ikpoba-Okha, Egor and drains, environments, and wash water in May, June and July (P < 0.05). This could be attributed to the fact that both the abattoir and the pond samples are contaminated from the handling and preparation process of the butchers who dress and slaughter their products in contact with the waste deposited around them thereby making the final products unsafe for consumption and favoring the proliferation of P. aeruginosa as those...
observed in the present study. Similar antibiotic resistance profile was also detected by Odjadjare et al.[5] where high level resistance was observed against the penicillins (90%–100%), cephems (70%), sulphamethoxazole (90%) and rifampin (90%). Also, Falodun and Adekanmbi[22] revealed 100% resistance to ceftazidime and piperacillin.

*P. aeruginosa* is being acknowledged as an increasing emerging organism causing opportunistic disease of clinical importance. A significant characteristic to public health it possesses is its reduced antibiotic susceptibility[8]. This reduced susceptibility is usually connected to a strenuous action of multiple antibiotic efflux pumps coupled with chromosomally mediated antibiotic resistance determinants (such as *mexXY, mexAB-oprM*) and the reduced permeability of the bacterial cellular envelopes by antimicrobial agents[3]. Aside intrinsic resistance, *P. aeruginosa* can easily develop acquired resistance resulting from the change in the genetic makeup in chromosomally encoded determinants or by the horizontal determinants transfer of antibiotic resistance genes[7]. Expansion of MDR by *P. aeruginosa* strains requires several different genotypic measures that include acquisition of horizontal dissemination of antibiotic resistance determinants and/or mutation[5]. Hyper-mutation enhances the choice of mutation-driven antibiotic resistance in *P. aeruginosa* strains consequently resulting in chronic infections, while the assembling of several diverse antibiotic resistance determinants in integrons enhances the acquisition of antibiotic resistance genes[7].

Antibacterial resistance is at present one of the significant threat to mankind as it constitutes alarming health crisis. Extensive uses of antibacterial agents in veterinary, human medicine and agricultural settings have led to the emergence of antibacterial-resistant organisms in different environmental settings including abattoir and aquaculture. Antibacterial resistance in aquaculture is a worldwide concern because antibacterial resistance genes can be disseminated easily from aquaculture and abattoir settings to other ecosystems and the food chain[13]. African catfish aquaculture has increased at a phenomenal rate through a continuous process of expansion, intensification, and diversification as a consequence of the Nigerian government sponsoring/financing small and medium scale enterprise. Risks of pathogenic bacterial isolates have also increased resulting in the increased use of antibacterial agents for therapy[23].

Antibacterial resistance in aquaculture and abattoir has significantly impacted the food chain and thus represents the risk to human and animal health[23]. Curbing antibacterial resistance, understanding of the sources, mechanisms and magnitude of antibacterial resistance and its significant impact on the food chain are important in designing, prioritizing control and monitoring programs that may generate data that would be relevant for performing implementation of antibacterial stewardship plans, quantitative microbial risk assessments, developing effective treatment regimen for the control of aquaculture and abattoir diseases and reducing risk to public health.

Similar investigation by Odjadjare et al.[5] on MAR index from effluent water ranged between 0.26 and 0.58. However, no clear pattern of MAR along the lines of isolate origin was observed. MDR has been described as acquired resistance to one agent in 3 or more antimicrobial classes, while extensive drug resistance has been described as resistance to one agent in all but 2 or fewer antimicrobial categories (*i.e.* bacterial isolates remain resistant to only 1 or 2 categories)[24].

It has been previously reported that antimicrobials are widely used in food animal production settings for treatment/prevention and for growth promotion[9,11]. Studies have also revealed that 50%–90% of drugs used to treat food producing animals are released into the environment either as metabolic or un-metabolized intermediates which, even though inactive, may go through transformation to the active form in the environment[7], consequently resulting in high selection of MDR *P. aeruginosa* from aquaculture and abattoir effluent.

The occurrence of MDR *P. aeruginosa* on food products could pose significant public health risks to consumers of such products. First, these include consumption of products contaminated with MDR bacteria which could result in infections as a consequence of antibiotic therapy which can be compromised due to resistant strain resulting in infections difficult to treat. However, it should be known that even if the products are cooked, the bacterial isolates might die off but it will not destroy determinants responsible for the resistance. Secondly, resistant pathogenic bacteria in animals can be transmitted to human via consumption of contaminated food products and resistance genes can also be disseminated to other bacteria via mobile genetic element such as integrons, plasmids, insertion sequence, transposons and gene cassette[2]. Finally, residual antibiotics in food animal products such as in the blood and liver can also result in the transmission of antibiotic resistant bacteria to the final consumer.

The potential pathogenic nature of *Pseudomonas* isolates is beyond doubt as different reports support findings in the present study. It was thus customary from the findings of our study that effluents and products from the abattoir and aquaculture environment could be an origin of dissemination of antibiotic resistant bacteria between human and animal population, and the environment. Therefore, public health workers must make efforts to regulate and monitor the bacterial parameters in the aquaculture and abattoir system to deter this trend to enhance qualitative production and prevent public health implication.

**Conflict of interest statement**

We declare that we have no conflict of interest.
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