Prevalence of Multidrug Resistant *Acinetobacter baumannii* in Eight Tertiary Hospitals in Southwestern Nigeria

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Abstract: The genus *Acinetobacter* currently contains 34 species, the vast majority of which are not regularly implicated in causing infection. However, incidences of hospital acquired infection with *Acinetobacter* species are increasing, mainly due to the rise in the number of infections caused by the species *Acinetobacter baumannii* in immune-compromised patients particularly in intensive care units (ICUs). The goal of this study is to investigate prevalence and resistance patterns of multidrug resistant *A. baumannii* strains isolated from clinical samples from tertiary hospitals in southwest Nigeria. The descriptive-cross sectional study was conducted in 8 major tertiary hospitals distributed within southwest Nigeria. Seventy-two strains of *A. baumannii* were isolated from clinical sources from April 2011 through May 2013. The identities of the isolates to species level were confirmed by standard biochemical methods using Oxoid Microbact™ Gram-negative identification system. The susceptibility patterns to 34 antimicrobial agents belonging to 13 classes of antibiotics were performed by disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Computer -aided Microbact software identified all the isolates as *A. baumannii* with each isolate been resistant to ≥3 classes of antibiotics. All the isolates were resistant to ≥14 antimicrobial agents tested, with 69 (95.8%) isolates resistant to 20-34 antimicrobial agents. All the isolates were also resistant to amoxicillin, amoxicillin-clavulanate, ampicillin, cefpodoxime, ceftazidime, ceftriaxone, cefuroxime, and cloxacillin. Significantly high rates of resistance were observed for erythromycin (98.6%); cephazolin (97.2%); aztreonam (93.1%); tetracycline (91.7%); cephalothin, trimethoprim-sulfamethoxazole (90.3%); kanamycin (88.5%); gentamicin, ticarcillin (86.1%); piperacillin, cefoxitin (87.5%), nitrofurantoin (81.9%); chloramphenicol (80.3%); ofloxacin (66.7%); and colistin (63.9%). High rates of carbapenem resistance were also recorded against meropenem (77.8%) and imipenem (56.9%). The least resistance was observed for azithromycin (37.9%); ciprofloxacin, polymyxin B (40.3%); and amikacin (43.1%). *A. baumannii* isolates from Southwest Nigeria showed unacceptably high rates of resistance to multiplicity of antimicrobial agents, including those not readily available in Nigeria. This study calls for a functional surveillance of *A. baumannii* antimicrobial resistance in Nigeria.

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Introduction:

Multidrug-resistant *Acinetobacter baumannii* (MDRAB) have emerged as a substantial public health problem worldwide. Its clinical significance over the last 15 years has been propelled by its remarkable ability to up-regulate innate resistance mechanism and acquires new resistance determinants with ease; tolerate wide range of pH, salinity, humidity, and unique ability to survive on almost all nutrient sources (Bergogne-BereZin and Towner, 1996). These abilities have allowed this pathogen to be ubiquitous in the hospital environment as well as the community and have made it one of the frontline pathogens threatening the current antibiotic era (Falagas and Karveli, 2007b). Also, the ability of *A. baumannii* to form biofilms has been reported to play a role in the process of colonization (Rajamohan et al., 2009). As such, *A. baumannii* has become a frequent colonizer of respiratory and digestive tracts, skin and throat (Rajamohan *et al*., 2009). Biofilms help the bacteria resist disinfection, allowing the participating cells to trade resistance genes, while facilitating the persistence of the pathogen (Rajamohan *et al*., 2009).

MDR *A. baumannii* have been reported to cause infections in immunocompromized, and debilitated patients, especially those in the intensive care units (ICUs). The most common presentation is pneumonia in mechanically ventilated patients in the ICUs (Montefour *et al*., 2008). Crude mortality rate of 30 – 75% has been reported for *Acinetobacter* pneumonia in hospitalized patients and even more in ventilator assisted patients (Paul *et al*., 2007). Apart from pneumonia, wound infection, bacteremia, urinary tract infection, secondary meningitis, native-valve infective endocarditis, peritonitis, keratitis, and osteomyelitis have been reported globally (Peleg *et*

al., 2008). Invasive procedures involving endotracheal tube, central venous catheter, urinary tract insertions, lumber puncture, myelography, ventriculography and ventriculoperitoneal shunt are the leading risk factors for infections. Mortality rate for central nervous system infection has been reported to be 20 to 27% in patients, in whom *A. baumannii* has been isolated from their cerebrospinal fluid (Seifert *et al*., 1995).

Acinetobacters can survive for >4 months in the environment, which is longer than 7 days survival period for *S. aureus* (Deitz *et al*., 1988). Because of this and aforementioned features, it has successfully involved in several outbreaks across the globe. In early 1980s, *A. baumannii* nosocomial outbreaks were described in South Europe, particularly in France, Germany, England, Netherlands, and Spain (Villegas and Hartstein, 2003; Fournier and Richet, 2006). The spread to Northern European countries like, Belgium and Germany has been linked with international transfer of colonized patients and airline travel (Peleg *et al*., 2006a). International *A. baumannii* clones known as European clones I, II and III have been reported in several European countries and also the United States (Nemec *et al*., 2004, van Dessel *et al*., 2004, Wroblewska *et al*., 2007). A data collected by National Nosocomial Surveillance Infection System from several hospitals in New York, USA from 1984 to 2003 on the prevalence of multidrug resistant *Acinetobacter* strains showed that the commonest manifestation was in ICU acquired pneumonia due to *A. baumannii,* which was found to be 4% in 1986 and 7% in 2003 (Gaynes and Edward, 2005). This substantial rise in United States was observed to have been contributed by the injured military personnel returning from war in Iraq and Afghanistan (Davis *et al*., 2005). Similar surveillance from 1997 to 2001 in South American countries like, Argentina, Colombia, Chile, and Brazil showed increase prevalence of MDR *A. baumannii* (Tognim *et al*., 2004). Back home in Nigeria, a study conducted by Iregbu et al. (2002) clearly revealed high prevalence of MDR *A. baumannii* found to be 50/58 (86.2%) in Lagos University Teaching Hospital (LUTH), Lagos. Also in a recent study conducted in the University College Hospital (UCH), Ibadan Nwadike et al. (2013) documented a prevalence of 11/14 (79.0%) for MDR *A. baumannii* specie over other species of Acinetobacters investigated.

The phenomena of emergence of MDR in this bacterium have also been traced to mutation in the drug target sites, acquisition of drug resistance genes, or emergence of new acquired mechanisms. The evolution of antibiotic resistance in Acinetobacters can be divided into two major eras: 1) before 1975, that marked the discovery of penicillin and awareness of this pathogen; and 2) after 1975, when there were substantial advances in invasive procedures in ICUs and discovery of powerful antibiotics. During this second period, new pattern of emergence has emerged and have been disseminated to many health care facilities. Currently, *Acinetobacter* infections are treated with aminoglycosides, cephalosporins (3rd and 4th generations), carbapenems, polymyxins, and Tigecycline either single or in combination and multidrug-, pandrug-, and extensively resistant *A. baumannii* have already emerged. A combined therapy of rifampin with carbapenems/polymyxins had given promising results but rifampin resistant strains have already been noticed. Alarmingly, there is paucity of information on the epidemiology and trends of antimicrobial resistance of *A. baumannii* from most African countries, including Nigeria.

This current study is centered on investigating the susceptibilities pattern of *A. baumannii* to spectrum of 34 antimicrobial agents belonging to 13 classes of antibiotics, some of which are not readily available in Nigeria. The results from this study will provide a scaffold for empiric prescription of antibiotics to patients with *A. baumannii* infections, as well as finding the current state of susceptibility of *A. baumannii* in southwest Nigeria.

Materials and Methods:

Study Population: Clinical specimens were collected from male and female patients attending 8 selected tertiary hospitals in southwest Nigeria. The samples were collected from microbiology laboratories of these various tertiary hospitals.

Sample Collection: The clinical specimens which include: mid-stream urine, wound swab, surgical swab, urethra swab, eye swab, blood and biopsy were collected from over 550 patients. The specimens collected were transported to pharmaceutical microbiology laboratory of University of Ibadan for standard biochemical analysis.

Isolates identification: All the isolates recovered were cultured on Eosin methylene blue (EMB) agar and MDR Leeds Acinetobacter medium (LAM). The identities of the isolates to species level were confirmed by standard biochemical methods using Oxoid Microbact™ Gram-negative identification system.

Antimicrobial Susceptibility Test: Antimicrobial susceptibility testing was performed by disk diffusion method on sensitivity test agar according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2010). A total of 34 antimicrobial agents from Oxoid Ltd (Fig. 1) were tested, consisting of 18 β-lactams/cephalosporins, 2 macrolides, 4 fluoroquinolones, 3 aminoglycosides, chloramphenicol, tetracycline, nitrofurantoin, nalidixic acid, trimethoprim-sulfamethoxazole, polymyxin B and colistin sulphate.

Results:

During the study period, a total of 72 clinical isolates of *A. baumannii* were recovered from 8 different tertiary hospitals (Table 1). The most common source of isolate derivation was from wound swab (20.8%) followed by surgical swab (18.1%), blood (16.7%). Least isolate derivation were from urethra swab and eye swab with 6.9% and 2.8% respectively.

The antimicrobial susceptibility profiles of *A. baumannii* isolates from these studied institutions are shown in Fig. 1. Amazingly, all the isolates were resistant to ≥14 antimicrobial agents tested, with 69 (95.8%) isolates resistant to 20-34 antimicrobial agents. All the isolates were resistant to amoxicillin, amoxicillin clavulanic acid, ampicillin, cefpodoxime, ceftazidime, ceftriaxone, cefuroxime, and cloxacillin. Significantly high rates of resistance were observed for erythromycin (98.6%); cephazolin (97.2%); aztreonam (93.1%); tetracycline (91.7%); cephalothin, trimethoprim-sulfamethoxazole (90.3%); kanamycin (88.5%); gentamicin, ticarcillin (86.1%); piperacillin, cefoxitin (87.5%), nitrofurantoin (81.9%); chloramphenicol (80.3%); ofloxacin (66.7%); and colistin (63.9%). High rates of carbapenem resistance were also recorded against meropenem (77.8%) and imipenem (56.9%). The least resistance was observed for azithromycin (37.9%); ciprofloxacin, polymyxin B (40.3%); and amikacin (43.1%).

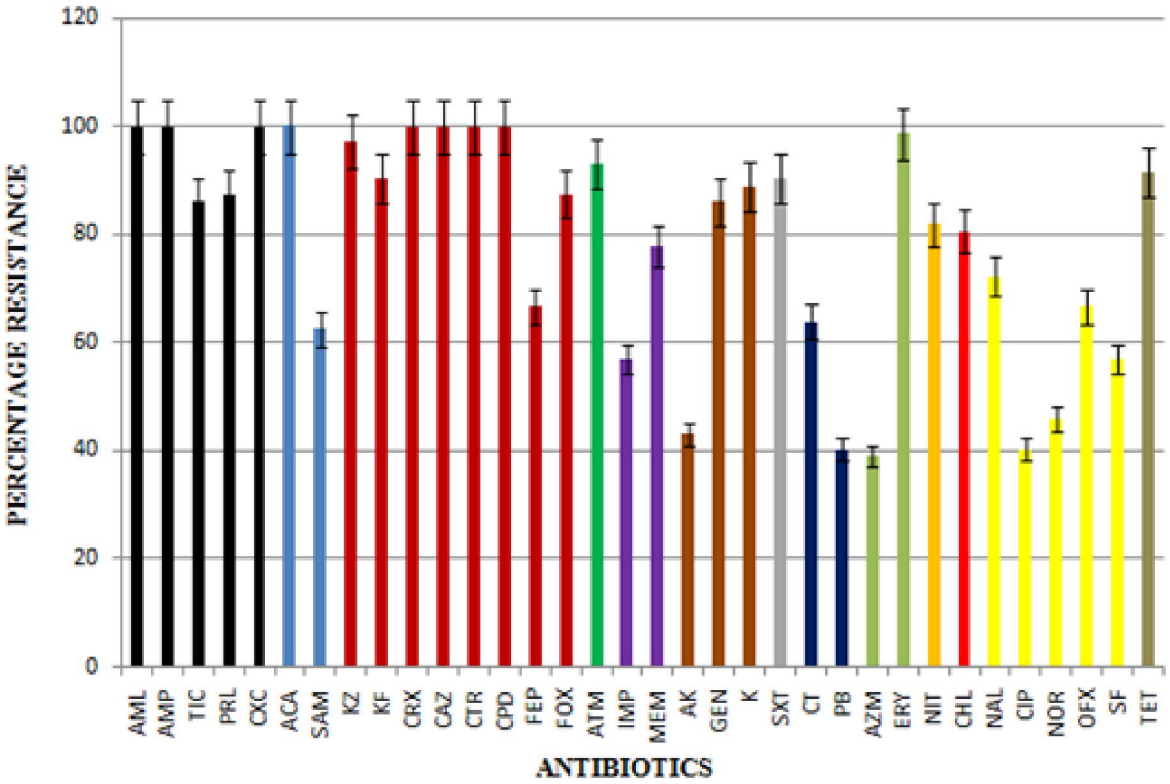

Fig: 1. In vitro susceptibilities pattern of 72 strains of *A. baumannii* expressing multidrug resistance against 34 spectrums of antimicrobial agents belonging to 13 classes of antibiotics with different colour bars according to CLSI 2011 classification. **AK**: Amikacin (30µg); **AML**: Amoxicillin (25µg); **AMP**: Ampicillin (25µg); **SAM**: Ampicillin-Sulbactam (30µg); **ACA**: Amoxicillin-clavulanate (30µg); **AZM**: Azithromycin (15µg); **ATM**: Aztreonam (30µg); **FEP**: Cefepime (30µg); **FOX**: Cefoxitin (30µg); **CPD**: Cefpodoxime (10µg); **CAZ**: Ceftazidime (30µg); **CTR**: Ceftriaxone (30µg); **CRX**: Cefuroxime (30µg); **KF**: Cephalothin (30µg); **KZ**: Cephazolin (30µg); **CHL**: Chloramphenicol (30µg); **CIP**: Ciprofloxacin (5µg); **CXC**: Cloxacillin (5µg); **CT**: Colistin (10µg); **ERY**: Erythromycin (5µg); **GEN**: Gentamicin (10µg); **IPM**: Imipenem (10µg); **K**: Kanamycin (30µg); **MEM**: Meropenem (10µg); **NAL**: Nalidixic acid (30µg); **NIT**: Nitrofurantoin (300µg); **NOR**: Norfloxacin (10µg); **OFX**: Ofloxacin (30µg); **PRL**: Piperacillin (30µg); **PB**: Polymyxin B (300units); **SF**: Sparfloxacin (5µg); **TET**: Tetracycline (30µg); **TIC**: Ticarcillin (75µg); **SXT**: Trimethoprim-Sulfamethozole (25µg).

Table 1: Distribution of *A. baumannii* isolates recovered from various clinical sources in this study.

Sources/Sites of *A. baumannii* isolates	Number of isolates	Percentage (%) of isolates
Wound swab	15	20.8
Surgical swab	13	18.1
Blood	12	16.7
Biopsy	9	12.5
Urine	8	11.1
Burns	8	11.1
Urethra swab	5	6.9
Eye swab	2	2.8
Total	72	100.0

Discussion:

This current study identified these isolates to specie level using computer aided Oxoid Microbact™ identification system. Antimicrobial resistance among *Acinetobacter* species has increased significantly in the past decades (Maragakis and Perl, 2008). The ability of *Acinetobacter* species to extensively resist antimicrobial agents may be explained in part by the organism's relatively impermeable outer membrane, selective pressure, and environmental exposure to a large reservoir of resistance genes (Bonomo and Szabo, 2006).

Definitions of multidrug-resistant *Acinetobacter* species vary, referring to a wide array of genotypes and phenotypes (Falagas et al., 2006). Two of the most common definitions of multidrug resistance are carbapenem resistance or resistance to ≥3 classes of antimicrobials (Falagas et al., 2006). This observation was made of our isolates as all the strains were resistance to ≥3 classes of antimicrobial agents investigated in this study. Strain that demonstrated resistance to all antimicrobial agents used in this study, including polymyxins, was also recorded. In this study all the strains exhibited resistance to ≥14 antimicrobial agents tested, with 95.8% showing resistant to 20-34 antimicrobial agents employed in this study. All (100%) strains were resistant to amoxicillin, ampicillin, amoxicillin-clavulanate, cefpodoxime, ceftazidime, ceftriaxone, cefuroxime, and cloxacillin used in this study (Fig. 1). In 1970s, *Acinetobacter* infections were treated with ampicillin, second generation cephalosporins, minocycline, colistin, carbenicillin and gentamicin (Iregbu et al., 2002). Today, most strains are resistant to ampicillin, cefotaxime, and chloramphenicol, with reports of 84% resistant to gentamicin in some institutions (Iregbu et al., 2002). This is consistent with the observation made in this study.

Among the class of penicillins used in this study as indicated by the black colour coded bars, all the strains (100%) were resistance to amoxicillin-clavulanate, ampicillin, and cloxacillin (Fig. 1). Similarly, remarkably high level of resistance was also recorded for ticarcillin (86.1%) and piperacillin (87.5%). This is comparable with results obtained by Ling et al., (2005) with resistance of 76% to ticarcillin and 78.9% to piperacillin.

Out of the two β-lactamase inhibitors used, amoxicillin-clavulanate did not improve the antimicrobial activity with all the isolates showing resistance. Similarly, relatively low level of susceptibility was also observed for ampicillin-sulbactam with resistance rate of 62.5%. This is in agreement with previous observation made by Higgins et al., (2004).

For the antibiotic class cephems indicated by the wine colour coded bars, all the isolates were resistance to cefuroxime the only second generation subclass of cephalosporin investigated. Similarly zero susceptibility rates were also recorded against the third generation subclass of cephalosporin, ceftazidime, ceftriaxone, and cefpodoxime. These observations were comparable to report made by Iregbu et al., (2002) against ceftriaxone and cefuroxime. For the first generation subclass, cephazolin and cephalothin, and subclass cephamycins, cefoxitin, significantly high resistance rates were noted. While 97.2% resistant rate was noticed for cephazolin, 90.3% was observed for cephalothin and 87.5% for cefoxitin. However, it is interesting to observe in this study, the emerging resistant pattern to cefepime. Cefepime a fourth generation subclass of cephalosporins has remained one of the most potent drugs against infections caused by *A. baumannii*. Amazingly, it is alarming to observe the relatively high level resistant of 66.7%, considering the relative low level of availability and use in these institutions. Generally, cephalosporins are β-lactam antibiotics with reportedly high antimicrobial activity and low toxicity. Increased resistance observed from *A. baumannii* isolates recovered from these institutions could partly be explained by high level production of extended-spectrum beta-lactamases (ESBLs) induced by selective pressure from broad-spectrum antimicrobial therapy. Apart from cefepime, which is not readily available across

drug counters, the other subclasses of cephalosporins used in this investigation were found to be prevalently prescribed in these institutions (unpublished observation).

Carbapenems remain the antibiotic of choice to treat *A. baumannii* and other Gram-negative infections due to both a wider spectrum of antibacterial activity and less frequent side effects (Fonseca et al., 2013). However, their overuse and misuse have selected for nosocomial isolates presenting intrinsic and acquired multidrug resistance determinants (Kuo et al., 2012a). It has been considered that resistance against carbapenems is, in itself, sufficient to define an *A. baumannii* as highly resistant (Fonseca et al., 2013). The result in this study on the carbapenems utilized revealed a 77% resistance rate for meropenem. This value contrasted the 63% reported in South Africa by Kock et al., (2013), but in tandem with result documented for *A. baumannii* investigated by Fonseca et al., (2013) in Brazil. Similarly, high level of resistance (56.9%) as indicated by purple colour coded bar (Fig. 1) was recorded against imipenem. This in a way contradicts 91.3% observed by Ramoul et al., (2013) in Algeria health care centers. This is a probable indication of the emergence of carbapenem-resistance strains of *A. baumannii* in Nigeria like other parts of the world. Surveillance studies have shown that the percentage of carbapenem-resistant isolates gradually increased over the last ten years in Europe, North America, and Latin America (Peleg et al., 2008). In other countries such as Tunisia and South Africa (Poirel et al., 2008), China, Taiwan, Singapore, Hong Kong, Japan, South Korea (Mendes et al., 2009), and Australia (Peleg et al., 2006), numerous outbreaks of carbapenem-resistant strains have been reported.

Aminoglycosides are usually used in combination with another active antimicrobial agent. However, many MDR *Acinetobacter* isolates showing intermediate susceptibility has been noted for amikacin. In this study, amikacin with 43.1% resistance rate, showed better activity against *A. baumannii* than gentamicin (86.1%) and kanamycin with 88.9%. Similar finding was reported by Nemec and Maixnerová, **(**2004) with amikacin, gentamicin and kanamycin having similar trend of 47%, 87% and 93% resistance rates respectively.

Polymyxins are group of polycationic peptide antibiotics, exhibiting potent efficacy against most Gram-negative bacteria (Benedict and Langlykke, 1947; Stansly et al., 1947). Among all the five chemical compounds (A–E) of polymyxins, only polymyxin B and E (colistin) are used clinically. In this study, 40.3% of the strains were resistant to polymyxin B while 63.9% were resistant to colistin. This increase is suggestive of overuse. In a recent study conducted by Hannan et al., (2014), in Pakistan all the strains of *A. baumannii* investigated were susceptible to polymyxin B and resistant to colistin. Reports from multiple investigators have also illustrated a 100% susceptibility to extensively drug resistant (XDR) *A. baumannii* strains (Kuo et al., 2012b; Lim et al., 2011). Colistin resistance has been reported from various regions of the world. In Spain, colistin resistance was found to be 40.6% (Arroyo et al., 2009), and 12% in Kuwait (Al-Sweih et al., 2011). In a study reported by Chang et al., (2012), 10.4% colistin resistance was documented. In another similar study by Rodriguez et al., (2010), colistin resistance was found to be 7.1%. Although the frequency of colistin resistance is low globally in contrast to our findings, it has been substantiated through *in-vitro* experiment that the rate of development of resistance among *Acinetobacter* to colistin is rapid (Tan et al., 2007). Colistin is now used in the treatment of infections caused by MDR and XDR Gram-negative organisms such as *Pseudomonas* and *A. baumannii* due to its relatively low neurotoxicity and nephrotoxicity as compare to Polymyxin B.

Fluoroquinolones a subclass of quinolones are among the most widely used antibiotic in clinical practice and are highly effective against most Gram-negative species. Currently, multiple studies have shown a steady increase in the resistance to fluoroquinolones in *Acinetobacter* species clinical isolates and more concretely in *A. baumannii* with resistance rate higher than 75% (Hujer et al., 2009; Chopra et al., 2010). In contrast to our findings, fluoroquinolones seem more active in comparison with other classes of antibiotics used in this study against the strains of *A. baumannii* recovered from these institutions. While 66.7% was resistant to ofloxacin, 56.9% was recorded against sparfloxacin and 45.8% against norfloxacin. The least resistance was observed for ciprofloxacin with resistant rate of 40.3%. The gradual increase in resistance observed for fluoroquinolones in this study is suggestive of a possible overuse of these groups of antibiotics in these institutions. Also worthy of note is the significant high resistance recorded against the other classes of antibiotics used in this study. For erythromycin, 98.6% of isolates were found to be resistance, 93.1% were noted for aztreonam, while 91.7% were observed for tetracycline. Others such as trimethoprim-sulfamethoxazole, nitrofurantoin, chloramphenicol, and nalidixic acid, 90.3%, 81.9%, 80.6%, and 72.2% were documented. Amazingly, azithromycin a class of macrolides used against Gram-positive organisms was the most remarkably active antibiotics with susceptibility rate of 61.1%, suggestive of none usage of this antibiotic in the treatment of infections caused by MDR *A. baumannii* in these institutions.

Conclusion:

A. baumannii isolates from these institutions showed unacceptably high rates of resistance to multiplicity of antimicrobial agents, including those not readily available in Nigeria. To deal with the increasing antimicrobial resistance of *A. baumannii*, measures for controlling overuse and abuse of antibiotics should be instituted in these hospitals. Antibiotics should only be prescribed if an infection is highly suspected. Physicians should choose an older generation of antibiotic according to the antimicrobial susceptibility results and reserve the powerful newer antibiotics. If the culture results are shown to be negative and no sign of infection, antibiotic therapy should be discontinued.

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