Bacteriological Review of Multi-Drug Resistance (MDR) Pathogens involved in Respiratory Tract Infections (RTIs) in Abeokuta, Nigeria

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ABSTRACT: Various Bacteria have being implicated as etiological agents of respiratory tract infections, often times these same bacteria colonize the nostrils mouth and throat, acting as normal flora, before adverse events make them become pathogenic. Our objective is to review bacteria agents isolated from various respiratory tract infections and their antibiotic susceptibility profile in Abeokuta, Nigeria. 130 cases of various respiratory conditions were retrospectively reviewed from May 2010 to December 2010, for culture and susceptibility profile. All samples were processed following standard Microbiological protocols and susceptibility was tested by Kirby-Bauer method for disk diffusion. An isolation rate of (29.0%) was recorded for pathogens (71.0%) for commensal (Normal flora). Distribution of pathogenic bacteria isolates by gram reaction showed that Gram negative bacilli (GNB) had a higher value with 22(58.0%) and Gram positive cocci (GPC) 16(42.0%). Female subjects recorded a higher isolation rate than males, 62.2% vs 37.8%, (P-value 1.98, Chi square test X^2). *Klebsiella pneumonia* recorded the highest occurrence rate with 15(40.5%), followed by *Streptococcus pneumonia* with 8(21.6%), the lowest rate was recorded by *Proteus spp* with 1(2.7%). Age range distribution was highest in 45-60(39.0%), 15-30 (37.5%) the lowest rate was recorded by 0-15 (21.0%). The antibiograms revealed that majority of the pathogens showed multi-drug resistant (MDR) pattern to most of the antibiotics used. Augumentin was the most broadly active antibiotic with 75.0% sensitivity to *Streptococcus pnuemoniae*, 100.0% sensitivity to *Streptococcus viridians*, and 100.0% sensitivity to *Staphylococcus aureus,* followed by Gentamycin, Ofloxacin also displayed good sensitivity with a rate of 73.3% to *Klebsiella pneumonia*, 75.0% sensitivity to *Pseudomonas aeruginosa.* The Cephalosporins performed poorly, and there was absolute resistance to Amplicillin and Tetracycline, while Amoxiclillin recorded very poor sensitivity against all isolates tested. The high β-lactamase resistance observed in all our isolates, particularly in GNB isolates even to the cephalosporin is in an indication of acquisition and dissemination of ESBL genes. The study showed the presence of multi-drug resistant (MDR) pathogens among the subjects with RTIs attending out-patient clinic in a tertiary hospital in Abeokuta, Nigeria and this calls for particular public health attention. We hereby advocate better surveillance and infection control programs in our health institutions for early detection and intervention of multi-resistant bacteria.

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1. INTRODUCTION

Respiratory tract infections are one of the commonest forms of human disease, world wide with global mortality rates estimated at about 7 million (Ozyilmaz et al., 2005).The human respiratory tract, particularly the upper respiratory tract is normally open to collonisation by diverse organisms particularly anaerobes with a ratio of upto 1: 100 aerobes to anaerobes (Murray, 1998), commonly encountered organisms include *Peptostretococcus*. *Actinomyces*, and *Fusobacterium spp*. Aerobes on the other hand which ideally exist as harmless commensiles include, Strptococcus *spp* (Viridans group), Staphylococcus *spp*, Enteriobacteriacea, *Corynobacterium* and *Niesseria spp* (Murray, 1998). Some of the aforementioned organisms are potentially pathogenic causing disease when they are accidentally introduced to sterile sites e.g. sinuses, middle ear and the brain or in immunocompromised patients. Often times respiratory tract infection are initiated by viral agents and lowed immunity gives rise to bacteria supper-infection, particularly with opportunistic pathogens normally colonizing the respiratory tract. Isolation of a potential pathogen alone is not sufficient to confirm diagnosis, particularly from

non sterile sites like the throat, involvement with the disease process through other clinical investigation has to corroborate with the condition to the exclusion of other bacteria before diagnosis can be confirmed (Carrol, 2002).

Epidemiologic studies have shown that bacteria agents of Respiratory tract infections (RTI) differ from region to region and from Lower and Upper RTI. For instance, in the Unites states, respiratory disease was the 4th leading cause of disability and the third leading cause of hospitalizations in Canada (Desalu et al., 2011). Group A Streptococcal infections have been reported to cause about half a million deaths world wide majority resulting from respiratory tract infections and acute rheumatic fever (Safar et al., 2011). Methicillin resistant *S. aureus* MRSA carriage from respiratory tract samples among hospital staff capable of noscomial infection has also been previously reported (Alli et al., 2011).

With changing antibiotic susceptibility profiles of various bacteria capable of causing severe RTI, the need for regular surveillance of the prevailing bacteria pathogens and their susceptibility profiles cannot be overemphasized. This study was conducted with the objective of determining the distribution pattern of bacterial agents of both upper and lower respiratory tract infections and their antibiotic susceptibility pattern in a South Western Nigerian Hospital.

2. MATERIALS AND METHODS

Study Design: The study is a retrospective review of bacteria isolation pattern of repiratory tract samples of all ages and gender and their respective antibiotic susceptibility pattern in a Tertiary hospital in Abeokuta, Ogun state, Nigeria. Samples submitted for processing included, Sputum, throat swab samples, and Pleural aspirate. The duration of the study was from May 2010 and December 2010 results were extracted from previous records of the laboratory register, all patient information were given the highest level of confidentiality in accordance to the Belmont report (Document of the U.S. Department of Health , Education and Welfare, 1979).

2.1. Bacteria isolation and identification:

All laboratory protocols were retrospectively done at the Microbiology Unit of the Federal Medical Center Abeokuta. Freshly collected samples were inoculated on Mac-Conkey, Blood and Chocolate agar, agar plates were incubated at 37^0C aerobically and chocolate agar plates were incubated under carboxiphyllic condition in a $C0_2$ jar at 37^0C for 24hrs. Isolates were identified using standard Microbiological protocols (Cheesbrough, 1996). Only strict pathogens or previously reported potential pathogens (Cheesbrough, 1996, Murray, 1998) were selected for antibiotic susceptibility testing, other non pathogenic bacteria were reported as commensal or normal flora in samples from non sterile sites.

2.2. Antibiotic susceptibility Testing

Antibiotic susceptibility was done by the Kirby Bauer Technique for disk-diffusion (Cheesbrough, 1996) using Multi-disc antibiotics (Abtek U.K.). Single disc used included Augumentin 50µg, Ceturoxime 30µg and Ceftazime 30µg (Oxoid). Isolates were considered as sensitive or resistant to an antibiotic according to the diameter of inhibition zone size interpretative chart (Clinical and Laboratory Standard Institute, 2006)

3. RESULTS ANALYSIS

3.1. Prevalence of MDR Pathogens among the subjects with RTIs in relation to gender

During the period under review, a total of 130 results were analyzed consisting of 49(37.7%) male and 81(62.3%) female subjects (Table 1). An isolation rate of 37(28.5%) was recorded for pathogens while commensal (Normal flora) recorded 93(71.5%). Distribution of pathogenic bacteria isolates by gram reaction showed that GNB had a higher value with 22(58.0%) and GPC 16(42.0%).

Table 1: Prevalence of MDR Pathogens among the subjects with RTIs

Sex	No. tested (%)	No. Positive (%)
Males	49(37.7)	14 (28.6)
Females	81(62.3)	23(28.4)
Total	**130(100)**	**37(28.5)**

3.2. Gender-related distribution of the various pathogenic bacteria isolates

Table 2 shows gender-related distribution of the various pathogenic bacteria isolates, *Klebsiella pneumoniae* recorded the highest occurrence rate with 15(40.5%), followed by *Streptococcus pneumoniae* with 8(21.6%), the lowest rate was recorded by *Proteus spp* with 1(2.7%), details of other isolates are shown in Table 2. Details of various gender distributions in relation to individual bacteria species are shown in detail in Table 2. Female subjects generally recorded a higher isolation rate of MDR pathogens than males (62.3% vs 37.7%, P-value = 1.98, Chi square test X^2) as shown in Table 2.

Table 2: Distribution of Pathogenic Bacteria isolates in relation to Gender

Isolates	No. (%)	No. Males (%)	No. Females (%)
Strepto. pneumoniae	8 (21.6)	3 (8.1)	5 (13.5)
Streptococcus viridians	3 (8.1)	1 (2.7)	2 (5.4)
Staphylococcus aureus	2 (5.4)	1 (2.7)	1 (2.7)
Klebsiella pneumoniae	15 (40.5)	5 (13.5)	10 (27)
Escherichia coli	4 (10.8)	2 (5.4)	2 (5.4)
P. aeruginosa	4 (10.8)	2 (5.4)	2 (5.4)
Proteus spp	1 (2.7)	0 (0.0)	1 (2.7)
Total	**37(100.0)**	**14(37.7)**	**23(62.3)**

3.3. Distribution of Pathogenic bacteria isolates in relation to age

Table 3 shows the bacteria distribution according to age range, the highest rate was recorded among patients in age range 45-60 with a rate of 39.0%, closely followed by age range 15-30 with 37.5% the lowest rate was recorded in age range 0-15 with 21.0%, details of the results are shown in Table 3. The isolation rate of MDR pathogens was not age dependent ($P > 0.05$).

Table 2: Distribution of Pathogenic bacteria isolates in relation to age

Age Range (years)	No. Tested	No. Positive (%)
0 - 15	17	4 (23.5)
15 - 30	24	9 (37.5)
31 – 45	48	10 (21.0)
46 – 60	23	9 (39.0)
61 and above	18	5 (27.8)
Total	**130**	**37(28.5)**

3.4. Bacteria occurrence rate according to Presumptive diagnosis

Table 4 shows bacteria occurrence rate according to Presumptive diagnosis. Occurrence rate of isolated bacteria pathogens according to presumptive diagnosis revealed that only 76(58.5%) subjects had data recorded for presumptive diagnosis and 23 (30.3%) were positive for bacterial pathogens. Of the 76 subjects, pulmonary tuberculosis (PTB) was the most frequently diagnosed, followed by unspecific upper respiratory tract respiration (URTI) 18(23.7%), lower respiratory tract infections were 12(15.8%), pneumonia and cough gave 9 (11.8%) each. Isolation rate according to diagnosis revealed that Pneumonia gave a rate of 66.7% isolation rate, followed by tonsislitis, URTI and bronchitis with 33.3% respectively and the lowest rate was recorded for PTB with 15.8% (Table 4).

Table 4: Bacteria occurrence rate according to Presumptive diagnosis

Diagnosis	No. examined (%)	No. Positive (%)
URTI	18 (23.7)	6 (33.3)
LRTI	12 (15.8)	3 (25.0)
Tonsilitis	6 (7.9)	2 (33.3)
Pneumonia	9 (11.8)	6 (66.7)
Persistent cough	9 (11.8)	2 (22.2)
PTB	19 (25.0)	3 (15.8)
Bronchitis	3 (4.0)	1 (33.3)
Total	**76(58.5)**	**23(30.3)**

3.5. Antibiotic sensitivity profile of isolated pathogenic bacteria from samples of respiratory tract infections

Table 5 shows antibiotic sensitivity profile of isolated pathogenic bacteria from samples of respiratory tract infections. Antibiotic susceptibility profile of tested bacteria isolates revealed that Augmentin was the most broadly active with 75.0% sensitivity to *Streptococcus pneumoniae*, 100% sensitivity to *Streptococcus viridians* , 100% sensitivity to *Staphylococcus aureus*, Gentamycin was also fairly active particularly among GNB with a sensitivity of 26.7% for *Klebsiella pneumoniae*, 75.0% sensitivity to *Pseudomonas aeruginosa*, and 100.0% to *Proteus spp*. Quinolone activity against tested isolates was also fair with Ofloxacin having a sensitivity rate of 73.3% to *Klebsiella pneumonia*, 75% sensitivity to *Pseudomonas aeruginosa* and 75.0% resistance to *Escherichia coli*. The Cephalosporins performed poorly with Cefuroxime recording a sensitivity of 33.3% against *Streptococcus pneumoniae* and resistance of 73.3% against *Klebsiella pneumoniae*, while 100.0% resistance was recorded by *Pseudomonas aeruginosa*. Absolute resistance was recorded by Ampicillin (100.0%) and Tetracycline (100.0%) to all isolates tested while very high resistance was recorded against Amoxicillin with *Streptococcus pneumoniae* recording 87.5% resistance details are shown in Table 5. It also revealed a multi-drug resistance (MDR) patterns, all isolates were resistant to ampicillin and tetracycline. *K. pneumoniae*, *E. coli*, *P. aeruginosa* and *Proteus* spp were resistant to amoxicillin, ampicillin and tetracycline. *P. aeruginosa* and *Proteus* spp were resistant to amoxicillin, ampicillin, erythromycin, tetracycline, Cefuroxime and Ceftazidime. Generally, the percentage sensitivity of isolates to the antibiotics tested ranged from 8.1% to 45.9% while the percentage resistance ranged from 54.1% to 100.0% (Table 5).

Table 5: Antibiotic sensitivity profile of isolated pathogenic bacteria from samples of Respiratory tract infections

Isolates	No. (%)	Antibiotics Sensitivity Profiles (%)								
		Amoxicillin	Ampicillin	Erythromycin	Tetracycline	Gentamycin	Ofloxacin	Augmentin	Cefuroxime	Ceftazidime
S. pneumoniae	8(21.6)	1(12.5)	0(0.0)	3(37.5)	0(0.0)	2(25.0)	Not tested	6(75.0)	3(37.5)	3(37.5)
S. viridians	3(8.1)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	1(33.3)	Not tested	3(100.0)	1(33.3)	2(66.7)
S. aureus	2(5.4)	1(50.0)	0(0.0)	1(50.0)	0(0.0)	1(50.0)	Not tested	2(100.0)	1(50.0)	1(50.0)
K. pneumoniae	15(40.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	4(26.7)	11(73.3)	4(26.7)	4(26.7)	10(10)
E. coli	4(10.8)	0(0.0)	0(0.0)	1(25.0)	0(0.0)	1(25.0)	1(25.0)	1(25.0)	1(25.0)	0(0.0)
P. aeruginosa	4(10.8)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(75.0)	3(75.0)	0(0.0)	0(0.0)	0(0.0)
Proteus spp	1(2.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)
Total	**37(100)**	**S**	**3(8.1)**	**0(0.0)**	**5(13.5)**	**0(0.0)**	**12(32.4)**	**15(40.5)**	**17(45.9)**	**10(27.0)**	**7(18.9)**
		R	**34(91.9)**	**37(100.0)**	**32(86.5)**	**37(100.0)**	**25(67.6)**	**22(59.5)**	**20(54.1)**	**27(73.0)**	**30(81.1)**

Keys: S = sensitive, R= resistant

4. DISCUSSION

The importance of pathogenic bacteria associated with respiratory tract infections cannot be overemphasized previous reports have implicated bacteria pathogens particularly *Streptococcus pneumoniae* as the commonest cause of pneumonia (ACCP, 2009). Another recent study done in Nigeria reported that respiratory tract infection accounted for 10% of the total emergency cases at Federal Medical center Ado-Ekiti (Desalu et al., 2011). Our study has attempted to reveal the association of pathogenic bacteria and various respiratory tract infections as well as their antibiotic susceptibility profile. An isolation rate of 28.5% was recorded for pathogenic bacteria; this is similar to other reports (Okesola and Ige, 2007).

Distribution of various species of bacteria revealed that *Klebsiella pneumoniae* was the most prevalent organism with 40.5%, followed by *Streptococcus pneumoniae* (21.6%), and the least prevalent isolate was *Proteus spp* with 2.7%. This is similar to a recent report at the University of Benin Teaching Hospital, Nigeria which also reported *Klebsiella pneumoniae* as the most prevalent bacteria isolate from RTI with a rate 30.16% (Egbe et al., 2011). Our report is not in agreement with that of Nweze et al. (2012) which reported *Haemophillus influenzae* as the most frequently isolated pathogen from respiratory tract of children in south eastern Nigeria. There was a significantly higher bacteria isolation rate in females than males P-value 1.98 ($P<0.05$, X^2). Age range distribution of bacteria isolates revealed that age group 46-60 years recorded the highest occurrence rate of pathogenic bacteria with 39.0%, followed by age group 16-30 years. From our study, this suffices to say that the elderly and the adolescent are most at risk of acquisition of a severe respiratory condition.

The study also highlights the occurrence rate of isolated pathogens in relation to clinical diagnosis, from our results PTB, was the most commonly diagnosed respiratory condition with 19(25.0%) of recorded cases followed by Upper respiratory tract infection URTI 18(23.7%) of cases. Similar trend has also been reported by Desalu et al. (2011). Pneumonia recorded the highest recovery rate of pathogenic isolates with a rate of 66.7% of all cases, this result is consistent with similar reports that have implicated pneumonia as one the most severe RTI across all ages (Carrol, 2002) and another report that highlighted pneumonia as a major LRTI in medical emergencies (Desalu et al., 2011). Majority of the pneumonia cases were lobar pneumonia, which has been attributed mainly to infections from *Streptococcus pneumoniae* (Adegboro, 2010). Our report of *Streptococcus pneumoniae* as the 2nd most frequently isolated pathogen hence supports this finding and is an indication for a review of the current immunization policy to include the newer pantavalent pneumococcal vaccine which will give protective coverage against the major serotypes of *Streptococcus pneumoniae* to at risk age groups.

Antibiotic susceptibility profile of isolated pathogens reveals that for Gram positive organisms, Augmentin was the most broadly active antibiotic with a sensitivity of 75% against *S. pneumoniae* and 100% against *Streptococcus viridians* and *Staphylococcus aureus*. This was closely followed by Ceftazidime with a susceptibility of 33.3% against *Streptococcus pneumoniae*, and 67.3% against *Streptococcus viridians* and 50% against *Staphylococcus aureus*,

high level of resistance was recorded by Amoxicillin and Erythromycin with rates of 12.5% and 37.5% respectively against *S. pneumoniae*, this records an unusually high resistance against β-lactam antibiotics and Macrolides, this is an indication of acquisition of β-lactamase resistance as well as possibly macrolide resistance genes by this group of organisms this is quite worrisome because resistance of this nature has been reported in *S. pneumoniae* elsewhere (Kattan et al., 2011), and our ability to properly control the further dissemination and spread of this resistant bacteria in both community and institutional setting cannot be guaranteed. Ako-Nai et al. (2005) presented a report in which 1.8% of staphylococcal isolates were resistant to gentamycin in a study Ibadan, Nigeria. Resistant to amoxicillin reported in this study is in agreement with previous reports by other investigators in a similar study (Adel-Rauf, 2004).

Gram negative pathogens displayed a higher resistance profile against tested antibiotics with 100% resistance to Ampicillin, Amoxicillin and Tetracycline, 73.4% resistance was recorded by *Klebsiella p* against Cefuroxime and Augmentin while 90.0% resistance was recorded by *K. pneumoniae* against Ceftazidime, this is a clear indication of Extended spectrum beta lactamase (ESBL) emergence among our *K. pneumoniae* isolates, this resistance trend was also recorded by other GNB isolates such as *Escherichia coli* and *Pseudomonas aeruginosa*. Thankfully, Ofloxacin displayed a fairly good susceptibility profile with *K. pneumoniae* recording 73.3% sensitivity, *Pseudomonas aeruginosa* 75.0% and *Escherichia coli* 25.0%. This is an indication that Quinolone resistance is yet to emerge in our environment despite the relative abuse of this antibiotic, although this statement cannot be substantiated because of the relatively small number of isolates tested, a more broad based survey is hereby recommended to actually justify our current observations.

Aminoglycosides have good activity against clinically important gram negative bacilli (Ullah et al., 2009). The aminoglycoside antibiotics include gentamcin, kanamycin, amikacin etc. These act by inhibiting bacterial protein synthesis. Gentamycin is routinely used synergistically with a beta-lactam antibiotic or vancomycin for empirical therapy in infective endocarditis (Nwadioha et al., 2010). Tetracycline is an antibiotic that inhibits bacterial growth. They are bacteriostatic and widely used as a broad- spectrum antibiotic with activity against Gram- positive and Gram- negative bacteria. Resistance to tetracycline is common and this is further confirmed from the results obtained in a study by Adedeji and Abdulkadir (2009). Resistance to tetracycline has developed because it is readily available in the country and has been widely misused (Adedeji and Abdulkadir, 2009). Resistance to tetracycline has been reported by Nkang et al. (2009a,b). Abubakar (2009) reported high rate of resistance to tetracycline and gentamicin in their study.

Proteus sp was susceptible to gentamycin and augmentin (100.0%) but resistant to all other antibiotics (100.0%). This is similar to what was reported by Ibiene et al. (2012). Also, this is at variance with what was reported by Mordi and Momoh (2009) and Okonko *et al.* (2010), who reported *Proteus* sp to be susceptibile to ofloxacin and ciprofloxacin. Sensitivity of *Proteus* sp to gentamicin, and its resistance to tetracycline reported by Mordi and Momoh (2009), Okonko *et al.* (2010) and Ibiene et al. (2012) is similar to the findings of this study. However, the *in vitro* sensitivity in this study did show gentamicin and augmentin to be the drug of choice for *Proteus* infections.

Twenty five percent of *E. coli* isolates in this study were sensitive to erythromycin, gentamycin, ofloxacin, augmentin and Cefuroxime. Uzeh *et al.* (2006) and Ajao and Atere (2009) reported that *E. coli* was sensitive to gentamicin. In a similar study by Kebira et al. (2009), 80% of the isolates were susceptible to gentamicin. Adedeji and Abdulkadir (2009) reported the isolates were generally highly susceptible to gentamicin (89%). Okonko et al. (2009a) reported 100% resistance to gentamicin and tetracycline by *E. coli* and *S. aureus* in a similar study. Bacteria of the Klebsiella genus may cause numerous infections in human, which are often treated with beta-lactam antibiotics (Amin et al., 2009).

Multi-drug resistance (MDR) pattern was also reported in this study, been resistant to more than 4 of the 9 antibiotics tested. This is comparable to the findings in previous studies by other investigators elsewhere in Nigeria. The problems of multi-drug resistance (MDR) have been the driving force for the development of newer quinolones (Amin et al., 2009). MDR and the presence of several virulence factors in the strains of many pathogens responsible for different diseases pose an increasing threat to the successful management of disease scourge. The data obtained in this study shows that the bacteria pathogens causing most RTIs are still susceptible to antimicrobial agents routinely used in the hospital though this is changing. Although the disc diffusion method was used to assess sensitivity and resistance and can be correlated clinically, further investigations employing the minimum inhibitory

concentrations (MIC) method will be needed to obtain more reliable results (Abubakar, 2009).

5. CONCLUSION

We have reported the bacteria isolation and antibiotic susceptibility pattern of respiratory tract infections, our report has highlighted the importance of *Klebsiella pneumoniae* and Streptococcus pneumonia, in the ateiology of various respiratory tract infections surprisingly we did not record any isolate of *Haemophillus influenzeae*, which has been reported as a commonly isolated pathogen in particularly LRTI (Cheesbrough, 1996; Nwenze et al., 2012). A major limitation to this study was our inability to culture and isolate *Mycobacterium tuberculosis* from our sputum samples, as this would have added insight into the role of this bacteria in the actual etiology of majority of our clinical PTB cases, this is however an area for further study. The high level of resistance to β-lactam resistance recorded in all our isolates, particularly in GNB isolates even to the more active cephalosporins in an indication of increased acquisition and dissemination of ESBL resistant properties possibly through plasmid mediated transfer from pathogenic to other commensal in our study setting. The data presented in this study are similar to those obtained in other cities in Nigeria and have shown the changing pattern in the types of pathogens causing RTIs and their resistance to many of the commonly available antibiotics, thus leading to the use of newer and more costly agents (Ako-Nai et al., 2005; Nwanze et al., 2007; Kolawale et al., 2009; Okesola and Oni, 2009; Abubakar, 2009; Okonko et al., 2009a,b; Nkang et al., 2009a,b; Ibiene et al., 2012; Adebayo-Tayo et al., 2012). We hereby advocate better surveillance and infection control programs in our institutional settings for early detection and intervention of multi-drug resistant bacteria emergence such as ESBL producing extra-intestinal enterobactraecea such as *K. pneumoniae* capable of severe respiratory diseases.

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