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Antimicrobial Susceptibility Pattern of Bacterial Isolates from Wound Infections at All Africa Leprosy, Tuberculosis and Rehabilitation Training Center, Addis Ababa, Ethiopia

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Abstract

Background: Antimicrobial resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals or other agents designed to cure or prevent the infection.

Objective: To isolate etiology of wound infections and determine their antimicrobial susceptibility pattern.

Methods: A cross-sectional study was conducted at ALERT Center from February to May 2017. Swabs from different types of wounds were taken and processed to isolate etiologic agents by using standard microbiological techniques. Antimicrobial susceptibility tests were performed by disc diffusion technique as per the standard modified Kirby-Bauer method.

Results: In this study 171 bacterial isolates were recovered from 188 specimens showing an isolation rate of 86.2%. The predominant bacteria isolated from the infected wounds were *Staphylococcus aureus* 96 (51.1%) followed by *Klebsiella pneumonia* 26 (15.2%), Escherichia coli 23 (13.4%). Out of 162 positive samples, 9 (5.5%) were mixed infections. *Staphylococcus aureus* exhibited highest sensitivity against *Clindamycin* (95.8%), *Gentamycin* (94.8%), *Chloramphenicol* (92.7%), *Ciprofloxacin* (89.6%) and *Cotrimoxazole* (84%). Gram-negative isolates, *E. coli, P. vulgaris, P. mirabilis, P. aeruginosa* and *Citrobacter* showed the highest sensitivity against Amikacin (100%). *E. coli* showed high resistance for Ampicillin (95.7%) and Augmentin (91.3%) whereas *P. vulgaris* showed 100% resistance for Ampicillin and 90.9% for Tetracycline.

Conclusion: High culture positivity rate of wound infections reported in the present study initiates many similar studies to be conducted on wound infection in the country. High level of drug resistance to the commonly prescribed drugs dictates a search for better choices.

Keywords: *Wound Infection; Bacterial Isolates; Drug Resistance Pattern; ALERT Centre; Ethiopia*

Introduction

Antimicrobial resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals or other agents designed to cure or prevent the infection. Thus the bacteria survive and continue to multiply causing more harm. Widespread use of antibiotics promotes the spread of antibiotic resistance [1,2].

Bacteria can acquire antibiotic resistance either by mutation or through an exchange of genetic material among same or closely related species. The sudden acquisition of resistance to antibiotics poses difficulties in treating infections. Resistance to several different antibiotics at the same time is, even more, a significant problem [1-3].

The probability of wound infections largely depends on the patients' systemic host defences, local wound conditions, and microbial burden. Wound develops into an infected state when the balance between the microorganism and the host shifts in favor of the microorganism. The conditions of antimicrobial therapy, both prophylactically and therapeutically, can only be defined when these factors are under control [2,3].

Hence, an on-going surveillance could play a significant role in the early recognition of a problem and, there is a need for early intervention for better management of wound infections. Exposure of subcutaneous tissue following a loss of skin integrity (i.e. wound) provides a moist, warm, and nutritious environment that is conducive to microbial colonization and proliferation [3-5].

Knowledge of the causative agents of wound infection and the extent of drug resistance of these isolates against different antimicrobial classes in a specific geographic region will, therefore, be useful in order to provide locally applicable data and to guide empirical therapy.

In Ethiopia, drug resistance pattern is highly increasing the time to time according to various studies due to misuses of antibiotic by the public. Hence this study is very essential to see the pattern of resistance and the result of this study will assist clinicians to prescribe the appropriate antibiotics and helps the patients in getting timely and appropriate treatment.

Materials and Methods

Study design and Study Area

A cross-sectional study was conducted from February to May 2017 at ALERT Center. ALERT Center is one of the specialized tertiary referral hospitals in the country. It is located in Addis Ababa at 7 km Southwest on the way to Jimma. ALERT main mission was to provide training for both genders in multiple aspects of Leprosy including prevention, treatment, and rehabilitation in an African context.

Sampling procedure

A structured and predesigned questionnaire was developed and used for collection of data on socio-demographic characteristics (age, sex, occupation and educational background of patients). Open wound swabs were aseptically obtained after the wound immediate surface exudates and contaminants were cleaned off with moistened sterile gauze and sterile normal saline solution. Dressed wounds were cleaned off with sterile normal saline after removing the dressing. The specimen was collected on sterile cotton swab by rotating with sufficient pressure. Double wound swabs were taken from each wound at a point in time to reduce the chance of contamination. The samples were transported to the laboratory after collection within 30 minutes.

Culture and Biochemical tests

Swabs collected from patients were streaked on a blood agar (5% sheep blood) and MacConkey agar (Oxoid) by the sterile inoculating loop. The plates were incubated at 35 - 37°C for 24 - 48 hours. Preliminary identification of bacteria was done based on colony characteristics of the organisms. Some colony characteristics like hemolysis on blood agar, changes in physical appearance in differential media and enzyme activities of the organisms. Biochemical tests were performed on colonies from pure cultures for identification of the isolates. Gram-negative rods were identified by performing a series of biochemical tests-Oxoid using: - Kliger Iron Agar (KIA), Indole test, Simmon's citrate agar, Lysine Iron Agar (LIA), urea and motility. Gram-positive cocci were identified based on their Gram-reaction, catalase and coagulase test results. Mannitol salt agar was used also as a differential media to differentiate coagulase positive from coagulase-negative *Staphylococci* (CoNS).

Antimicrobial susceptibility testing (AST)

Susceptibility testing was performed by Kirby-Bauer disk diffusion technique (27) according to criteria set by Clinical and Laboratory Standard Institute (CLSI) 2016. The inoculum was prepared from the pure culture by picking parts (3 - 5) of similar test organisms with a sterile wire loop and suspended in sterile normal saline. The density of suspension to be inoculated was determined by comparison with opacity standard on McFarland 0.5 Barium sulphate solution. The test organism was uniformly seeded over on Mueller-Hinton agar (Oxoid) surface and exposed to antibiotic diffusing from antibiotic-impregnated paper disks into the agar medium, and then incubated aerobically at 37°C for 16 - 18 hours. Diameters of the zone of inhibition around the discs were measured to the nearest millimetre using a clipper and classified as sensitive, intermediate, and resistance according to the standardized table supplied by CLSI 2016. Only the conventional antibiotics regularly available for frequent use in the study area was considered for this study and all the disks that have been used for the test were from Oxoid. The following antimicrobial agents were employed:- Penicillin (10 μg) Ceftriaxone (30 μg), Clindamycin (10 μg), Erythromycin (15 μg), Gentamycin (10 μg), Ciprofloxacin (5 μg), Tetracycline (30 μg), Ampicillin (10 μg), Augmentin (30 μg), Amikacin (30 μg), Cefepime (30 μg), Cotrimoxazole (25 μg), Chloramphenicol (30 μg) and Ceftazidime (30 μg).

Data Analysis

Data entry and analysis was performed by using SPSS statistical software version 20. The descriptive statistics were calculated for each variable using frequencies and crosstabs.

Ethical Consideration

All ethical considerations and obligations were duly addressed. Ethical approval was obtained from Department Ethics and Research Committee (DERC) of Addis Ababa University (AAU), College of Health Sciences (COHS), Department of Medical Laboratory Sciences. Permission was also obtained from ALERT Center for data collection. Written informed consents were obtained from each individual after the purpose of the study explained. For children, consent was obtained from the parent or guardian of the child.

Result

Socio demographic characteristics

A total of 188 study participants were enrolled in this study. Among these, 72 (38.3%) were females and 116 (61.7%) males. The ages of the participants ranged from 1 year to 83 years with mean age of 31.8 ± 17.02 (Table 1). In this study, wounds were collected from different body sites (Table 2). Most of the causes of the wound were identified (Table 3). Wound infection was the highest 120 (68.3%) in patients of age group 15 - 44 followed by 32 (17.0%) age groups of 45 - 64. Age was classified based on Provisional Guidelines on Standard International age classification (31).

Variables	Characteristics	Frequency (%)
Sex	Male	116 (61.7)
	Female	72 (38.3)
Age	≤ 14	24 (12.8)
	15 - 44	120 (68.3)
	45 - 64	32 (17.0)
	≥ 65	11 (5.9)
Level of education	Illiterate	41 (21.8)
	Elementary	65 (34.6)
	High school	42 (22.3)
	College and above	32 (17.0)
	Under age	8 (4.3)
Occupation	Government employee	21 (11.2)
	Private enterprise	33 (17.6)
	Day labourer	28 (14.9)
	Merchant	4 (2.1)
	House wife	22 (11.7)
	Farmer	7 (3.7)
	No job	26 (13.8)
	Under age	17 (9.0)

Table 1: *Socio demographic characteristics of study participants.*

Wound site	Frequency (%)
Leg	67 (35.6)
Foot	39 (20.7)
Arm	26 (13.8)
Finger	18 (9.6)
Face	13 (6.9)
Scalp	12 (6.4)
Back	10 (5.4)
Chest and abdomen	3 (1.6)
Total	188 (100%)

Table 2: *Site of wound infection.*

Causes	Frequency (%)
Accident	92 (48.9)
Operation (Surgical)	46 (24.5)
Unknown causes	26 (13.8)
Burn	17 (9.1)
Animal bites	7 (3.7)
Total	188 (100)

Table 3: *Causes of wounds infection.*

Isolated bacterial profile

Out of the 188 swabs taken 162 (86.2%) were culture positive for bacterial pathogens, while 26 (13.8%) culture showed no growth. Out of 162 positive samples, 9 (5.5%) were mixed infections and a total of 171 bacterial isolates were identified. Among the isolates, *Staphylococcus aureus* 96 (56.1%) was predominant. *Klebsiella pneumoniae* 26 (13.8%) was the most frequently isolated Gram-negative bacteria followed by *E. coli* 23 (12.2%). *Pseudomonas aeruginosa* (3.5%) and only one *Citrobacter* spp. (0.6%) was isolated. In our study, there were a total of 9 mixed bacteria isolated. The proportion of each bacterial isolate to the total isolates and Percentage frequency of mixed bacterial isolates from wound infection is presented in table 4.

Bacterial isolates	Frequency	Percentage
Staphylococcus aureus	96	56.1
Klebsiella pneumonia	26	15.2
Escherichia coli	23	13.4
Proteus vulgaris	11	6.4
Proteus mirabilis	8	4.7
Pseudomonas aeruginosa	6	3.5
Citrobacter spp.	1	0.6
Total	171	100
Mixed culture		
S. aureus and *E. coli*	3	33.3
S. aureus and *K. pneumoniae*	3	33.3
E. coli and *P. vulgaris*	3	33.3

Table 4: *Distribution and frequency of bacterial isolated from wound infection.*

Antimicrobial susceptibility patterns of different bacterial isolates

Antibiotic susceptibility of the isolated organisms was determined by standard Kirby-Bauer disk diffusion method. *Staphylococcus aureus* exhibited highest sensitivity against Clindamycin (95.8%), Gentamycin (94.8%), Chloramphenicol (92.7%), Ciprofloxacin (89.6%) and Cotrimoxazole (84%). In this study, *S. aureus* showed resistance for Penicillin (66.7%) and Tetracycline (46%) only.

Among Gram-negative isolates, *E. coli, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa* and *Citrobacter* showed the highest sensitivity against Amikacin (100 %) and for *K.pneumoniae* Amikacin showed 96.2% sensitivity. *E. coli* showed high resistance for Ampicillin (95.7%) and Augmentin (91.3%) whereas *P. vulgaris* showed 100% resistance for Ampicillin and 90.9% for Tetracycline (Table 5).

Multi-drug Resistance pattern

The higher rate of MDR (100%) was seen among *Citrobacter* spp., *P. mirabilis,* and *E. coli* but the lower rate of (20%) MDR isolates seen among *P. aeruginosa.*

Isolated Organisms	AST	Antibiotics													
		P	E	DA	CIP	GEN	CHL	COT	TE	AMK	CRO	CAZ	CFP	AMP	AUG
S. aureus (n = 96)	S	26.0	67.7	95.8	89.6	94.8	92.7	84.0	45.8	-	-	-	-	-	-
	I	7.3	6.3	1.1	2.1	1.0	1.0	0	7.3	-	-	-	-	-	-
	R	66.7	26.0	3.1	8.3	4.2	6.3	16.0	46.9	-	-	-	-	-	-
E .coli (n = 23)	S				73.9	69.6	73.9	43.5	8.7	100	65.2	78.3	78.3	0	4.3
	I				4.4	4.3	0	0	8.7	0	0	4.3	0	4.3	4.3
	R				21.7	26.1	26.1	56.5	86	0	34.8	17.4	21.7	95.7	91.3
P. vulgaris (n = 11)	S				72.7	63.6	45.5	54.5	9.1	100	30.0	70.0	90.0	0	20.0
	I				-	9.1	10.0	0	0	0	0	0	0	0	30.0
	R				27.3	27.3	45.5	45.5	90.9	0	70.0	30.0	10.0	100	50.0
P. mirabilis (n = 8)	S				62.5	62.5	25.0	25.0	0	100	50.0	62.5	62.5	12.5	25.0
	I				0	0	0	12.5	25.0	0	0	0	0	0	25.0
	R				37.5	37.5	75.0	62.5	75.0	0	50.0	37.5	37.5	87.5	50.0
K. pneumoniae	S				77.0	65.4	57.7	57.7	19.2	96.2	61.5	73.1	73.1	7.7	11.5
	I				3.8	7.7	7.7	3.8	27.0	3.8	0	0	0	0	84.7
	R				19.2	26.9	34.6	38.5	53.8	0	38.5	26.9	26.9	92.3	3.8
P. aeruginosa (6)	S				83.3	83.3	-	-	-	100	-	83.3	100	-	-
	I				16.7	0	-	-	-	0	-	0	-	-	-
	R				0	16.7	-	-	-	0	-	16.7	-	-	-
Citrobacter spp. (n = 1)	S				0	0	0	0	0	100	0	0	0	0	0
	I				0	0	0	0	0	0	0	0	100	0	0
	R				100	100	100	100	100	0	100	100	0	100	100

Table 5: *Percentage of antimicrobial susceptibility pattern of bacteria isolated from wound infections.*

Key: P: Penicillin; E: Erythromycin; DA: Clindamycin; CIP: Ciprofloxacin; GEN: Gentamycin; CHL: Chloramphenicol; COT: Cotrimoxazole; TE: Tetracycline; AMK: Amikacin; CRO: Ceftriaxone; CAZ: Ceftazidime; CFP: Cefepime; AMP: Ampicillin; AUG: Augmentin; S: Sensitive; I: Intermediate; R: Resistance AST: Antimicrobial Susceptibility Testing

	No. (%) of resistance							
Bacterial isolates	**R0**	**R1**	**R2**	**R3**	**R4**	**R5**	**R6-10**	**MDR**
S. aureus (n = 96)	15 (15.6)	34 (35.4)	25 (26.0)	12 (12.5)	3 (3.1)	4 (4.2)	3 (1.6)	47 (83.9)
K. *pneumoniae* (n = 26)	-	3 (11.5)	5 (19.2)	5 (19.2)	-	3 (11.5)	10 (38.5)	23 (88.4)
E. *coli* (n = 23)	-	-	3 (13.0)	7 (30.4)	3 (13.0)	3 (13.0	7 (30.4)	23 (100)
P. *vulgaris* (11)		1 (9.1)	2 (18.2)	1 (9.1)	2 (8.2)	-	5 (45.5)	10 (90.9)
P. *mirabilis* (n = 8)	-	-	1 (12.5)	1 (12.5)	2 (25.0)	-	4 (50.0)	8 (100)
P. *aeruginosa* (6)	5 (83.3)	-	1 (16.7)	-	-	-	-	1 (20)
Citrobacter spp. (n = 1)	-	-	-	-	-	-	1 (100)	1 (100)
Total	20 (7.3)	38 (22)	37 (13.6)	26 (9.5)	10 (5.8)	10 (5.8)	30 (17.5)	113 (66.1)

Table 6: *Multi-drug resistance pattern of bacteria isolated from patients with infected wounds.*

Key: *R0: No Resistance to Antibiotic; R1: Resistance to 1 Antibiotics; R2: Resistance to 2 Antibiotics; R3: Resistance to 3 Antibiotics; R4: Resistance to 4 Antibiotics; R5: Resistance to 5 antibiotic; R 6-10: Resistance to 6-10 Antibiotics*

Discussion

Of the 188 clinical samples collected from patients with cases of wound infections, bacteria have been identified in 162 patients giving an isolation rate of 86.2%. Though the prevalence rate of wound infections in the present study was within the reported range, it was relatively the same prevalence rates of 87.3%, and 70.5% reported in similar studies conducted in Southwest and North East Ethiopia; respectively [5,7]. This study also has similar prevalence rate with studies conducted in Nepal, Nigeria and India at the rate of 80%, 82% and 89.5 % respectively [15,16,18]. Both local and abroad studies showed similar bacterial isolates in the range of 70.5% to 89.5%, this shows similarity may be due to following Standard operating procedures strictly for bacterial isolation.

The type and the relative frequencies of bacteria causing wound infections vary greatly among studies. In the present study, among 171 bacterial isolates, 96 (56.1%) were Gram-positive, i.e. *S. aureus* and 75 (43.9%) were Gram-negative. Among Gram-negative bacterial isolated, *K. pneumoniae* was found in 26 patients (15.2%), *E. coli* in 23 (13.4%) and *P. vulgaris* 11 (6.4%) patients. In this study, *S. aureus* and *K. pneumoniae* were the major bacteria associated with wound infection. The same has been reported by Araya G., *et al.* [32], Esebelalie., *et al* [33]. *E. coli* as a third predominant isolate following S. aureus and K. pneumoniae has been documented by Mama M., *et al.* and Shriyan., *et a*l [4,5].

The same study conducted in Ethiopia had shown *E. coli* as the first most prevalent. However, in our study, *K. pneumoniae* 26 (15.2%) was the predominant Gram-negative bacteria. Variation in the distribution of microbial agents between different geographical locations and regions within the same country may be responsible for this diversity. The possible reason for the high frequency of *S. aureus* is that this bacteria commonly found in human skin as normal flora. Wherever this bacterium gets breaks on skins and soft tissue they can easily disseminate. Cross-contamination of the wound from nasal colonization by *S. aureus* could be one possible explanation for high isolation rate of *S. aureus*. The importance of preventing cross-contamination in hospital environments was well explained by Onwubiko., *et al* [17]. In our study, *P. aeruginosa* was among the least isolated bacteria and this might be due to only 17 (9.0%) of wound swabs were taken from burn patients whereas; it was highly prevalent in most other studies [7,21,34].

The prevalence rate of mixed infections (5.3%) observed in this study was lower than 34.6% reported in the previous study by Anil., *et al* [21]. This may be due to the difference in identification methods that are known to influence the relative prevalence of bacteria which makes the comparison of results difficult.

Based on CLSI guideline 2016 we have used selected drugs for *Pseudomonas aeruginosa* which were available in the study area during the study period. Among drugs guided by CLSI 2016, we have utilized Gentamycin, Ciprofloxacin, Ceftazidime, Amikacin and Cefepime. In the present study, *P. aeruginosa* showed high sensitivity for most drugs, 100% for Amikacin and Cefepime, 83.3% for Gentamycin, Ciprofloxacin and Ceftazidime. There was no resistance bacterium isolated in our study for selected drugs. The relatively low level of resistance to these drugs may be, these drugs had been in the market for a relatively low availability most of the time as compared to drugs such as tetracycline, ampicillin and erythromycin. Our result was similar with a study conducted in Jima, Ethiopia by Mama., *et al.* [5] but not concurrent with results documented in Nepal by Anil., *et al.* and Salu Rai [20,38], this might be due to variation in geographical location and drug consumption trend.

Given that the majority of therapy for wound infections is empiric and that bacteria associated with wound infections are demonstrating increasing anti-microbial resistance, continuously updated data on antimicrobial susceptibility patterns would be beneficial to guide empiric treatment. In our study, both Gram-positive and Gram-negative bacteria were tested for drug susceptibility against a panel of eight drugs for Gram-positive and eleven drugs for Gram-negative bacteria. The number of drugs tested against bacteria isolated from wound infections in the present study was more or less the same number and family of drugs tested in previous studies in Ethiopia [5,7,34,35]. This may play a vital role to identify if there is a shift in a drug resistance pattern for the similar drugs used in previous studies.

The overall drug resistance rates of Gram-negative bacteria isolates ranged from 3.8% for Augmentin and 10% for Cefepime to 100% for Ampicillin, and 90% for Tetracycline. This figure demonstrates that Ampicillin and Tetracycline as a single agent for empirical treatment of wound infections would not cover the majority of wounds infected by Gram-negative bacteria in the study area. High level of drug resistance to Ampicillin and Tetracycline in the present study was compatible with results of similar studies conducted locally [5,7,35,36] and from abroad [21,23,24]. Availability of these anti-microbial agents without a prescription and inappropriate dosing schedules may explain the isolation of high level of drug resistance against these drugs and other drugs such as Penicillin.

S. aureus showed an average resistance rate of 22.2% to most of the antimicrobial drugs tested which is relatively similar with previous studies done locally by Mama., et al. and Mulugeta., *et al* [5,7]. In the present study, *S. aureus* showed high resistance for Penicillin 66.7% and this is concurrent with the study conducted locally by Hailu., *et al.* [33] but lower than a study conducted by Mama., *et al* [5]. A study conducted in Nigeria by Onwubiko N., *et al*. [17], the resistance rate of Penicillin for *S. aureus* was very low, i.e. 7.1 % only. From various drugs used in our study for *S. aureus, Clindamycin* (95.8%), Gentamycin (94.8%), Chloramphenicol (92.7%) and Ciprofloxacin (89.6%) showed high sensitivity. This finding has an agreement with a study conducted locally in Jimma and Dessie by Mama., *et al*. and Mulugeta., *et al* [5,7]. It has also shown an agreement with a study conducted in Karnataka by Kaup., *et al* [6]. In the current study, Tetracycline (49.6%) showed slightly high resistance which was lower than the study conducted by Mulugeta K., *et al*. [7] but it was similar with the study conducted in Karnataka by Kaua., *et al* [6].

K. pneumoniae, the first most common Gram-negative bacterium isolate was sensitive to Amikacin (96.2%) and Ciprofloxacin (77.0%) and was intermediate for Augmentin (84.7%). The average resistance rate for this isolate was 44.2% and it was comparable with the results documented from previous studies by Derese., *et al*. and Zarrin., *et al* [36,37].

The second most common Gram-negative isolate in our study was *E. coli* 23 (13.4%). It was highly sensitive for Amikacin (100%) and this result is the same with the study conducted by Mama., *et al.* [5] and showed low resistance to Gentamycin (3.2%), Ciprofloxacin (2.7%), Cefepime (2.7%), Ceftazidime (2.1%), and Ceftriaxone (4.3%). High resistance was observed for Ampicillin (95.7%), Augmentin (91.3%) and Tetracycline (86%). This resistance rate observed in our study was similar with a study conducted in Southwest- Ethiopia and South India [5,37].

P. vulgaris and *P. mirabilis* showed high sensitivity for Amikacin (100%). Both isolates showed sensitivity for Cefepime, 90%, and 62.5% respectively. *P. vulgaris* showed high resistance for Ampicillin 100% and Tetracycline (90%) whereas *P. mirabilis* showed a resistance rate of 87.5% and 75% for Ampicillin and Tetracycline respectively. These results were comparable with various studies conducted in Addis Ababa, Jimma, Mekele, India [5,13,19,35].

Overall MDR rate of isolated bacteria in this study was 66.1%. This finding was similar with MDR rate reported by Mulugeta., *et al.* [7] but lower than 95.5%, 85 %, 82.1% resistance rate reported by Mulu., *et al*, Mama., *et al*. and Sewunet., *et al.* respectively [5,27,34] and Mohammad., *et al*. in Nepal [16].

Conclusion

High culture positivity rate of wound infections reported in the present study initiates many similar studies to be conducted on wound infection in the country. High level of drug resistance to the commonly prescribed drugs dictates a search for better choices.

Conflict of Interests

The work does not have any financial and/or non-financial competing interest. The authors declare that there is no conflict of interests regarding the publication of this paper. The study was approved by Department Ethics and Research Committee (DERC) of Addis Ababa University (AAU), College of Health Sciences (COHS), Department of Medical Laboratory Sciences. Permission was also obtained from ALERT Center for data collection.

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