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Research Article

THE BACTERIAL PROFILE OF THE POSTOPERATIVE WOUND INFECTION IN A SOUTH INDIAN HOSPITAL

KB. Jnaneshwara[1], R. Eshwar Singh[1], R. Lava[1] and BV. Murlimanju[2]*

[1]Department of Microbiology, J.J.M. Medical College, Davangere, Karnataka, India.
[2]Department of Anatomy, Kasturba Medical College, Mangalore, Manipal University, Manipal, Karnataka,India.

ABSTRACT

Aim: Postoperative wound infection is a severe problem in the surgical specialties, which can cause mortality, morbidity and economic burden. Objectives of the present study were to study the frequency of various types of bacteria and their antibiotic susceptibility in the postoperative infected wounds at a South Indian hospital. **Methods:** The present study included one hundred cases of samples of infected postoperative wounds which were subjected to the culture and sensitivity. The bacteria were diagnosed by the standard microbiological methods. **Results:** The present study observed that there were about 111 bacteria which were isolated from the postoperative wound samples. Among them, 32.4% were gram positive cocci and the remaining 67.6% were gram negative bacilli. The most common bacteria isolated was E. coli (24.3%) followed by the S. aureus (21.6%) and Pseudomonas spp (20.6%). All among the gram positive cocci showed uniform susceptibility to the vancomycin. The E. coli showed maximum susceptibility to amikacin and chloramphenicol. Ampicillin was the most ineffective drug against the gram negative bacilli. Klebsiellaspp displayed maximum resistance to the antibiotics including aminoglycosides, fluoroquinolones and third generation cephalosporins. Pseudomonas spp exhibited maximum susceptibility to polymyxin B. C**onclusion:** We suggest that it is advisable to scrutinize the postoperative wound infection in each and every hospital to evolve the control strategies. Using the suitable antibiotics, taking aseptic precautions and keeping good hygiene have to be strictly applied to all the surgical cases to reduce the postoperative wound infections.

Keywords: Postoperative wound, E. coli, Antimicrobial susceptibility, Multidrug resistance.

1. INTRODUCTION

The infections which occur during the hospital staywerenot present orin an incubating stage,at the time of admission to the hospital,are considered as nosocomial. They are hospital acquired and cause financial burden to the patients. It has been reported that the estimated cost of management of nosocomial infections is about 4.5 billion dollars and in spite of that the death frequency is about 88,000 every year in the United States.[1]The infection of operation wounds are most likelyhospital acquired and constitute about 1/5th of all the nosocomial infections.The operation wound infections are the second common hospital acquired infections after the urinary system infections.[2]Theoperation wound can get infected either by a bacteria, which may be found in the operatingroom or by abacterial flora, which are found in the patient's own body.[3]Sometimes the hand becomes the greatest danger of infection as it comes in contact with the tissues much more frequently than the instruments.[4]It was hundred years ago, the Streptococcus was the most frequent pathogen encountered in the surgical wounds. Twenty years ago the Staphylococcus was the main organism.These days, the gram negative bacilli have replaced the Staphylococcus.[3]

The wound infection and other postoperative infections continue to be a problem even though the antibiotics have reduced their frequency. This is because of the widespread usage of the antibioticswhich has developed the resistant bacterial types.Inspite of the better advances made in the aseptic precautions, antimicrobial agents, sterilization and operation techniques, the postoperative wound infection still continues to be the major problem in the surgical specialties. This would lead tolengthy hospital stay,[5]expensive treatment, disability and death,which is due to the surgical management. In this context, isolation of the microorganism and studying its antimicrobial profile helps in the better management of postoperative wound infection. The department of microbiology serves very vital function in diagnosingand treating thehospital acquired infections, which will guide the physician in regard to choose the accurate antimicrobial. Due to all these implications, the presentinvestigation was performed to isolate and study the antimicrobial susceptibility of various isolates.The goal of the present investigation was to study the frequency of different bacterial types and susceptibility pattern to the antibiotics in the postoperative infected wounds in a South Indian hospital.

2. MATERIALS AND METHODS

The present study was performed at the microbiologydepartment of our institution.The present study included a total of 100 cases of postoperative wound infection. Among them 54 were from men and 46 were from women. The age group of the patients ranged between 3- 84 years. Most of the patients were in the age group of 16 through 30 years (45%), 25% was 31 through 45 years and 46-60 years (16%) in that order. The cases were from the teaching hospital of our institution which is located in South India.The wound infections other than the postoperative cases and the cases involving endoscopic procedures,incision and drainage of abscess, urethral dilations, slough excision and split skin graft were excluded from the present study. The patient should have a sutured wound in order to be considered in the present investigation. The samples were collected from the depth of the wound with strict aseptic precautions with the help of dry sterile cotton swab sticks for bacteriological examination.[6]Two culture swabs from the each sample were obtained, one for the direct smear study and the other for aerobic culture which was immediately sent to the laboratory for investigation.

On day 1, direct microscopic examination was done by gram stain to look for pus cells and the bacteria.The first swab was used for making smear by rolling the swab stick on a clean glass slide, which was heat fixed. Gram stained smear was examined under the microscope and the bacteria were segregated intococci and bacilli, gram positive orgram negative. This report was then correlated with the growth in the culture plates after 18-24 hours. The second swab was inoculated on sheep blood agar and MacConkey agar as well as nutrient broth and incubated at 37^{0}C for 18-24 hours under the aerobic conditions. The culture media were procured from Hi- Media laboratory and prepared as per manufacturer's recommendation as well as inputs from Mackie and McCartney.[7]

On day 2, identification of the growth was performed and themorphology of the colonies on the blood agar and McConkey agar were studied. Smear from the colonies were prepared and stained with gram stain and segregated into gram positive and negative after the microscopic examination. The bacteria were further microbiologically segregated by using the relevant biochemical and physiological tests.[8,9] The antibiotic susceptibility was studied by using the Kirby-Bauer disc diffusion method. Three to five isolated colonies of similar morphology were inoculated and the incubation was done for 2-8 hours, until the turbidity of the broth is matched.The antibiotic discs were placed on the agar plate within 15 minutes of inoculation by using a sterile needle and pressed firmly against the plate. The plates were inverted and incubated for 18-24 hours at 37^{0}C.

On the day 3, the final identification of the bacteria was made taking into account of the various biochemical and physiological tests. Antibiotic susceptibility pattern were reported by measuring the zone of inhibition with a millimeter scale. The antibiotic disc was reported as susceptible, intermediate and resistant, based on the criteria provided by NCCLS.[10] The catalase test was done to differentiate Staphylococcus (catalase positive) from Streptococcus and Enterococcus (both are

catalase negative). Enterococcus was identified presumptively by inoculating into 6.5% NaCl broth (salt tolerance) and observing the turbidity. If there was no turbidity, it was considered as Streptococcus. Enterococcus was further identified by heat tolerance (which is surviving at 60^{0}Cfor 30 minutes).
The identification of the gram negative bacilli was also performed. Oxidase positive isolates, which were suspected to be Pseudomonas spp, were further identified by positive Citrate utilization test. Oxidase negative isolates were presumptively identified as Acinetobacter and Enterobacteriaceae members. Acinetobacter was identified following an oxidative reaction or inert reaction when inoculated into O/F medium (Oxidative/ Fermentative medium). The Enterobacteriaceae was differentiated by lactose into lactose fermenters and non-lactosefermenters.Lactose fermenters were subjected to motility and IMViC test and production of H_2S was observed on T.S.I agar. The non-lactose fermenters were inoculated into urease medium. Proteus spp were presumptively identified by Urea hydrolysis and further confirmed by Phenyl alanine deaminase positive test.

3. RESULTS

In the 100 cases of the present study, about 111 bacteria were observed accounting for 1.1 bacteria per case as an average. All these 111 isolates were aerobes and facultative anaerobes. The grams stain of all direct smears correlated well with the growth on culture. Of these 111 isolates, 36 (32.4%) were gram positive cocci and 75 (67.6%) were gram negative bacilli. E. coli was isolated most frequently (24.3%) followed by S. aureus (21.6%), Pseudomonas spp (20.7%), Klebsiellaspp (13.5%) and Coagulase negative Staphylococci (7.2%). The patterns of bacteria isolated in the present study group are represented in Figure 1.Out of the 100 clinical samples, 85 samples (85%) yielded growth of single bacterium (monomicrobial/pure growth), 13 samples (13%) yielded growth of more than one bacterium (polymicrobial/mixture) and 2 samples (2%) yielded no growth. Staphylococcus Aureus was the most common bacteria (91.7%) isolated in pure culture, which is followed by Pseudomonas sp (82.6%). In mixed growth the common bacteria isolated were E. coli (33.3%) and Klebsiellaspp (33.3%). Coagulase negative Staphylococci (100%) and Acinetobacter (100%) were isolated exclusively in pure culture. Enterococcus spp and Proteus spp were isolated equally in both pure growth and mixtures, while Citrobacterspp and Enterobacterspp both were isolated only in mixed cultures (50% each). The bacterial isolates in pure growth and mixtures in post-operative wound infection of the present study are represented in Table 1.
Out of the 100 clinical samples taken, 49 were from the abdomen, 45 were from limbs, 5 were from groin and 1 was from the neck region.The frequency of bacteria of postoperative wound infection in relation to site of operation is represented in Table 2. Out of 111 isolates obtained from postoperative wound infection, 58 isolates were found to be from the abdominal surgery (52.2%) followed by 47 isolates from limb surgery (42.3%).S. aureus was resistant to penicillin in 95.8% isolates,ciprofloxacin (91.7%), cephalexin (79.2%) and erythromycin (79.2%). Susceptibility of S. aureus was 100% to Vancomycin followed by Cloxacillin (79.2%) and Amikacin (50%).Among Enterococcus spp, resistance to Penicillin was 100% followed by Cloxacillin (75%). All of themexhibited the susceptibilityto Vancomycin. The susceptibility pattern of gram positive cocci has been represented in Table 3. Susceptibility of E. coli was more to Amikacin (63%) and Chloramphenicol (59.3%), but resistance was highest to Ampicillin (96.3%) followed by Cotrimoxazole (85.2%), Sparfloxacin (81.5%), Ciprofloxacin (77.8%), Gentamicin (77.8%), Cephotaxime (77.8%) and Ceftazidime (74.1%).Klebsiellaspp showed greater resistance to almost all the antibiotics. The resistance was 100% to Ampicillin followed by Cotrimoxazole (93.3%), Gentamicin (86.7%), Sparfloxacin (86.7%), Ciprofloxacin (80%), Cephotaxime (73.3%), Ceftazidime (73.3%), Chloramphenicol (66.7%) and Amikacin (66.7%). The susceptibility patterns of gram negative bacilli are given in table nos. 4 &5. The Pseudomonas spp showed maximum susceptibility to Polymyxin B (87%) followed by Amikacin (56.5%), Ceftazidime (52.2%), Piperacilin (52.2%) and Carbenicillin (39.1%). The susceptibility pattern of Pseudomonas spp. is given in table no 6.

DISCUSSION

There are several predisposing causesto the emergence of postoperative infected wounds.The patient characteristics which favor the postoperative wound infections include coincident remote site infections or colonization, use of systemic steroids, diabetes mellitus, and history of cigarette smoking, obesity and old age.

The poor nutrition, transfusion of certain blood products before and after the surgery, preoperative hospitalization can also add into it. The environmental factors can prevent phagocytic cells from functioning efficiently by lowering tissue oxygen tension (PO_2). The lowered PO_2 inhibits the phagocytosis and will enhance the growth of anaerobic microorganisms.[11]For the most nosocomial wound infections, the endogenous flora of the patient which is present in the body surface and the visceramay become the source of infection.[12]The operating surgeon can decrease the chances of postoperative wound infectionby using the drains appropriately, avoiding excessive cautery and not performing intestinal anastomoses if there is any possibility of ischemia.

Perhaps Streptococcus pyogens wasthe most important cause of the hospital acquired infectionsearlier is now hardly ever encountered as it is highly susceptible to the antibiotics. Tetanus spores can survive in the dust for a very long time. Hospital acquired tetanus is usually a result of faulty sterilization techniques or other lapses in asepsis.[13] It has been reported that the balance between the host immunity and bacterial virulence has been altereddue to the use of higher antibacterial agents.[14]

In the present study,the gram negative bacillary infections (67.6%) had a high prevalence than those due to gram positive (32.4%) in the postoperative wound infections.This predominance of gram negative bacteria in the postoperative wound infection in the presentinvestigation is in conformity with the findings of Agarwal et al.[15]and Anvikar et al.[3]E.coliwas the commonly isolated strain in the presentinvestigation, accounting for 24.3% of all the isolates. The present surgery observed that 22 out of 27 isolates of E.coli were from the postoperative wound infection following an abdominal surgery. S. Aureus was the next accounting for 21.6% of all the isolates. Among them 24 isolates of S. Aureus were isolated from the limb surgery. Agarwal et al.[15]described the predominance of E.coli (31.31%) followed by the S. Aureus (29.29%) in the postoperative wound infections. The pseudomonas species was accounting for 20.7% of all the isolates. This is similar to Anvikar et al.,[3] in their study the isolation of pseudomonas spp. was 25%. But the isolation of Klebsiella spp. in the present study (13.5%) is lesser compared to Anvikaret al.[3]which showed Klebsiella spp. (28.8%) as the emerging hospital acquired pathogen.

Amikacin, cloxacillin and vancomycin were the most effective antimicrobial agents against the gram positive cocci, in the present investigation. The higher susceptibility of S.aureus to the cloxacillinwas 79.2% in the present study, which is similar to that of Kowli et al.,[16]where it was 76%. The rapid emergence of S.aureus resistant to variety of antibiotics is of a great concern. The resistance to Penicillin is 95.8% followed by Ciprofloxacin (91.7%), Cephalexin (79.2%), Erythromycin (79.2%) and Gentamicin (70.8%). This may be due to the increasing use of betalactam antibiotics and Gentamicin. Lack of antibiotic policy is one more probable contributing factor. Amikacin showed maximum efficacy against E.coli (63%) followed by Chloramphenicol (59.3%), while resistance to 3rd generation cephalosporin ranged from 74.1% to 77.8%. The values of the present study are similar with the observations of Arya M et al.[17]These findings of the present study are similar to that of Anvikar et al.[3]Against Pseudomonas spp., Polymyxin B showed maximum sensitivity of 87% followed by Amikacin (56.5%). These data are lesser compared to that of Kumar AP et al.[18]in which sensitivity to Polymyxin B was 100%.The present study observed the less effectiveness of the Fluoroquinolones. The resistance to Sparfloxacin and Ciprofloxacin were 91.3% and 65.2% respectively. Resistance to Cephotaxime was 87%.These observations of resistance pattern to many antibiotics aresimilar to the findings ofArya M et al.[17]

Table 1: Distribution of the bacterial isolates (n=111) in pure growthand mixtures in postoperative wound infection

Bacteria (n=111)	Pure (n1=85)	Mixture (n2=26)
E. coli (n=27)	18 (66.7%)	9 (33.3%)
S. aureus (n=24)	22 (91.7%)	2 (8.3%)
Pseudomonas spp (n=23)	19 (82.6%)	4 (17.4%)
Klebsiellaspp (n=15)	10 (66.7%)	5 (33.3%)
*CONS (n=8)	8 (100%)	Nil
Enterococcus spp (n=4)	2 (50%)	2 (50%)
Acinetobacterspp (n=4)	4 (100%)	Nil
Proteus spp (n=4)	2 (50%)	2 (50%)
Citrobacterspp (n=1)	Nil	1 (100%)
Enterobacterspp (n=1)	Nil	1 (100%)

*CONS-Coagulase negative Staphylococci

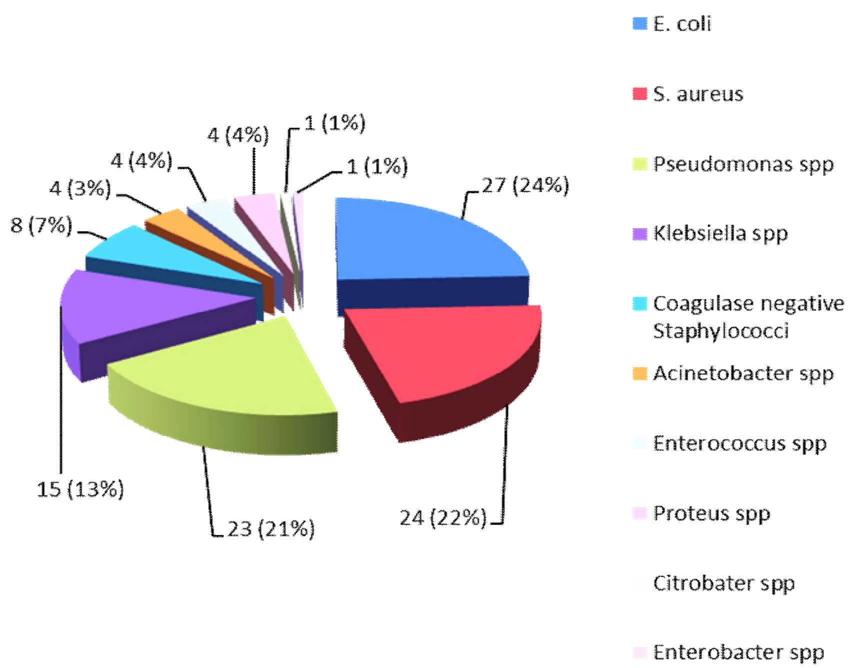

Fig. 1: Showing the frequency of bacterial isolation in postoperative wound infection in the present study (n = 111).

Table 2:Bacteriology of the postoperative wound infection in relation to site of operation

Bacteria	abdomen (n=58, 52.25%)	limbs (n=47, 42.34%)	groin (n=5, 4.5%)	neck (n=1, 0.9%)
E. coli (n = 27)	22	3	2	-
S. aureus (n = 24)	9	13	2	-
Pseudomonas sp (n = 23)	11	11	-	1
Klebsiellasp (n = 15)	5	10	-	-
Coagulase negative Staphylococci (n=8)	3	4	1	-
Enterococcus sp (n = 4)	2	2	-	-
Acinetobactersp (n = 4)	2	2	-	-
Proteus spp (n = 4)	2	2	-	-
Citrobactersp (n = 1)	1	-	-	-
Enterobactersp (n = 1)	1	-	-	-

Table 3: Susceptibility pattern of gram positive cocci in postoperative wound infection

	S. Aureus (n=24)		***CONS (n=8)**		**Enterococcus spp. (n=4)**		
Susceptibility	S	R	S	R	S	R	
penicillin	1 (4.3%)	23 (95.7%)	2 (25%)	6 (75%)	0 (0%)	4 (100%)	
cloxacillin	19 (79.2%)	5 (20.8%)	4 (50%)	4 (50%)	3 (75%)	1 (25%)	
erythromycin	5 (20.8%)	19 (79.2%)	3 (37.5%)	5 (62.5%)	2 (50%)	2 (50%)	
cephalexin	5 (20.8%)	19 (79.2%)	1 (12.5%)	7 (87.5%)	2 (50%)	2 (50%)	
amikacin	12 (50%)	12 (50%)	5 (62.5%)	3 (37.5%)	2 (50%)	2 (50%)	
gentamicin	7 (29.2%)	17 (70.8%)	2 (25%)	6 (75%)	1 (25%)	3 (75%)	
ciprofloxacin	2 (8.3%)	22 (91.7%)	2 (25%)	6 (75%)	2 (50%)	2 (50%)	
cotrimoxazole	7 (29.2%)	17 (70.8%)	0 (0%)	8 (100%)	2 (50%)	2 (50%)	
vancomycin	24 (100%)	0 (0%)	8 (100%)	0 (0%)	4 (100%)	0 (0%)	

*CONS-Coagulase negative Staphylococci; S-Susceptible; R-Resistant

Table 4: Susceptibility patterns of gram negative bacilli (E. coli, Klebsiella sp. and Proteus sp.) in postoperative wound infection

	E. coli (n=27)		Klebsiella spp. (n=15)		Proteus spp. (n=4)	
Susceptibility	**S**	**R**	**S**	**R**	**S**	**R**
Ampicillin	1 (3.7%)	26 (96.3%)	0 (0%)	15 (100%)	0 (0%)	4 (100%)
Cefotaxime	6 (22.2%)	21 (77.8%)	4 (26.7%)	11 (73.3%)	0 (0%)	4 (100%)
Ceftazidime	7 (25.9%)	20 (74.1%)	4 (26.7%)	11 (73.3%)	2 (50%)	2 (50%)
Amikacin	17 (63%)	10 (37%)	5 (33.3%)	10 (66.7%)	2 (50%)	2 (50%)
Gentamicin	6 (22.2%)	21 (77.8%)	2 (13.3%)	13 (86.7%)	1 (25%)	3 (75%)
Ciprofloxacin	6 (22.2%)	21 (77.8%)	3 (20%)	12(80%)	1 (25%)	3 (75%)
Sparfloxacin	5 (18.5%)	22 (81.5%)	2 (13.3%)	13 (86.7%)	1 (25%)	3 (75%)
Cotrimoxazole	4 (14.8%)	23 (85.2%)	1 (6.7%)	14 (93.3%)	0 (0%)	4 (100%)
Chloramphenicol	16 (59.3%)	11 (40.7%)	5 (33.3%)	10 (66.7%)	1 (25%)	3 (75%)

S-Susceptible; R-Resistant

Table 5: Susceptibility patterns of Acinetobacter sp., Citrobacter sp. andEnterobacter sp. (gram negative bacilli) in postoperative wound infection

	Acinetobacter sp. (n=4)		Citrobacter sp. (n=1)		Enterobacter sp. (n=1)	
Susceptibility	S	R	S	R	S	R
Ampicillin	0 (0%)	4 (100%)	0 (0%)	1 (100%)	1 (100%)	0 (0%)
Cefotaxime	2 (50%)	2 (50%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)
Ceftazidime	2 (50%)	2 (50%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)
Amikacin	3 (75%)	1 (25%)	0 (0%)	1 (100%)	1 (100%)	0 (0%)
Gentamicin	2 (50%)	2 (50%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)
Ciprofloxacin	1 (25%)	3 (75%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)
Sparfloxacin	1 (25%)	3 (75%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)
Cotrimoxazole	1 (25%)	3 (75%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)
Chloramphenicol	3 (75%)	1 (25%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)

S-Susceptible; R-Resistant

Table 6: Susceptibility pattern of Pseudomonas spp. in postoperative wound infection

	Pseudomonas spp. (n=23)	
Susceptibility	S	R
Cefotaxime	3 (13%)	20 (87%)
Ceftazidime	12 (52.2%)	11 (47.8%)
Amikacin	13 (56.5%)	10 (43.5%)
Gentamicin	8 (34.8%)	15 (65.2%)
Ciprofloxacin	2 (8.7%)	21 (91.3%)
Sparfloxacin	20 (87%)	3 (13%)
polymyxin B	9 (39.1%)	14 (60.9%)
Carbenicillin	12 (52.2%)	11 (47.8%)

S-Susceptible; R-Resistant

CONCLUSION

The postoperative wound infection is the commonest nosocomial infection only after the urinary tract infection. The present microbiological study has determined the bacteriology (aerobes and facultative anaerobes) of postoperative wound infection. The most common bacteria isolated in pure culture were S. aureus followed by Pseudomonas spp. The majority of E.coli isolates were from infections following abdominal surgery. The gram positive cocci showed uniform susceptibility to Vancomycin. Cloxacillin was the second best drug effective against gram positive cocci. S. aureus displayed maximum resistance against Penicillin, Ciprofloxacin and Erythromycin. E.coli showed maximum susceptibility to Amikacin and Chloramphenicol. Klebsiella spp. displayed maximum resistance to all the antibiotics, including Aminoglycosides, Fluoroquinolones and third generation Cephalosporins. Pseudomonas spp. exhibited maximum susceptibility to Polymyxin B and displayed maximum resistance to Sparfloxacin and Cephotaxime. We suggest that it is necessary to implement urgent measures for restriction of nosocomial infections. Judicious use of antibiotics, strict asepsis and proper hygiene should be applied. We believe that the data of the present study may provide useful guidelines for choosing the effective therapy against the isolates from postoperative infected wound. It is advisable to scrutinize the postoperative infected wound in each and every hospital to evolve the control strategies.

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