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Antibiotic Susceptibility Pattern of Bacterial Isolates from Patients of Respiratory Tract Infection at 43 Centers in Punjab, Pakistan

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Abstract

Respiratory tract infections are the most common diseases that are associated with social burden for the patient. In most cases, treatment is started before the culture sensitivity. Empiric therapy is based on symptoms and the medical practitioner's experience. Antibiotic resistance has become a serious health problem in the world, and also in Pakistan. This study was conducted to ascertain the current scenario of bacterial susceptibility in respiratory tract infection in order to optimize empiric therapy among people of all groups of ages in Punjab province located in Pakistan. Forty three (43) centers were selected randomly in major cities of Punjab. The numbers of patients investigated in these centers were 311. The study was undertaken from January 2012 to December, 2012. This period spans all seasons in the study areas. Out of 311 bacterial isolates, *Klebsiella* species have been isolated in 41 cases (13.06%), *Escherichia coli* 74 (23.57%), *Acinetobacter* 43 (13.69%), β-hemolytic *streptococcus* 15 (4.78%), *Pseudomonas* species 111 (35.35%), *Streptococcus pneumonia* 10 (3.18%) and *Staphylococcus aureus* in 17 cases (5.41%). Gram-positive bacteria were isolated from 42 patients (13.51%) while Gram-negative bacteria were isolated from 269 patients (86.49%). On an average 30 antibiotics of various classes have been tested for the susceptibility of gram-positive bacteria and gram-negative bacteria. 66.25% of the antibiotics tested showed susceptibility to the gram-positive bacteria. Vancomycin showed maximum sensitivity (100%) to gram-positive bacteria followed by Linezolid (97.44%) and Fusidic acid (83.34%). On the other hand 33.44% of the antibiotics tested showed sensitivity to the gram-negative bacteria. Cefoperazone/Sulbactam showed maximum sensitivity (91.39%) to gram-negative bacteria followed by Imipenem (72.75%) and Pipercillin/Tazobactam (71.60%).

Keywords: Respiratory tract; Antibiotics; Pneumonia; Bronchitis; Drug side effects

Introduction

Respiratory infections occur more often than they are reported, and are responsible for more duration of bed disability, restricted activity, stay away from job and school than the other reported acute illnesses. Respiratory tract infections account for more than 40% of days of disability secondary to acute illness and pneumonia and influenza are among ten leading causes of death in general population, and 80-90% of the deaths occur in the elderly (persons >65 years of age) [1,2]. The actual number is much higher because this figure does not include persons died of pneumonia, with other conditions (e.g., HIV, alcohol and tobacco-related diseases) [3].

Among bacterial infections, respiratory tract infections range from pharyngitis and otitis media to acute bronchitis and pneumonia. Respiratory tract infections are the most common diseases that are associated with social burden for the patient. In most cases, treatment is started before the culture sensitivity.

Financial influence of respiratory infections in 1999 was $25.6 billion [4]. These figures largely underestimate the economic impact of outpatient treatment of respiratory tract infections. Statistics reported by the National Health Interview Survey evaluate that 182 million incidents of respiratory tract infection occurs in which no medical attention is solved. Often, individuals with respiratory tract infections try over-the-counter medicines for relief of their symptoms, and only seek medical treatment when these efforts become useless. OTC medications in population, contribute towards the $456,000,000 annually to treat respiratory infections [3,4].

In Pakistan since July 29, 2010 to December 22, 2011, approximately 12,887,440 of acute respiratory infection cases are reported. Patient consultations have been reported in DEWS (Disease Early Warning System) from the flood affected districts four provinces in Pakistan which is about 23% of total consultations [5] as described in following Table 1.

Diseases	Total
Acute diarrhea	1,346,646 (8%)
Bloody diarrhea	84,324 (0.5%)
Acute Respiratory Infection	3,443,184 (20%)
S. Malaria	802,606 (5%)
Skin Diseases	775,032 (4%)
Unexplained fever	612,683 (3%)
Total (All consultations)	17,584,383

Table 1: Respiratory infection cases.

Antibiotic resistance has become a serious health problem in the world, and also in Pakistan. The United States Centre for Disease

Prevention and Control (CDC) calls antibiotic resistance one of its "top concerns". This is because over the last decade almost all types of bacteria have become less susceptible to antibiotic treatment when it is really needed.

Gram-negative organisms produce β-lactamases, and this is a major factor in the resistance of the semi synthetic broad-spectrum β-lactam antibiotics. Aminoglycosides are inactivated by phosphorylation, acetylation, adenylation and the necessary enzymes are found in both gram-negative and gram-positive plasmids and several on transposon. Plasmid-mediated modification of protein binding site on the 50S subunit is the basis of resistance to erythromycin, fluoroquinolones and decreased binding due to a point mutation in the DNA gyrase. Plasmid- mediated resistance to tetracyclines is an important example of reduced drug accumulation; these are also found in both gram-positive and gram-negative bacteria [6]. The resistance of *S. aureus* to erythromycin and other macrolides, and fluoroquinolones, is due to an energy-dependent efflux. Inhibitors such as pumps may be useful additional antibiotics [7]. Disturbing development of resistance has been found in the *Staphylococci*, one of the most common causes of hospital systemic infections, a number of species that are now resistant to almost all currently available antibiotics [8].

Nature has bestowed microorganism with devilishly effective adaptive mechanisms for putting down the best treatment strategies, and we try to remove them. This challenging situation has been reviewed thoroughly [9].

Dosing errors are common in antibiotics administration. Excessive dosage can cause significant side effects, while too little can lead to treatment failure, and lead to antibiotic resistance [10].

Antibiotic resistance is unavoidable and emerges mostly when a patient is given empiric therapy. This is mainly due to insufficient research work on antibiotic sensitivity patterns. The present study is an attempt to find out the current status of antibiotic sensitivity pattern of common bacterial isolates at Punjab level. The aim of this study is to optimize empiric therapy in Respiratory tract infections.

Methods

The study involved major cities of Punjab (Pakistan). Forty three (43) centers were selected randomly in major cities of Punjab. The patients of all groups of ages were included in study. The study aim and objectives were explained to all the participating patients through laboratory staff and it was assured that all the information obtained from them will be kept confidential. Based on this, consent and verbal approval granted. Similarly, the administrative heads of selected laboratory centers were assured that all information of patients will remain confidential. We therefore identified the laboratory centers in Punjab both in hospitals and in community. The numbers of patients investigated in these centers were 311. Selected laboratory centers were asked to collect specimens from patients suffering from respiratory tract infections. Specimens were as follows: Throat swab; Sputum; Bronchial washing; Tracheal secretion.

Specimens were enclosed within specialized containers. A transport medium (Amies Transport medium) was also present within these containers. These were recapped tightly. After collection, each specimen was sent to their central laboratory for culture sensitivity test. The samples were inoculated into sheep blood agar, MacConkey agar and chocolate agar medium and incubated at 37°C for 48 h. Antimicrobial susceptibility testing was performed using disc diffusion method.

$BaSO_4$ turbidity standard is used for inoculums which is equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension). The swab is inoculated into the dried surface of a Mueller-Hinton agar plate by streaking it over the entire sterile surface of agar medium. The anti-microbial discs are dispensed onto the surface of the inoculated agar plates by using automated disc dispenser. Pathogens were identified after 48 h of incubation [11]. Selected Laboratory centers strictly follow CLSI (Clinical and Laboratory Standards Institute) previously known as NCCLS (National Committee for Clinical Laboratory).

Only positive culture sensitivity reports were selected. The data was collected on properly designed Performa and also in the form of photocopies of original reports.

Statistics

Minimal statistics (only percentage and average) is applied in Microsoft Excel 2007 in data entry and result interpretation.

Results

In total 311 respiratory pathogens were isolated in Punjab during study period of January 2012 to December 2012. Seven types of bacteria were isolated. Out of 311 bacterial isolates, *Klebsiella* species have been isolated in 41 cases (13.06%), *Escherichia coli* 74 (23.57%), *Acinetobacter* 43 (13.69%), β-Hemolytic *Streptococcus* 15 (4.78%), *Pseudomonas* species 111 (35.35%), *Streptococcus pneumoniae* 10 (3.18%) and *Staphylococcus aureus* in 17 cases (5.41%). Gram-positive bacteria were isolated from 42 patients (13.51%) while Gram-negative bacteria were isolated from 269 patients (86.49%) (Table 2).

S. No.	Name of Bacterium	No. of Isolates	Percentage
Gram-positive			
1	*Streptococcus pneumoniae*	10	3.18%
2	*Staphylococcus aureus*	17	5.41%
3	B-Hemolytic *Streptococcus*	15	4.78%
	Total	42	13.51%
Gram-negative			
4	*Acinetobacter*	43	13.69%
5	*Pseudomonas aeruginosa*	111	35.35%
6	*Klebsiella* species	41	13.06%
7	*Escherichia coli*	74	23.57%
	Total	269	86.49%
	Grand Total	311	100%

Table 2: Prevalence of respiratory tract bacteria.

Generally patients of age more than 50 years are more susceptible to respiratory tract infections due to less immunity. Among the patients of age group less than 15 years β-hemolytic *Streptococcus* has highest

prevalence of 27% followed by *Staphylococcus aureus* (24%). While among the patients of age group 15-25 years Escherichia coli has maximum prevalence of 20% followed by *Pseudomonas aeruginosa* (14%). Among the patients of age group 25-50 years β-hemolytic *Streptococcus* has highest prevalence of 47% followed by *Pseudomonas aeruginosa*. While among the patients of age group more than 50 years, *Acinetobacter* has maximum prevalence of 65% followed by *Klebsiella* which has prevalence of 63% (Table 3).

S. No.	Name of Bacterium	<15 Years		15-25 Years		25-50 Years		>50 Years	
		No. of Patients	% age of Patients	No. of Patients	% age of Patients	No. of Patients	% age of Patients	No. of Patients	% age of Patients
1	*Klebsiella* species	4	10%	5	12%	6	15%	26	63%
2	*Escherichia coli*	13	18%	15	20%	18	24%	28	38%
3	*Acinetobacter*	---	---	6	14%	9	21%	28	65%
4	B-Hemolytic *Streptococcus*	4	27%	1	7%	7	47%	3	20%
5	*Pseudomonas aeruginosa*	8	7%	16	14%	36	32%	51	46%
6	*Streptococcus pneumoniae*	1	10%	1	10%	3	30%	5	50%
7	*Staphylococcus aureus*	4	24%	2	12%	3	18%	8	47%
	Total	34	11%	46	15%	82	26%	149	48%

Table 3: Prevalence of respiratory bacteria in different age groups. On an average 30 antibiotics were tested against 7 isolated respiratory pathogens.

Klebsiella species showed maximum susceptibility to Cefoperazone/ Sulbactam and Carbapenems. 100% of *Klebsiella* species were susceptible to Cefoperazone/ Sulbactam and more than 91% to Imipenem and Meropenem. *Escherichia coli* showed the maximum susceptibility to Carbapenems, Cefoperazone/Sulbactam and Pipercillin/Tazobactam. More than 94% of *Escherichia coli* were susceptible to Imipenem, Meropenem and Cefoperazone/Sulbactam. However 89.7% of *Escherichia coli* were susceptible to Pipercillin/ Tazobactam. *Acinetobacter* showed maximum susceptibility to Cefoperazone/Sulbactam. 84.38% of *Acinetobacter* were susceptible to Cefoperazone/Sulbactam. β-hemolytic *Streptococcus* showed maximum susceptibility to Ampicillin, Amoxicillin, Amoxicillin/ Clavulanic acid, Ampicillin/Sulbactam and Ceftazidime. 100% of β-hemolytic *Streptococcus* was susceptible to Ampicillin, Amoxicillin, Amoxicillin/Clavulanic acid, Ampicillin/Sulbactam and Ceftazidime. However 91.67% β-hemolytic *Streptococcus* were susceptible to Doxycycline. *Pseudomonas aeruginosa* showed maximum susceptibility to Pipercillin/tazobactam, Cefoperazone/Sulbactam and Carbapenems.

More than 85% of *Pseudomonas aeruginosa* were susceptible to Pipercillin/tazobactam, Cefoperazone/Sulbactam and Imipenem. However >80% of *Pseudomonas aeruginosa* were susceptible to Meropenem and Amikacin. *Streptococcus pneumoniae* showed maximum susceptibility to Penicillins, Cephalosporins, Carbapenems, Tigecycline and Vancomycin. 100% of *Streptococcus pneumoniae* were susceptible to Penicillins, Cephalosporins, Carbapenems, Tigecycline and Vancomycin. However 83.33% of *Streptococcus pneumoniae* were susceptible to Doxycycline. *Staphylococcus aureus* showed maximum susceptibility to Vancomycin, Linezolid and Fusidic acid as 100%, 92.31% and 83.33% respectively.

Twenty nine (29) antibiotics were tested against gram-positive respiratory pathogens. 66.25% of the antibiotics tested showed sensitivity to the gram-positive bacteria as shown in Table 4. Vancomycin showed maximum sensitivity (100%) to gram-positive bacteria followed by Linezolid (97.44%) and Fusidic acid (83.34%).

S. No.	Name of Antibiotic	Staphylococcus aureus	Streptococcus pneumoniae	β-hemolytic Streptococcus	Average percentage
1	Vancomycin	100.00%	100.00%	100.00%	100.00%
2	Linezolid	92.31%	100.00%	100.00%	97.44%
3	Fusidic acid	83.34%	66.67%	100.00%	83.34%
4	Cefipime	46.15%	100.00%	100.00%	82.05%
5	Cefoperazone	46.15%	100.00%	100.00%	82.05%
6	Cefotaxime	46.15%	100.00%	100.00%	82.05%

7	Cefuroxime	46.15%	100.00%	100.00%	82.05%
8	Amoxicillin/Clavulanic acid	40.00%	100.00%	100.00%	80.00%
9	Ceftazidime	40.00%	100.00%	100.00%	80.00%
10	Ceftriaxone	40.00%	100.00%	100.00%	80.00%
11	Cefaclor	40.00%	100.00%	100.00%	80.00%
12	Imipenem	40.00%	100.00%	100.00%	80.00%
13	Meropenem	40.00%	100.00%	100.00%	80.00%
14	Cephalexin	40.00%	100.00%	80.00%	73.33%
15	Cephadrine	40.00%	100.00%	80.00%	73.33%
16	Ampicillin/Sulbactam*	16.67%	100.00%	100.00%	72.22%
17	Amoxicillin*	13.33%	100.00%	100.00%	71.11%
18	Ampicillin*	13.33%	100.00%	100.00%	71.11%
19	Oxacillin	30.77%	100.00%	66.67%	65.81%
20	Doxycycline	20.00%	83.33%	91.67%	65.00%
21	Moxifloxacin	46.15%	25.00%	75.00%	48.72%
22	Erythromycin	30.77%	75.00%	38.46%	48.08%
23	Levofloxacin	40.00%	50.00%	30.77%	40.26%
24	Ofloxacin	40.00%	50.00%	30.76%	40.25%
25	Azithromycin*	0.00%	75.00%	38.46%	37.82%
26	Ciprofloxacin	40.00%	37.50%	30.77%	36.09%
27	Gentamicin*	40.00%	0.00%	66.67%	35.56%
28	Enoxacin*	33.33%	50.00%	11.11%	31.48%
29	Trimethoprim/ Sulphamethoxazole*	33.33%	25.00%	7.69%	22.01%
	Average	40.62%	80.60%	77.52%	66.25%

Table 4: Anti-bacterial susceptibility of gram-positive bacteria. *Antibiotic tested in less than 20% of either of Gram-positive isolates. All data is interpreted using (M100-S21 Vol. 31 No. 1) breakpoints.

Thirty four (34) antibiotics were tested against gram-negative respiratory pathogens. 33.44% of the antibiotics tested showed sensitivity to the gram-negative bacteria as shown in Table 5. Cefoperazone/Sulbactam showed maximum sensitivity (91.39%) to gram-negative bacteria followed by Imipenem (72.75%) and Pipercillin/Tazobactam (71.60%).

S. No.	Name of Antibiotic	Escherichia coli	Pseudomonas aeruginosa	Klebsiella species	Acinetobacter	Average percentage
1	Cefoperazone/Sulbactam	94.29%	86.92%	100.00%	84.38%	91.39%
2	Imipenem*	95.71%	86.11%	92.11%	17.07%	72.75%
3	Pipercillin/Tazobactam*	89.71%	89.90%	97.05%	9.76%	71.60%
4	Meropenem*	95.71%	82.08%	91.43%	17.07%	71.57%
5	Amikacin*	76.06%	80.00%	97.37%	9.76%	65.80%

6	Tobramycin*	47.14%	62.16%	68.42%	17.07%	48.70%
7	Gentamicin*	47.89%	66.67%	73.68%	2.50%	47.68%
8	Aztreonam*	36.00%	51.43%	88.89%	9.09%	46.35%
9	Cefipime*	35.21%	66.36%	80.00%	0.00%	45.39%
10	Ciprofloxacin*	33.80%	57.66%	71.05%	2.44%	41.24%
11	Levofloxacin*	30.99%	51.40%	71.43%	9.76%	40.89%
12	Moxifloxacin*	32.84%	53.09%	71.42%	3.13%	40.12%
13	Cefoperazone*	28.79%	55.85%	71.05%	0.00%	38.92%
14	Ofloxacin	28.57%	50.45%	71.05%	4.88%	38.74%
15	Ampicillin/Sulbactam*	22.22%	25.00%	100.00%	0.00%	36.81%
16	Ceftazidime	29.58%	29.73%	71.05%	2.44%	33.20%
17	Cefotaxime*	26.76%	29.09%	71.05%	0.00%	31.73%
18	Enoxacin*	0.00%	24.00%	100.00%	0.00%	31.00%
19	Cefixime*	0.00%	50.00%	71.43%	0.00%	30.36%
20	Ceftriaxone*	25.35%	20.91%	71.05%	0.00%	29.33%
21	Amoxicillin*	12.50%	100.00%	0.00%	0.00%	28.13%
22	Nitrofurantoin*	100.00%	0.00%	0.00%	0.00%	25.00%
23	Doxycycline*	12.86%	12.05%	32.43%	39.47%	24.20%
24	Cefuroxime*	19.72%	6.54%	67.57%	0.00%	23.46%
25	Trimethoprim/ Sulphamethoxazole*	20.00%	14.41%	42.86%	0.00%	19.32%
26	Amoxicillin/Clavulanic acid*	15.49%	12.50%	47.36%	0.00%	18.84%
27	Cefaclor*	11.27%	2.44%	54.05%	0.00%	16.94%
28	Cephalexin*	8.45%	2.22%	30.56%	0.00%	10.31%
29	Cephadrine*	8.45%	2.35%	25.00%	0.00%	8.95%
30	Ticarcilin/Clavulanic acid*	0.00%	20.00%	0.00%	0.00%	5.00%
31	Ampicillin*	12.50%	0.00%	0.00%	0.00%	3.13%
32	Minocyclin*	0.00%	0.00%	0.00%	0.00%	0.00%
33	Naladixic acid*	0.00%	0.00%	0.00%	0.00%	0.00%
34	Pipemedic Acid*	0.00%	0.00%	0.00%	0.00%	0.00%
	Average	32.29%	37.98%	56.75%	6.73%	33.44%

Table 5: Anti-bacterial susceptibility of gram-negative bacteria. *Antibiotic tested in less than 20% of either of Gram-negative isolates. All data is interpreted using (M100-S21 Vol. 31 No. 1) breakpoints.

Overall 42.23% of the bacterial isolates showed susceptibility to the antibiotics tested as shown in Table 6. Imipenem was tested against more than 25% of each of gram-positive and gram-negative isolates. Bacterial isolates showed highest susceptibility of 76.38% to Imipenem. Susceptibility to Meropenem, Cefipime was 75.79% and 63.72% respectively. Among orally available antibiotics, susceptibility to Moxifloxacin was 57.56%, but as the prevalence of gram-negative bacteria is more than gram-positive bacteria, Cefuroxime (52.75%) is best option for empiric therapy in respiratory tract infection which is cheaper than Moxifloxacin.

Antibacterial Susceptibility Pattern				
S. No.	**Name of Antibiotics**	**Percentage Sensitivity of Gram-negative Isolates**	**Percentage Sensitivity of Gram-positive Isolates**	**Percentage Sensitivity of all Isolates**
1	Imipenem	72.75%	80.00%	76.38%
2	Meropenem	71.57%	80.00%	75.79%
3	Cefipime	45.39%	82.05%	63.72%
4	Cefoperazone	38.92%	82.05%	60.49%
5	Doxycycline	24.20%	91.67%	57.93%
6	Moxifloxacin	40.12%	75.00%	57.56%
7	Gentamicin	47.68%	66.67%	57.18%
8	Cefotaxime	31.73%	82.05%	56.89%
9	Ceftazidime	33.20%	80.00%	56.60%
10	Ceftriaxone	29.33%	80.00%	54.66%
11	Ampicillin/Sulbactam	36.81%	72.22%	54.51%
12	Cefuroxime	23.46%	82.05%	52.75%
13	Vancomycin*	0.00%	100.00%	50.00%
14	Linezolid*	0.00%	100.00%	50.00%
15	Fusidic Acid*	0.00%	100.00%	50.00%
16	Amoxicillin	28.13%	71.11%	49.62%
17	Amoxicillin/Clavulanic acid*	18.84%	80.00%	49.42%
18	Cefaclor*	16.94%	80.00%	48.47%
19	Cefoperazone/Sulbactam*	91.39%	0.00%	45.70%
20	Cephalexin*	10.31%	73.33%	41.82%
21	Cephadrine*	8.95%	73.33%	41.14%
22	Ampicillin*	3.13%	71.11%	37.12%
23	Ciprofloxacin	41.24%	30.77%	36.00%
24	Levofloxacin	40.89%	30.77%	35.83%
25	Pipercillin/Tazobactam*	71.60%	0.00%	35.80%
26	Ofloxacin	38.74%	30.76%	34.75%
27	Oxacillin*	0.00%	65.81%	32.91%
28	Amikacin*	65.80%	0.00%	32.90%
29	Tobramycin*	48.70%	0.00%	24.35%
30	Aztreonam*	46.35%	0.00%	23.18%
31	Enoxacin*	31.00%	11.11%	21.06%
32	Azithromycin*	0.00%	38.46%	19.23%
33	Erythromycin*	0.00%	38.46%	19.23%
34	Cefixime*	30.36%	0.00%	15.18%

35	Trimethoprim/Sulphamethoxazole*	19.32%	7.69%	13.50%
36	Nitrofurantoin*	25.00%	0.00%	12.50%
37	Ticarcilin/Clavulanic acid*	5.00%	0.00%	2.50%
	Total	31.58%	52.88%	42.23%

Table 6: Anti-bacterial susceptibility pattern. *Antibiotic tested in less than 20% of either of Gram-positive and Gram-negative isolates. All data is interpreted using CLSI (M100-S21 Vol. 31 No. 1) breakpoints.

Discussion

Resistance is an extremely scary situation for both patients and health care workers. Drug side effects, allergic reactions and diarrheal infections result from inappropriate use of antibiotics. There is need of surveillance studies of antibiotic susceptibility at national level at regular intervals. Physician should be well aware of susceptibility patterns in a particular region before prescribing antibiotics. Empiric therapy should be based on most recent study.

The present study showed that certain antibiotics were more effective and cheaper than other antibiotics, e.g., in *Streptococcus pneumonia*, Amoxicillin and Ampicillin are effective and more economical than Vancomycin, Linezolid and Tigecycline.

Health care providers, hospital administrators and policy maker must work together in reducing unnecessary antibiotic use, emphasis and implement intervention system. Government of Pakistan should review their policies regarding rational use of antibiotics and restrict over the counter sale of antibiotics. Hygenicity and sterility of medical equipment should not be compromised at any cost.

From the data, the antibiotics having low susceptibility profile (i.e. Trimethoprim/Sulphamethoxazole) should not be prescribed (in empiric therapy) and dispensed; further the manufacturing should be hold for ample period of time to regain its susceptibility back. On the other side, the prescriber should prefer the antibiotics with good susceptibility profile (i.e., Imipenem).

Conclusion

This study was conducted to ascertain the current scenario of bacterial susceptibility in respiratory tract infection to optimize empiric therapy among people of all groups of ages in Punjab province located in Pakistan.

The present study concluded that Imipenem which is a broad spectrum antibiotic is most suitable as an empirical therapy in respiratory tract infections in hospital setting followed by Meropenem and in general practice Doxycycline is most effective as oral antibiotic followed by Moxifloxacin.

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