English title (font 22 times new Romans)

A Thesis

Submitted in Partial Fulfillment of the Requirements for the

Master degree

In Pharmaceutical Sciences
(specialty)

By

Student Name

Bachelor (or Master) of Pharmaceutical sciences, year
Teaching assistant, name of Department
Faculty of Pharmacy, Ain Shams University

Year

title

A Thesis

Submitted in Partial Fulfillment of the Requirements for the

Master (or philosophy) degree
In Pharmaceutical Sciences
(specialty)

By

Student name

Teaching Assistant, Microbiology and Immunology Department
Faculty of Pharmacy, Ain Shams University

Under Supervision of

Dr. Name, PhD
Professor of Microbiology and Immunology,
Faculty of Pharmacy, Ain Shams University

Dr. Name, PhD
Professor of Microbiology and Immunology,
Faculty of Pharmacy, Ain Shams University

Dr. Name, Ph.D
Associate Professor of Microbiology and Immunology,
Faculty of Pharmacy, Ain-Shams University

Year

Acknowledgments

First, I thank "**Allah**" for granting me the power to accomplish this work.

I would like to express my deepest thanks to **Prof. Dr. Name**, Professor of Microbiology and Immunology, and founder of the Microbiology and Immunology Department, Faculty of Pharmacy, Ain Shams University, for her valuable scientific supervision, constructive advice and continuous guidance throughout the work.

My deepest gratitude and appreciation are expressed to **Prof. Dr. Name**, Head of Microbiology and Immunology Department and Former Vice Dean for community service and environmental affairs, Faculty of Pharmacy, Ain Shams University, for his divine support and for kindly supplying the laboratory facilities whenever needed. His constructive criticism, guided me immensely throughout the work and during the revision of the thesis.

I am also greatly indebted to **Dr. Name**, Associate Professor of Microbiology and Immunology Department, Faculty of Pharmacy, Ain Shams University, for suggesting the topic of research and for providing continuous scientific supervision and follow up. His valuable time and big effort are greatly appreciated. I also thank him for providing guidance especially throughout the genetics part in this study.

I would also like to thank my dear **colleagues** and to all the **workers** at the Faculty of Pharmacy, Ain Shams University for their help and support during this work. Besides, I would like to thank all the **staff members** at Sadr Al-Abbasiyya Hospital, for their help during collection of specimens.

Finally, my deepest everlasting thanks and appreciation are for my beloved **parents** for their continuous support and encouragement throughout my life.

والحمد لله رب العالمين.....

Student Name

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List of Abbreviations

AAC(6')-Ib	aminoglycoside 6'-N-acetyltransferase type Ib enzyme
aac(6')-Ib	Gene coding for aminoglycoside 6'-N-acetyltransferase type Ib
aac(6')-Ib-cr	Gene coding for aminoglycoside 6'-N-acetyltransferase type Ib ciprofloxacin resistant variant
AC	Accession code
API	Analytical profile index
ATS	American Thoracic Society
BLAST	Basic Local Alignment Search Tool
bp	Base pair
CAP	Community-acquired pneumonia
cfu	Colony forming unit
CLSI	Clinical and Laboratory Standards Institute
CTX-M	CTX-M extended spectrum β-lactamase enzyme
ctx-m	Gene coding for CTX-M extended spectrum β-lactamase
DDST	Double disc synergy test
EDTA	Ethylene diaminetetraacetic acid
EMB	Eosin methylene blue
ESBL(s)	Extended spectrum β-lactamase(s)
ICU	Intensive care unit
IDSA	Infectious Diseases Society of America
LB	Luria Bertani

LRTI(s)	Lower respiratory tract infection(s)
MDR	Multiple drug resistant
MH	Mueller-Hinton
MIC	Minimum inhibitory concentration
NCBI	National Center for Biotechnology Information
OMP	Outer membrane protein
ORF	Open reading frame
PBP(s)	Penicillin binding protein(s)
PCR	Polymerase chain reaction
PER	*Pseudomonas* extended resistance
qnr	Gene coding for quinolone resistance
rpm	Round per minute
RTI(s)	Respiratory tract infection(s)
rRNA	Ribosomal ribonucleic acid
SDS	Sodium dodecyl sulphate
SHV	SHV extended spectrum β-lactamase enzyme
shv	Gene coding for SHV extended spectrum β-lactamase
SOB	Super optimal broth
SOC	Super optimal broth with catabolite repression
T_a	Annealing temperature
TAE	Tris-acetic acid-EDTA
TE	Tris-EDTA
TEM	TEM extended spectrum β-lactamase enzyme

tem	Gene coding for TEM extended spectrum β-lactamase
T_m	Melting temperature
Tris	Trishydroxymethylaminomethane
tRNA	Transfer ribonucleic acid
TSI	Triple sugar iron agar
URTI(s)	Upper respiratory tract infection(s)
VEB	Vietnamese extended spectrum β-lactamase

List of Figures

List of Tables

Abstract

(font 12, times new Romans)

In this study, …..

Abstract

Introduction

(font 12, times new Romans)

The goal of this study is to determine ……. Therefore, the protocol of the study included the following:

1.

2.

3.

4.

5.

Literature Review

1. Heading 1

1.1. heading 2

1.2. Epidemiology

Literature conclusion (4-5 scentences)

Aim of the study

So, the aim of this study was to elucidate (2-3 scententes.

Materials and Methods

Materials

1. Microorganisms

1.1. Clinical bacterial isolates

.

1.2. Standard bacterial strains

2. Chemicals

The different chemicals used in this study and their sources are listed in table 1.

Table 1 Chemicals used in this study and their sources

Name	Source
Acetic acid (glacial)	El-Nasr Chemicals Co. (ADWIC), Egypt
Agarose A	Bio Basic Inc, Canada
Barium chloride dihydrate	El-Nasr Chemicals Co. (ADWIC), Egypt

3. Antimicrobial agents

The antimicrobial agents incorporated in the growth culture medium to increase plasmid copies per bacterial cell and/or to recover the transformants of *E. coli* DH5α harboring plasmids are listed in table 2.

Table 2 Antimicrobial agents used in this study

Antimicrobial Agent	Dosage Form (Conc. or amount)	Source
Amikacin	Amikacin® vial (500 mg/ 2 ml)	Amoun, Cairo, Egypt
Ampicillin	Epicocillin® vial (1 g)	Eipico, Cairo, Egypt
Ceftriaxone	Ceftriaxone® vial (250 mg)	Sandoz, Cairo, Egypt
Ciprofloxacin	Ciprofloxacin® infusion (200 mg/ 100 ml)	Amriya Pharm IND, Alexandria, Egypt

3.1. Blood agar

Blood agar was prepared by aseptically adding sterile human blood to sterile molten nutrient agar adjusted at 50ºC to a final concentration of 10% (v/v) and poured into sterile petridishes. The human blood was obtained from the central blood bank of Al-Demerdash Hospital, Cairo, Egypt.

3.2. Chocolate agar

It was prepared as blood agar except that the medium was heated while it was gently swirled until the color becomes chocolate brown before being poured into sterile petridishes.

3.3. Luria Bertani (LB) broth

Tryptone	10.0 g
Yeast extract	5.0 g
NaCl	10.0 g
Distilled H_2O ad.	1000 ml

3.4. Luria Bertani (LB) agar

LB broth	1000 ml
Agar-agar	15.0 g

3.5. Super optimal broth (SOB) medium (Hanahan, 1983)

Tryptone	20.0 g

Yeast extract	5.0	g
NaCl	0.58	g
KCl	0.19	g
Distilled H_2O ad.	1000	ml

After autoclaving add:

$MgCl_2$ (1 M)	10.0	ml
$MgSO_4$ (1 M)	10.0	ml

3.6. Super optimal broth with catabolite repression (SOC) medium (Hanahan, 1983)

Glucose	3.6	g
SOB medium	1000.0	ml

3.7. Glycerol stock

Tryptone	10.0	g
Yeast extract	5.0	g
NaCl	10.0	g
Glycerol 96%	200	ml
Distilled H_2O ad.	1000	ml

This medium was used for long term preservation of isolates at -20ºC.

Methods

4. Collection of specimens

Clinical specimens were obtained from the microbiology laboratory of Sadr Al-Abbasiyya Hospital. They were collected in clean, dry, wide-neck containers. Specimens were directly streaked on blood agar, chocolate agar (Cheesbrough, 2006) and MacConkey agar plates at the hospital and then transported within 1 hour to the microbiology lab, Faculty of Pharmacy, Ain Shams University for incubation and further study. **The whole study was approved the Faculty of Pharmacy ethics committee Nr…..(May 2019)**

5. Isolation and purification of clinical pathogens

The plates were incubated overnight at 37ºC (Cheesbrough, 2006). An isolated bacterial colony from the obtained growth was purified by streaking on the surface of culture plates. The distinctive characters of the pure colonies were recorded.

6. Categorization of the collected clinical isolates

The collected clinical isolates were categorized according to their Gram reactions. Fresh pure colonies of the test isolate were used to prepare a heat fixed smear which was subsequently stained by Gram technique. The Gram reactions and the microscopical characters of the test isolates were recorded.

7. Antimicrobial susceptibility testing by disk diffusion method

The Kirby-Bauer disk diffusion method was used to determine the susceptibility of the clinical isolates to antimicrobial agents and it was carried out as recommended by the Clinical and Laboratory Standards Institute (CLSI) (CLSI M2-A9, 2006).

a) Inoculum preparation

Freshly (18 to 24 hours incubation period) isolated colonies of the test isolate, grown on Mueller Hinton agar, were suspended in isotonic saline. Turbidity was adjusted to match 0.5 McFarland standard suspension as follows: the inoculum and the 0.5 McFarland suspension were prepared in identical screw-capped tubes and visual comparison was done in adequate light against a white card with contrasting black lines.

b) Inoculation of Mueller Hinton agar plates

Mueller-Hinton agar was prepared according to the manufacturer's directions and autoclaved. The agar was allowed to cool to 50°C then poured into sterile glass flat-bottomed petridishes to a depth of approximately 4 mm. Optimally within 15 min after adjusting the turbidity of the inoculum, a sterile swab was dipped in the adjusted isolate suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab. A dry Mueller-Hinton agar plate was inoculated by streaking the swab over the entire agar surface. Streaking was repeated two more times, after rotating the plate approximately 60° each time to ensure an even distribution of the inoculum. Finally, the rim of the plate was swabbed.

c) Application of antimicrobial disks to inoculated plates

The disks containing the antimicrobial agents were transferred to the surface of the inoculated plate using a sterile forceps and gently pressed. No more than eight disks were placed on one 120-mm plate, or more than four disks on a 90-mm plate. The plates were inverted and incubated at 37°C for 16 to 18 hours.

d) Reading the plates and interpreting the results

After incubation, the plates were first examined for even growth and circular uniform inhibition zones. For some isolates, the test was repeated when the growth was too light or too heavy or had any other defects. The plates were held inverted above a black background and the inhibition zone diameters were measured in

millimeters, recorded and interpreted by referring to the standard table shown in appendix. The susceptibilities of the clinical isolates were recorded as susceptible, intermediate or resistant to the tested antimicrobial agents.

8. Statistics

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Results

1. Isolation, categorization and antibiogram analysis of the total collected clinical bacterial isolates

1.1. Isolation and categorization of the collected isolates

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1.2. Antibiogram analysis of the total collected isolates

1.2.1. Overall results of all tested isolates

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Table 3 Summarization of antibiogram analysis results of the total collected bacterial isolates against different tested antimicrobial agents

Antimicrobial Agent	Sensitive	Resistant	P-value
Amikacin			
Amoxicillin			
Co-amoxiclav			
Cefadroxil			
Cefuroxime			
Ceftriaxone			

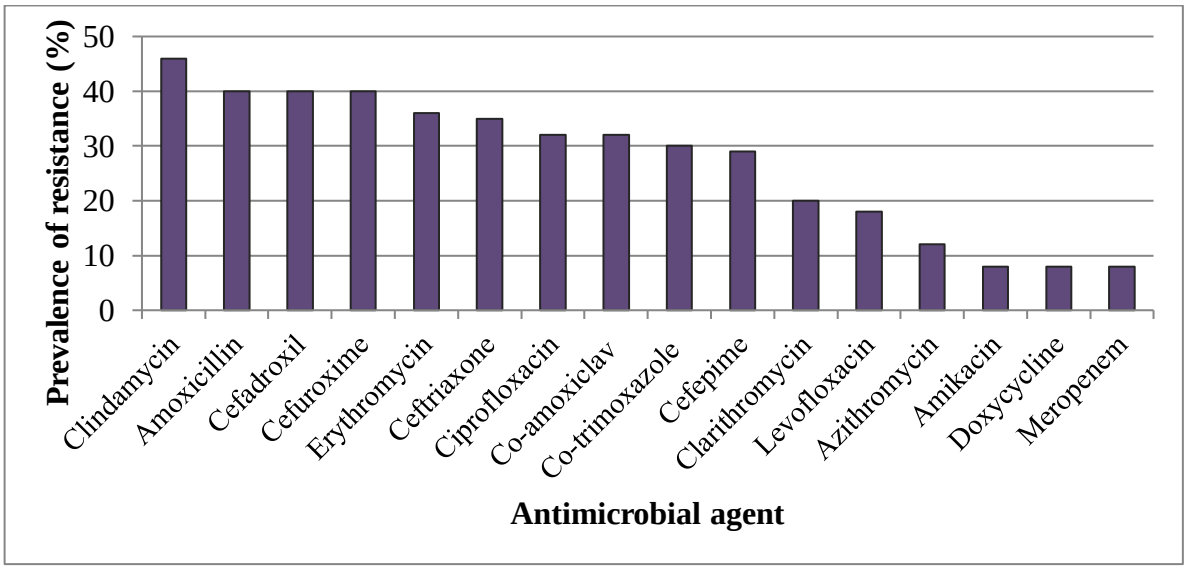

Figure 1 Prevalence of resistance to different antimicrobial agents among the total collected bacterial isolates
Total number of tested isolates is 235 isolates

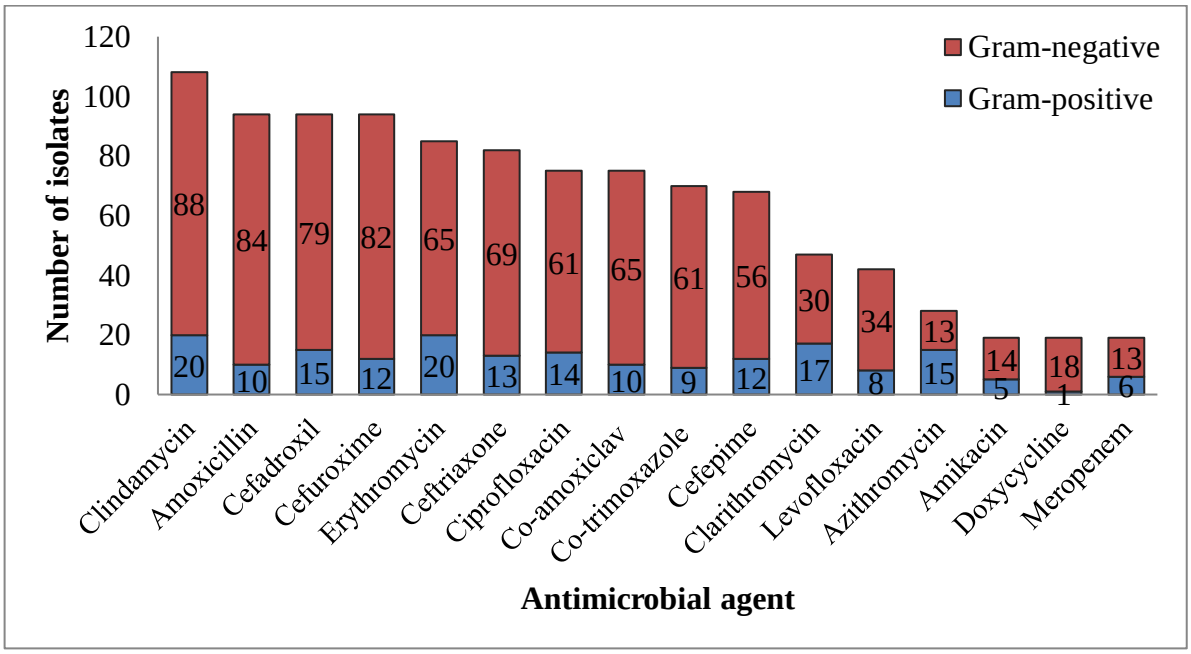

Figure Distribution of resistance among the total Gram-positive and negative collected bacterial isolates against different tested antimicrobial agents Total number of tested isolates is 235 isolates

2. Identification of the multiple drug resistant isolates

Out of the…..

Discussion

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Conclusion

- fdsfds.
- sgssfg.
- gfgf
- fgfdhg.
- fgfg
- fggf

Limitations (if available)

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Recommendations

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Work perspectives

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Summary

The present study was concerned (aimed at or focused on) with ……..

Therefore, to achieve this aim, ……

.

References

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Ahmad, S., N.F. Al-Juaid, F.Q. Alenzi, E.H. Mattar and O.-S. Bakheet (2009). "Prevalence, antibiotic susceptibility pattern and production of extended-spectrum beta-lactamases amongst clinical isolates of *Klebsiella pneumoniae* at Armed Forces Hospital in Saudi Arabia." *Journal of the College of Physicians and Surgeons--Pakistan.* **19**(4): 264-265. https://doi.org/04.2009/JCPSP.264265

Guidelines:

1. **References are arranged alphabetical**
2. **References should be updated and include those published in the last 4-5 years (30-35% of the total number of references**
3. **Include DOI of the references (whenever available)**
4. **All reference must be cited in the appropriate positions within the text.**

APPENDIX

NCBI Resources How To

Nucleotide Nucleotide Advanced

Display Settings: GenBank Send:

Klebsiella pneumoniae subsp. pneumoniae plasmid pKPS29 beta-lactamase SHV-1 gene, partial cds

GenBank: KM052217.1

FASTA Graphics

Go to:

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  TITLE     Direct Submission
  JOURNAL   Submitted (21-JUN-2014) Microbiology and Immunology, Faculty of
            Pharmacy, Ain Shams University, Organization of African Unity St.,
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NCBI Resources How To

Nucleotide Nucleotide Advanced

Display Settings: GenBank

Klebsiella pneumoniae subsp. pneumoniae plasmid pKPS29 TEM-1 beta-lactamase gene, partial cds

GenBank: KM052218.1

FASTA Graphics

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  TITLE     Direct Submission
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NCBI Resources How To

Nucleotide Nucleotide Advanced

Display Settings: GenBank

Escherichia coli plasmid pECAC-10 aminoglycoside-(6')-N-acetyltransferase AAC(6')-Ib (aac6') gene, partial cds

GenBank: KM052219.1

FASTA Graphics

Go to:

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ACCESSION   KM052219
VERSION     KM052219.1  GI:686689811
KEYWORDS    .
SOURCE      Escherichia coli
  ORGANISM  Escherichia coli
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
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  TITLE     Direct Submission
  JOURNAL   Submitted (21-JUN-2014) Microbiology and Immunology, Faculty of
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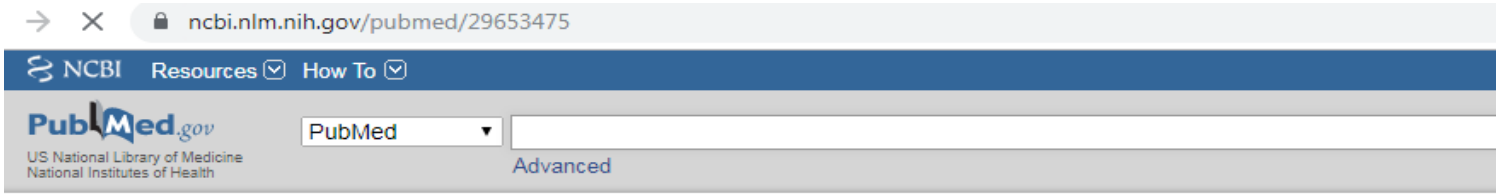

Format: Abstract

Send to

Microb Drug Resist. 2018 Nov;24(9):1316-1325. doi: 10.1089/mdr.2017.0354. Epub 2018 Mar 13.

Plasmid-Mediated Quinolone Resistance in Gram-Negative Pathogens Isolated from Cancer Patients in Egypt.

Hamed SM[1], Aboshanab KMA[2], El-Mahallawy HA[3], Helmy MM[4], Ashour MS[5], Elkhatib WF[2].

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5 5 Department of Microbiology and Immunology, Faculty of Pharmacy, Al-Azhar University , Cairo, Egypt .

Abstract

Fluoroquinolones (FQs) are the drugs of choice for prophylaxis of bacterial infections in immunocompromised cancer patients. This study aimed to investigate FQ resistance and the prevalence of plasmid-mediated quinolone resistance (PMQR) determinants in 239 Gram-negative isolates collected at a tertiary care cancer hospital in Cairo, Egypt. Disc diffusion and broth microdilution tests showed that 70.7% of the isolates were nonsusceptible to ciprofloxacin (MIC_{50} = 64 µg/ml). Polymerase chain reaction (PCR) revealed that 53.6% of the isolates carried at least one PMQR determinant, of which 23.4% were susceptible to ciprofloxacin. The most prevalent gene, aac(6')-Ib-cr, was identified in 36.8% of the isolates, while qnr genes were harbored by 31.0% (qnrS, 24.3%; qnrB, 7.1%, and qnrA, 0.4%). The oqxAB genes were only detected in Klebsiella sp. isolates (92.5%). PMQR determinants were more likely detectable among isolates recovered from pediatric patients than adults (59.3% vs. 43.8%) and were significantly associated with ceftriaxone and gentamicin resistance. A combined genetic analysis using random amplified polymorphic DNA-PCR and enterobacterial repetitive intergenic consensus-PCR showed that most of the qnr-positive isolates were not clonal. Findings of the current study raised concerns about the efficacy of prophylactic use of FQs in cancer patients in our region. It also demonstrates the possible role of PMQR-positive ciprofloxacin-susceptible isolates in the dissemination of resistance to other antimicrobial agents and the urgent need to reconsider the existing FQ breakpoints defined by the Clinical and Laboratory Standards Institute.

كلية الصيدلة
قسم .....

عنوان الرسالة باللغة العربية
كلية

رسالة مقدمة
لاستكمال متطلبات الحصول علي درجة الماجستير(او دكتور الفلسفة) في العلوم الصيدلية
تخصص (.....)

إعداد

اسم الباحث
بكالوريوس (او ماجستير) العلوم الصيدلية، سنه المنح
معيدة او باحث بقسم ..... بكلية الصيدلة - جامعة عين شمس (الجهة البحثية)

تحت إشراف:

أ.د.....
أستاذ.....
كلية الصيدلة – جامعة عين شمس

أ.د.....
وأستاذ .....
كلية الصيدلة – جامعة عين شمس

الدكتور .....
أستاذ مساعد (مدرس) .....
كلية الصيدلة - جامعة عين شمس

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