ISSN(Online): 2319-8753
ISSN (Print) : 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 5, May 2016

Genetic Identification of Some Clinical Strains of *Enterobacter* from Hospitals' Contamination

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ABSTRACT: Bacterial contamination still as one of the most important medical problems. Six isolates of *Enterobacter* have been studied in this research, taken from public health lab., from Misan province, and identified there by biochemical tests, as primary identification, for the level of genus or species, whereas the applying of PCR technique and sequencing, led to accurate identification till the level of strains.

KEY WORDS: *Enterobacter*, Enterobacteriaceae.

I.INTRODUCTION

The cross- infection from patient to patient or from hospital personnel to patients, represents constant hazards [1].
One of the important causes of contaminations is *Enterobacter*.
According to the authors of[2], it belongs to the family Enterobacteriaceae and has a general features; straight rods, 0.6–1.0 ×1.2–3.0 μm, motile by peritrichous flagella (generally 4–6), Gram negative,facultatively anaerobic and ferment the glucosewith production of acid and gas.
Parodi*et al*[3] reported that *Enterobacter* species are a cause of nosocomial meningitis among neurosurgery patients increasingly, but riskfactors for these infections are not well defined.

It had been demonstrated by Thiolas*et al* [4]that *Enterobacteraerogenes* is an agent of hospital-acquired infection which exhibits a remarkable resistance toβ-lactam antibiotics during therapy and among five successive isolates of *E. aerogenes* investigated, four presented resistant phenotypes during successive treatments byimipenem and colistin.
In the study of [5] it had been showed that of 183 patients, 86 (47%) had bacteremia werecaused by broad-spectrum cephalosporin-resistant *Enterobacter* species, their infections were classified as resistant.

Fiore *et al*[6] considered the *Enterobactersakazakii* as an emerging pathogen connected to neonatal as cases of necrotizing enterocolitis and meningitis due to contaminated powdered infant formula using,and it can also be found in a broad range of foods and in water, in a different of areas including houses and hospitals .
Neonatal nosocomial infections had been reviewed by Mussi-Pinhataand doNascimento[7] showed that in neonatal intensive care units, sepsis and pneumonia were frequently diagnosed, and listed *Enterobacter cloacae* as one of causes.
For the above importance and others, present study aimed for identification of some *Enterobacter* isolates from hospitals' contamination at genetic level as a confirmation for biochemically routine work at hospitals laboratories.

II. MATERIAL AND METHODS

Isolation and cultivation of bacteria. Swabs have been taken from Public health lab, Microbiology unit, which belong todifferent places at two hospitals in Misan province, as shown in table (1)

ISSN(Online): 2319-8753
ISSN (Print) : 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 5, May 2016

Table (1): Sources and characteristics of samples, showed different places at the 2 selected hospitals.

Isolate number	Hospital	description
E1	Alsader	Artificial kidney/hemodialysis bed 2
E2	Alsader	Burns/room 1/ the ground
E3	Alsader	Premature(pediatrics)/ Incubator 8
E4	Kalaatsalih	Neonatal room/ walls and floor
E5	Kalaatsalih	Neonatal room/ bed
E6	Kalaatsalih	Incubator 1

Blood agar (LAB M, UK) Chocolate agar and MacConky agar (Salucea, Netherlands) have been prepared according to the instructions of manufacturing companies as possible.

Swaps have been cultured on the media and incubated at 35±2ºC for 24hr. Different colonies have been sub cultured by streaking for purification [8], [9].

Morphological characterization. Selected colonies have been characterized by shape , color, consistency, size, and fermentation of lactose according to [10], [11].Gram staining has been done by the following of (Syrbio, Jorden) company instructions.

Biochemical tests. Different biochemical tests have been carried out according to[11]. The API 20E strep (Biomerieux, France) has been applied for more identification according to instructions of manufacturing company.

Genetic identification.

Genomic DNA extraction. Cultures of isolates have been activated on nutrient broth for 18- 24 hr., centrifuged at 7000- 8000 cycle/min. for 10-15 min., washed with distilled water at the same as preparing to the procedure of the genomic DNA mini kit (Geneaid, Taiwan) as possible, with some changes in concentrations and time for the addition materials.

Detection of genomic DNA by gel electrophoresis. Method of electrophoresis by using 0.8% agarosehas been done according to Sambrook and Russell [12] as possible due to facilities.

Identification by Polymerase Chain Reaction (PCR). The primers of Lane [13]for 16SrDNA which were B27F(5-AGAGTTTGATCCTGGC-3)and U1492R(5-GGTTACCTTGTTACGACTT-3)were used in this study, with PCR program selected from[14], as well as adaptation by [15] table (2). TheProkić*et al.* [16]amplification steps were abstracted with little changes.

Table (2): PCR amplification program, showed the steps with their temperatures, times and cycles number.

Step	Temperature	Time	No. of cycle
Initial denaturation	92 °C	2 min	1
Denaturation	94 °C	30 sec	30
Annealing	51.8 °C	45 sec	
Extension	72 °C	1.5 min	
Final extension	72 °C	5 min	1

Detection of 16S rDNA by gel electrophoresis. Agarose of 2% was used as well as the steps of Sambrook and Russell [12] methods in order to detect the 16S rDNA gene, with some differences required.

Purification, sequencing and manipulation of data for PCR product. The obtained product of PCR has been sent for Bioneer Company laboratories/ Korea, for purification and sequencing. Then data have been manipulated.

ISSN(Online): 2319-8753
ISSN (Print) : 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

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III. RESULTS

Isolation and cultivation. Six isolates have been selected and identified by biochemical tests andmorphological characterization; as found in table (3), three of them have been identified to the level of genus, while the others have been identified to the level of strain.

Table (3): selected isolates with primitive identification, showed different *Enterobacter*genusand species,that were identified by morphological characterization and biochemical tests

Isolatenumber	Name
E1	*Enterobactersakazaki*
E2	*Enterobactercloacea*
E3	*Enterobactercloacea*
E4	*Enterobacter*spp
E5	*Enterobacter*spp
E6	*Enterobacter*spp

Morphological characterization.
Colonies characteristics. Isolates were circular, opaque, convex, smooth, shiny, moderate in size, lactose fermenter.

Gram staining. Staining has shown that isolates are Gram negative rod bacteria.

Biochemical tests. Isolateshas showed same results in biochemical tests, which ranged from positive to negative reactions as found in the table (4).

Table (4): Results of manually biochemical tests for isolates, showed same responses to the tests among the different isolates

Test \ Isolate	E1	E2	E3	E4	E5	E6
Indole	-	-	-	-	-	-
Urease	-	-	-	-	-	-
Citrate utilization	+	+	+	+	+	+
Voges- Proskauer	+	+	+	+	+	+
Motility	+	+	+	+	+	+
Catalase	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-

; +: positive, -: negative, E1-E6: number of isolates.

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ISSN (Print) : 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

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API 20E system: The results of biochemical tests of this system have showed some resembles and differences in the response to the reactions of the tests among different isolates, as shown in the table (5).

Table (5): Results of biochemical tests of API 20E system for isolates, showed some different responses to the tests among the different isolates,

Isolate / Test	E1	E2	E3	E4	E5	E6
ONPG	+	+	+	+	+	+
ADH	+	+	+	+	+	+
LDC	-	-	-	-	-	-
ODC	+	+	+	+	+	+
CIT	+	+	+	+	+	+
H2S	-	-	-	-	-	-
URE	-	-	-	-	-	-
TDA	-	-	-	-	-	-
INO	-	-	-	-	-	-
VP	+	+	+	+	+	+
GEL	-	-	-	-	-	-
GLU	+	+	+	+	+	+
MAN	+	+	-	+	+	+
INO	+	-	-	-	-	+
SOR	-	+	+	-	+	+
RHA	+	-	-	+	-	-
SAC	+	+	+	+	-	+
MEL	+	+	+	-	-	-
AMY	-	+	+	+	+	+
ARA	-	+	+	+	+	+
OX	-	-	-	-	-	-

; + positive, - negative, E1-E6: number of isolates, ONPG: (Ortho NitroPhenyl-ßDGalactopyranosidase ,

ISSN(Online): 2319-8753
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International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 5, May 2016

ADH:Arginine DiHydrolase, LDC: Lysine DeCarboxylase, ODC: Ornithine DeCarboxylase,CIT: Citrate utilization, H2S: H2S production, URE: Urease, TDA: TryptophaneDeAminase, IND: Indole production, VP: acetoin production(VogesProskauer) ,GEL: Gelatinase ,GLU: Fermentation / oxidation (Glucose) , MAN: f / o(Mannitol) , INO: f / o(Inositol), SOR: f / o (Sorbitol), RHA: f / o (Rhamnose), SAC: f / o(Saccharose), MEL: f / o (Melbiose), AMY: f/ o (Amygdalin), ARA: f/o (Arabinose),OX:Cytochrome-Oxidase

Genetic identification.

Genomic DNA extraction and detection. Electrophoresis technique has revealed clear isolated DNA bands for the all isolates as found in the figure (1).

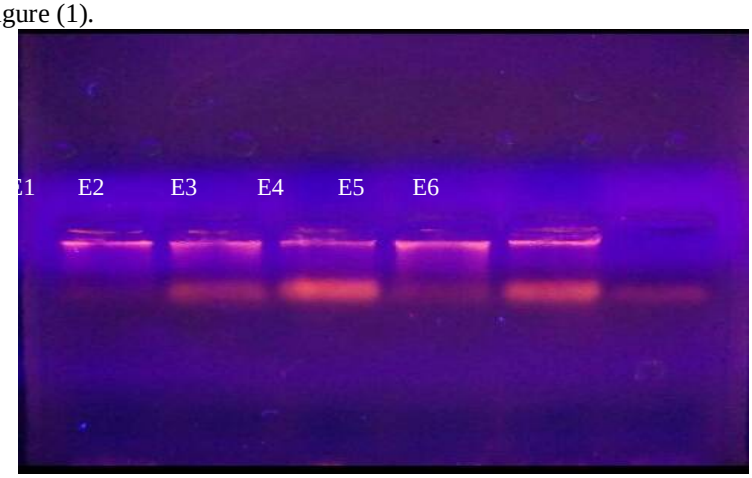

Figure (1): Gel electrophoresis, showed the isolated genomic DNA bands; E1- E6: number of isolates.

Amplifying of the 16S rDNA gene by (PCR) Technique. By using a universal primer, results have obtained the required band of 16S rDNA for each isolates along with electrophoresed ladder in the region of 1500bp, as in the figure (2).

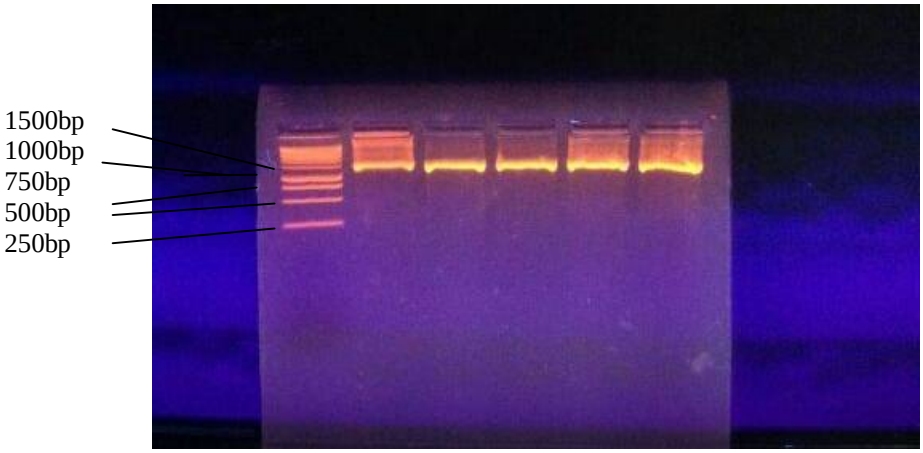

Figure (2): Gel electrophoresis for PCR technique, showed the band of 16s rDNA in all isolates, E1-E5: numberof isolates, L: DNA ladder of 1Kb.

Sequencing. The six isolates were identified to the level of strain, as in the table (6).

ISSN(Online): 2319-8753
ISSN (Print) : 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

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Vol. 5, Issue 5, May 2016

Table (6): Sequencing of isolates after data manipulation, showed different strain of *Enterobacter*

Isolate	Sequence	Original strain
E1	GNGNCTTAAACATGCAAGTCGAACGGTAACAGGAAGCAGCT TGCTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTG GGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTA GCTAATACCGCATAACGTCGCAAG ACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGC CCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAG GCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACT GGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGT GGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCA GCGGGGAGGAAGGCGATAAGGTTAATAACCTTGTCGATTGA CGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCA GCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACT GGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTG AAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCA GGCTAGAGTCTTGTAGAGGGGGGGTAGAATTCCAGGTGTAG CGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAA GGCGGCCCCCTGGACACAGAC	*Enterobactersakazakii* gene for 16S rRNA
E2	GTNTTANCCCTTGCAGTCGAACGGTAACAGGAAGCAGCTTGC TGCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGA AACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCT AATACCGCATAACGTCGCAAG ACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGC CCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAG GCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACT GGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGT GGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTATGAAGAAGGCCTTCGGGGTGTGAAGTACTTTCA GCGGGGAGGAAGGCGATAAGGTTAATAACCTTGTCGATTGA CGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCA GCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACT GGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTG AAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCA GGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGC GGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAG GCGGCCCCCTGGACA	*Enterobacter cloacae* strain EC7
E3	GGCNNCCTTTNNATGCAAGTCGAGCGGTAGCACAGGAAGCT TGCTCCCGGGTGACGAGCGGCGGACAGGGGAGTAATGTCTG GGAAACTGCCTGATGGAGGGGGATAACTACTGTGAACGGGA GCTAATACCCCATAACATCGCAAGA GCACAAAGGGGGACCTCCTGGCCCCTTGCCACCTTATGTGCC CCCATGAGATAATTTACTATGAGGGGGGGCATCTCACTCCCT AGACGACGATCCCTTGTTTTGCTAAGAGGAAACCAGCCACTC TGTAACTGAGACTCCCCCCACACT CCCACGGACGCCTCAATGGGGAATATTGCATTTGCGCGCCAG CCTTATTCACCATGGCTCTTGTGTTAAGAATGCCGGGGGGGG GGGGGGGGTCTTTTCATCGTTAGGAAGNGCCAAATTTAATAA CCTTGTGGATTGTCCTTCCCGCAA ATACCACCCTCTATTTTCTTGTCTCCAGCCGCTGTTATTCCCG ACGGTGC	*Enterobacter cloacae* strain GG78
E4	GATGGGCGGCGGCCTACCATGCAGTCGAGCGGCAGCACGAA GTAGCTTGCTCCTTTGCCGGCGAGCGGCGGACGGGTGAGTAA	*Enterobacterludwigii* strain hswX32

ISSN(Online): 2319-8753
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Vol. 5, Issue 5, May 2016

	TGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAA ACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGG GGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGAT TAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCC TAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGA CACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATT GCACAATGGGCCCAAGCCTGATGCACCCATGCCGCGTGTTTG AATAAGGCCTTCGGGGTGTAAAGTATTTTCTCCGGGGGGAGA GGGGGTTGGGGGTGCAGCACCCGTCGCATAGGTCTCTAACAA GCAAAAACACCCTTGCAAA	
E5	CGGCGNNANTTNTTTNTACCTGACAANTCGAGCGGAAGATG GGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACA CGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAAC CGGGGCTAATACCGGATGCTTGTTT GAACCCCATGGTTCAAACATAAAAGGTGGCTTCGGGTACCAC TTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTTAGGTAA TGGCTCACCAATGCACACATGCGTATCCTACCAGAGAGGGTG ATCGGCCACACTGGGACTGAGACT CCGCCCACACTCCTGCGGGAGGCAGTAGGTGGAATCTTCCGC AATGGACGAACGTCTGAAGGATCACGCCTGGGGGGGGCTTA CGGTTTTCAGAGGCTTTTCCTCTTTTGTTACTTAAAAAGTGTA CGATTCTTTTCGGGGGTGGTCTTG ACCTTCTTTAACCAGTATTTCTTTCATT	*Enterobacter cloacae* strain TR20T2
E6	TNNGNGGCGCTACCNTGCAGTCGAGCGGATGAGTGGAGCTT GCTCCATGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGA ATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTA ATACCGCATACGTCCTACGGGAGAAA GTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGT CGGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGAC GATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAAC TGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA ATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGT GTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGG AGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACC AACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGG TAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTA AAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCC GGGCTCAACCTGGGAACTGCATCCAAAACTGGCGAGCTAGA GTATGGCAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAAT GCGTAGATATAGGAAGGAACACCAGTGGCGAAG	*Enterobacterasbu riae* strain IHB B 1070

E1-E6: number of isolate,**bp: base pairs**

IV. DISCUSION

Nasser *et al*[1] found that*Enterobacters*pp was one of the most prevalent bacterial contaminants of the environment of Intensive Care Unit and the highest rates (19.11%) of contamination by bacteria had been found on the walls and the floor, suggested that could be managed mostly by strict application of sterilization measures.

Jalaluddine*et al*[17] demonstrated that *Enterobacteraerogenes* is a cause of nosocomial infections also, like other members of the family Enterobacteriaceae, and studied the epidemiology of this bacterium because of the incidence in the hospital of Saint-Pierre university in Brussels was higher at 1995 than of the preceding and the following years among the 33patients, the ratio (1:2) of colonization and infection, as well as (39%) as the high crude mortality rate among the infected patients.

As in agreement with Alhilfi[18], the results of PCR technique in this research showed that amplified region is found in 1500 bp approximately, for all six isolates, because the 16S rDNA appears in this region in all bacteria.

ISSN(Online): 2319-8753
ISSN (Print) : 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 5, May 2016

Accurate identification of bacterial isolatesin hospitals, is an essential task for the microbiological control, andthe analysis of small subunit ribosomal RNA gene (16S rDNA) sequence is usually used for the of bacterial identification and classification [19].

Present study has showed that the compatibility of the biochemical and molecular identification for the three isolates E1, E2 and E3, while the incompatibility appeared for E4, E5 and E6 in which biochemical test couldn't reach to the final identification of the isolates, even for the species, whereas in molecular methods, the isolates have been identified to the strain level. It may be belonged to the high sensitivity and accuracy for the molecular tests, which get in a close agreement with the authors in [20]who concluded that16S rRNA gene sequencing was useful in ascertaining the clinical relevance of the Enterobacteriaceae strains which isolated fromthe bone marrow transplant recipient stool with diarrhea.

WhereasAhmadi*et al*[21] reported that PCR is less time consuming because it takes less than 24 h to complete while bacterial identification by conventional microbiological and biochemical methods needs for more than 48h.

Patel [22] reported that sequencing of the 16S rDNA gene an important tool for phylogenetic relationships determination between bacteria and it is a powerful mechanism in patients for identifying new pathogens with suspected bacterial disease, and recently is being applied for routine identification of bacterial isolates in the clinical laboratory.

V. CONCLUSION

We have concluded that hospitalsmight be considered as one of contamination sources by bacteria, especially members of enterobacteriaceae, which may need for more care and attention. On another side there is a necessity in coupling of biochemical tests with molecular technique in order to reach an accurate identification of bacteria.

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ISSN(Online): 2319-8753
ISSN (Print) : 2347-6710

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Vol. 5, Issue 5, May 2016

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