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Studying the pattern of phenotypic resistance to some antibiotics for local isolates of *Enterobacter cloacae* and it's evolutionary relationship with global isolates

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Keywords: *Enterobacter cloacae*,HSP60 gene , PCR , evolutionary relationship .

Summary :

26 isolates of *Enterobacter cloacae* from 264 specimen from different clinical sources and from inpatients for ages ranged from one Week to 73 years from 15/12/2016 to 18/7/2017collected from two hospitols of AL-Diwaniyah city . The isolation and diagnosis process was based on the results of phenotypic and biochemical tests and the Vitek system .The sensitivity of the isolates for antibiotics were tested against 15 antibiotic using disk diffusion method , The results showed an absolute resistance (100%) for Amoxicillin, Cephalothine and Doxycycline, while their sensitivity to Amikacin , Gentamicin, Tetracycline, Nitrofurantoin and Nalidixic acid were various, they resisted it at percentage of 11.53% , 34.61% , 73.07% , 34.61% and 23.07% respectively . For the rest of the Cephalosporin group (Cefoxitin, Cefotaxim and Ceftriaxone) , the isolates were resisted them with percentage 65.38%, 57.69% and 34.61%, respectively , While all isolates (100%) were sensitive to Chloramphenicol, Ciprofloxacin, Impenem and Meropenem . Twenty-four isolates (92.30%) were able to produce β-lactamase . PCR was used to amplify *HSP60* gene which used to determine the sequence of nucleotides and draw the phylogenic tree and find the relationship between the current isolates of this study with global isolates.

Background

Enterobacter cloacae are saprophytic microorganisms, live in digestive system as normal flora and can be isolated from patients suffering from diarrhea , urinary tract infection and bloodstream infection[1].It gram negative bacteria , bacilli , motile ,facultative anaerobic and opportunistic can transform to pathogens[2]. *E. cloacae* is able to resist multi drugs which are coded with chromosome as resistance of penicillin and the first three generation of cephalosporin or extra genetic elements as plasmides[3,4].Many of these bacteria are able to produce extended spectrum β-lactamases enzymes which hydrolysis penicillin . monobactam , carbapenems and cephalosporin and coded with plasmids mostly[5]. *Enterobacter* sp. Have gene called *hsp60* which used as tool for molecular identification of this genus and make DNA sequencing and study the evolutionary relationship and

compare it with global isolates[6].because of pathogenic of *E. cloacae* and reducing local studied about it ,this study aims to appraising the phenotype resistance of this bacteria and it's capable to produce β-lactamases enzymes besides draw phylogenic tree of it .

Materials and Methods

Samples Collection : 264 sample were collected from different clinical sources and from inpatients in AL-Diwaniyah women's and children's educational and AL-Diwaniyah General Educational hospitals for ages ranged from one Week to 73 years for the period from 15/12/2016 to 18/7/2017 ,include 90 samples of stool from patients with diarrhea , 135 samples of urine (58 samples took from urine catheter sacs and 77 samples took directly) besides 38 swaps from urine catheter tube and one sample from blood of patient suffering from septicemia .

The Isolation and Identification :

All samples cultured on routine media like Macconkey agar and Blood Agar (Difco, USA) and incubated at 37c for 20-24 h. the results of culture used to identify *E.cloacae* besides biochemical tests , gram stain . Vitek 2 Compact system (Biomerieux , USA) was used for final identification .

Susceptible of Antibiotic Test :

Disc Diffusion Method was used to make the test depend on Kirby and Bauer (1966)by culture *E. cloacae* on blood agar , and select pure bacterial colony to culture it on Muller Hinton agar (Biolife, Italy)with swaps then put the discs of antibiotic including Amoxicillin, Cephalothine, Doxycycline, Amikacin , Gentamicin, Tetracycline, Nitrofurantoin, Nalidixic acid, Cefoxitin, Cefotaxim , Ceftriaxone , Chloramphenicol, Ciprofloxacin, Impenem and Meropenem (6 discs of antibiotic for each petri dish), the dishes incubated for 16-18 h at 35±2 °C , then reading of the results depend on inhibition diameters and compare it with CLSI[7] .

Production of β- lactamase Enzymes Test:

Strips test of β- lactamase Enzymes were used for quick detection about the ability of *E. cloacae* to produce these enzymes which are responsible on beta lactam drugs resistant in gram negative besides the positive .One pure well grown colony was transferred with sterile loop and streaked on the moisture test strip , if the color of the strip changes into reddish pink during five minute , that's mean it's positive interaction .

polymerase chain reaction Assay(PCR):

DNA Extraction :

Presto Mini gdna Bacteria Kit (Geneaid , South Korea) used to extract the nuclear acid DNA depend on the steps of the procedure which are found with the kit .DNA is kept in refrigerator at 2-8 °C to use in genetic assay .

Amplifying of Heat Shock Protein gene (*HSP60*):

HSP60 amplified by using polymerase chain reaction mixture which contains from : Prime Taq Premix (GeNet Bio , South Korea) , primers of *HSP60* (table 1), DNA template and free nuclease water by using thermal cycles program(table 2).

Table 1. primer used in this study

gene	PCR primer	Primer sequence 5' _3'	Tm °C	PCR product (nt)	Reference
HSP60	Hsp60-F	GGT AGA AGA AGG CGT GGT TGC	61.8	341	8
	Hsp60-F	ATG CAT TCG GTG GTG ATC ATC AG	60.6		

Table 2. thermal cycles program of Hsp60 gene primer

Initial denaturation		94˚C for 5 min.
Cycle conditions	**denaturation**	94˚C for 30 sec.
	annealing	57.5˚C for 30 sec.
	extension	72˚C for 60 sec.
Final Extension		72˚C for 5 min.
Cycles No.		30

Agarose Gel Electrophoresis :

Method of Sambrook[9] used to Electrophoresis PCR product of *HSP60* gene by using Agarose salts , Tris Borate EDTA (TBE), ethidium bromide and Loading Dye at 80V/1 H , then UV ray used to observe the bands and compare it with ladder .

DNA sequencing and evolutionary relationship :

21 samples of PCR produces of *HSP60* gene of *E. cloacae* sent to Macrogene Co. (south Korea) to make DNA sequencing for this bacteria , nucleotides exhibited on gene bank for getting accession number for each isolate , then drew phylogenic tree by using NCBI-Blast Alignment.

RESULTS and DISCUSSION :

Isolation and Identification :

26 isolates of *E. cloacae* from 264 samples from different sources including urine , stool , catheter system of urine and blood were got in this study depending on their cultural characters, microscopic , biochemical and finally using of Vitek 2 Compact system which can diagnose this bacteria in high accuracy reach to 100% nearly and it could divide our bacteria into two Subspecies (table 3).

From result we found that the rate of our isolation of *E. cloacae* was 9.84% including 5.3% from urine , 1.8% from stool for patients with diarrhea , 1.13% for each of catheter sacs and swaps of catheter tube and 0.37% from blood of patient suffering from bacteremia .The total rate of isolation was similar to [10] who get 9.61% of this bacteria .The catheter systems are gates to entrance *Enterobacter i*nto blood stream[11], and the role of *Enterobacter* as causing of diarrhea is common because these bacteria are opportunistic and can transfer from normal flora to be pathogenic and striking the human body and its tissues especially when make surgical operations

or put the urine catheter systems for a long time which can contribute in spreading of bacteria and causing Nosocomial infections[12].The ability of *E. cloacae* for coexisting out of intestine was recorded from [13] who consider that *Enterobacter* spp. are one of ten common bacterial genus causing bacteremia in hospitals specially for immunocompromised patients besides the National Nosocomial Infections Surveil-lance System (NNIS) consider that *Enterobacter* sp. responsible on 5-7% of human infection which were acquired from hospitals in USA from 1976 -1989.

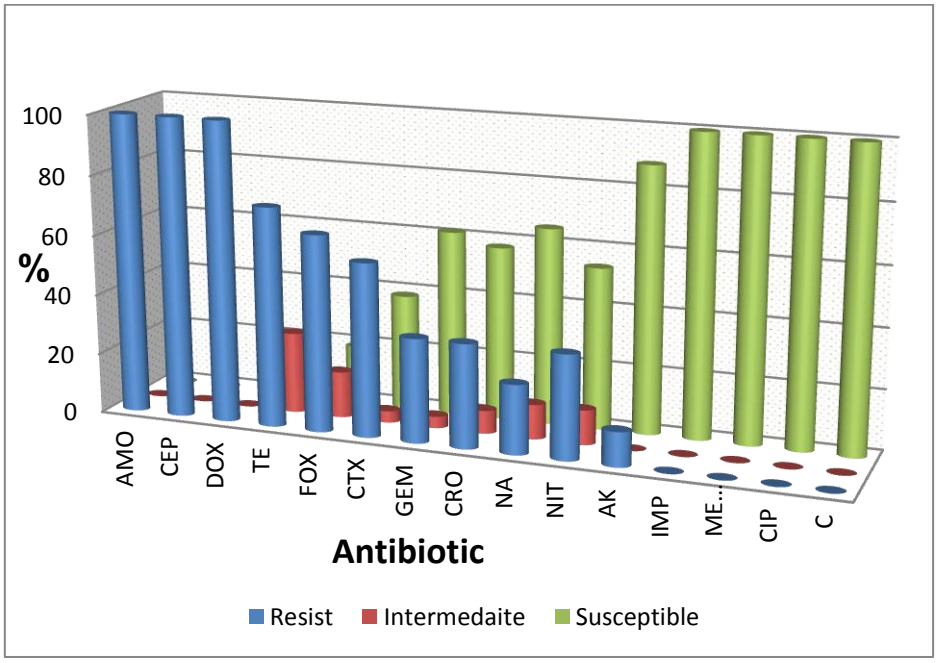
Table 3.subspies of Isolates in this study and it's source

No. of isolate	Source of isolate	Name of isolate
1	Urine	*E. cloacae* subspecies *cloacae*
2	Catheter tube	*E. cloacae* subspecies *cloacae*
3	Stool	*E. cloacae* subspecies *cloacae*
4	Urine	*E. cloacae* subspecies *dissolvens*
5	Urine	*E. cloacae* subspecies *cloacae*
6	Catheter sac	*E. cloacae* subspecies *cloacae*
7	Blood	*E. cloacae* subspecies *dissolvens*
8	Urine	*E. cloacae subspecies cloacae*
9	Urine	*E. cloacae subspecies cloacae*
10	Urine	*E. cloacae subspecies cloacae*
11	Stool	*E. cloacae subspecies cloacae*
12	Catheter tube	*E. cloacae* subspecies *dissolvens*
13	Urine	*E. cloacae* subspecies *cloacae*
14	Urine	*E. cloacae* subspecies *cloacae*
15	Stool	*E. cloacae* subspecies *cloacae*
16	Catheter sac	*E. cloacae* subspecies *dissolvens*
17	Urine	*E. cloacae* subspecies *cloacae*
18	Urine	*E. cloacae* subspecies *cloacae*
19	Stool	*E. cloacae* subspecies *dissolvens*
20	Urine	*E. cloacae* subspecies *dissolvens*
21	Catheter tube	*E. cloacae* subspecies *cloacae*
22	Stool	*E. cloacae* subspecies *cloacae*
23	Urine	*E. cloacae* subspecies *cloacae*
24	Catheter sac	*E. cloacae* subspecies *dissolvens*
25	Urine	*E. cloacae* subspecies *dissolvens*
26	Urine	*E. cloacae* subspecies *cloacae*

***E. cloacae* Susceptible of Antibiotic** :

The results of Susceptible of Antibiotic of *E. cloacae* (Figure 1) showed that all isolates (100%) were resisted to amoxcillin , cephalothin and doxcyclin while their sensitivity to Amikacin , Gentamicin, Tetracycline, Nitrofurantoin and Nalidixic acid were various, they were resisted it at percentage 11.53% , 34.61% , 73.07% , 34.61% and 23.07% respectively . For the rest of the Cephalosporin group (Cefoxitin, Cefotaxim and Ceftriaxone) , the isolates were resisted them with

percentage of 65.38%, 57.69% and 34.61%, respectively . All isolates (100%) were sensitive to Chloramphenicol, Ciprofloxacin, Impenem and Meropenem which mean their successful drugs to treat the infections caused by *E.cloacae.*



AMO=Amoxcillin , CEP=Cephalothin, Dox= Doxcyclin , TET= Tetracycline , FOX=Cefoxtin , CTX= Cefotaxime , GEM=Gentamicin , CRO= Ceftriaxone , NA= Nalidixic acid , NIT=Nitrofurantoin , AK=Amikacin , IMP= Imipenem ,MEM=Meropenem , CIP= Ciprofloxacin , C= Chloramphenicol

Figure 1 : Susceptible of Antibiotic in *E. cloacae* isolates

The absolute resistance to Amoxcillin in *E. cloacae* record from number of researcher as [14,15],and it is due to the ability of these bacteria to produce β-lactamases enzymes which coded by chromosome besides these enzymes are common in species of *Enterobacter* genus so that give it selective character under treatment pressure [2]. β-lactamase class A can resolve beta lactam ring of penicillin and make it inactive toward bacteria , while high production of β-lactamase class C can resolve penicillin and cephalosporin[2,16].The partial resistance of *Enterobacter* to the third generation of cephalosporin like Cefotaxime and Ceftriaxone due to chromosomal AmpC cephalosporinase enzymes [16], or happening mutation effect on β-lactamase class A include CTX-M which has extended spectrum in cephalosporin lysis[17], or because of ability of *E. cloacae* to produce carbapenemases class A enzymes(it has 2 group of these enzymes IMI-2 and NMC-A) which are common in enterobacteraceae and it can analyze most of beta lactam antibiotic like penicillin , cephalosporin , carbapenem and aztreonam[18]. Resistance of our isolate to aminoglycoside especially

gentamicin due to modify enzymes for aminoglycoside like acetyltransferases and adenyltransferases besides of the exchange of plasmids and genetic transfer elements increase of bacterial resistance to these antibiotic[2].

The bacterial resistance to tetracyclins in our isolates are clear (absolute resistance to doxcyclin and 73.07% to tetracycline and may be due to ribosomal protection or by using efflux pumps coded by genes carried on chromosome or plasmids especially in gram negative to export tetracyclins out of bacterial cell and protect it from bacteriostatic effects of tetracyclins, besides of the ability of mutation existing in targets of these antibiotic[19,20]. Most of the isolates of *E. cloacae* were sensitive to quinolones (Ciprofloxacin and Nalidixic acid) which were used in this study . The active effect of these antibiotic especially Ciprofloxacin was recorded from[21,22]who got sensitivity rate (100 , 96) % respectively . Mechanism of quinolones' effect is inhibit duplication and transcriptions of DNA by binding with DNA gyrase the essential aim for quinolones in gram negative bacteria[23],while [24] showed that the increasing resistance to quinolones as Nalidixic acid is due to chromosomal mutations or caused by plasmids carried genes called *QnrA* gene although anther studies showed that *QnrA* geneis unable to resist ciprofloxacin we found it alone and this may explain the reason of the sensitivity of all our isolates to Ciprofloxacin.

Carbapenems like Imipenem and Meropenem besides Chloramphenicol as antibiotic from phenicol group proved their activity to kill all *E. cloacae* isolates which did not show any resistance towards these bacteria ;therefore it is a successful antibiotic to the treatment of infection with *E. cloacae ,* and this result agree with [25]. Chloramphenicol is considered from extend spectrum and bacteriostatic antibiotic which can inhibit protein synthesis in bacteria by binding with ribosomal subunit 50 S and interaction with peptidyl transferase and blocking the elongation of peptide chain[23]. Because of high toxicity of chloramphenicol , recede use of this antibiotic for human in forth-going nations and rarely use for systematic infections but can use locally[26]and there is a warning when using in pregnancy and people who have allergy to it , besides chloramphenicol can cause anemia because of the killing effect on normal flora which provide the human body with amount of vitamin K [27] .

Among all our isolates of *E. cloacae* we found just 9 isolates (34.61%) resistant to nitrofurantoin . Resistance of *E. cloacae* to nitrofurantoin depend on using of efflux pumps to get out this antibiotic from bacterial cell [28]. Nitrofurantoin can use to treat many infection caused by gram negative especially intestinal infection and urinary tract infections (UTI) because of its effect on bacterial DNA , and it is considered as safely drug in many countries including USA to treat UTI in pregnant women depending on laboratories results and experiments on animals upon injected with nitrofurantoin [29].

β-lactamase production :

strips saturated with chromogenic cephalosporins used to test the ability of *E. cloacae* isolates to produce β-lactamase enzymes . Positive results depend on the changes of strip color to reddish pink (figure 1) as a result of refraction amide

bond of beta lactam ring by β-lactamase and changes color of nitrocefin due to high sensitivity of this material to β-lactamase which are common in gram negative and positive . Our results for this test showed that 24 isolates (92.30 %) were produced β-lactamase while two isolates (one from female has diarrhea and anther from urine catheter sac for inpatient at fractions unit) were unable to produce these enzymes . The production of β-lactamase test which is called sometimes (nitrocefin test) has high accuracy , speed and sensitivity for β-lactamase or hydrolysis of beta lactam ring and this sensitivity is due to easily dissolving of nitrocefin in these enzymes and iodine or pH indicator responsible on color change of β-lactamase strips[30].

Figure 1 : Strips test of β- lactamase: (upper strip= (-) result , bottom strip = (+) result

Amplification of *HSP60* Gene :

Monoplex PCR were used to amplify *hsp60* gene which can use to identify *Enterobacter* genus and to find DNA sequencing to study evolutionary relationship among our positive isolates with global isolates of *E. cloacae* by using NCBI-Gene Bank Global .PCR technique showed all our isolates were followed *Enterobacter* spp. (figure 3 , A and B). *HSP60* gene has more accuracy than *16SrRNA* gene because the ability of *16SrRNA* for recognize among close species of enterobacteraceae especially *Enterobacter* spp. is weak compared with *hsp60* gene[31].

Finding evolutionary relationship of locally isolates of *Enterobacter* spp. :

We get nucleotides sequencing for 21 isolates of *E. cloacae* which sent to Macrogene company in South Korea , then offered in NCBI-Gene Bank database for getting accession number for each isolate (table 4).Phylogenic tree were drawn by using MEGA6 program and analysis of *HSP60* sequences of *E. cloacae* (figure 3). The similarity rate of our isolates comparing with global isolates were in range between 99-100 % , and most of our isolates were similar to USA and South Korea isolates besides number of our isolates were similar with another global isolates from Tanzania , Switzerland and China.

Table 4. Nucleotides Sequences and Accession No. of our isolates

No. of Sequence	source	Accession No.
1	Urine	MH119313
2	Catheter tube	MH119314
3	Stool	MH119315
4	Urine	MH119316
5	Urine	MH119317
6	Catheter sac	MH119318
7	Urine	MH119319
8	Urine	MH119320
9	Urine	MH119321
10	Stool	MH119322
11	Urine	MH119323
12	Stool	MH119324
13	Catheter sac	MH119325
14	Urine	MH119326
15	Urine	MH119327
16	Stool	MH119328
17	Urine	MH119329
18	Urine	MH119330
19	Catheter sac	MH119331
20	Urine	MH119332
21	Urine	MH119333

The differences among the isolates of same species may be due to the structural differences among these isolates . In study of Population Genetics of the Nomenspecies *E. cloacae* achieved by [8] showed differences among bacterial groups of *E. cloacae* due to differences of specific amino acids which found in heat shock protein coded by *HSP60* e.g. *E. cloacae* subspecies *cloacae* have specific proteins in the position 430 and 466 differ from proteins which are found in the position 430 of *E. cloacae* subspecies *dissolvens* .Using of *HSP60* gene which is called *groEL* or *cpn60* is successful and a useful application for classification of bacteria besides of its ability to analyze the evolutionary relationships and draw phylogenic tree [32].

Conclusions: *E. cloacae* are multi drugs resistance as amoxillin , cephalothin and Doxcyclin and the best drugs for treatment infections caused by this bacteria are chloramphenicol , ciprofloxacin ,impenim and meropenem besides this study proved that most of *E. cloacae* able to produce β-lactamases enzymes which increase its virulence. We find high identity among our isolates and the global isolate by using nucleotides sequences and their phylogenic tree .

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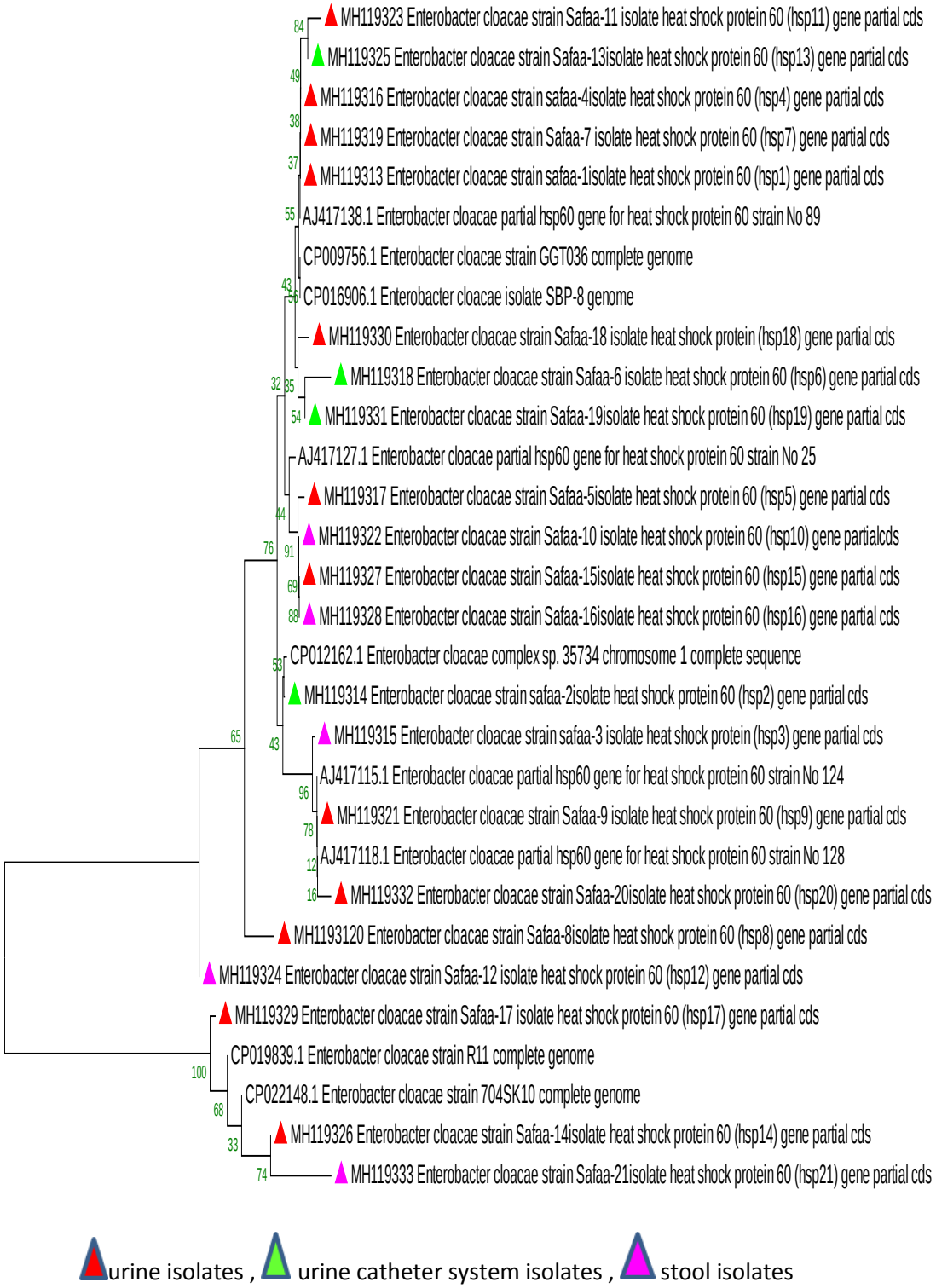

Figure 3 : Phylogenic tree of *E. cloacae* isolated in this study

REFERENCES :

1•Kose , S. ; Esra O.; Zeynep G. ; Gulgun A. ; Halide T. ; Neval A. ; Onder E. and Recep O. (2016). *Enterobacter Cloacae* Sepsis Outbreak in Neonatal Intensive Care Unit Due to Contaminated Total Parenteral Nutrition Solution. Journal of Pediatric Researches . ; 3(2):109-12.

2•Mezzatesta, M.L.; Gona F. and Stefani S. (2012). *Enterobacter cloacae* complex: clinical impact and emerging antibiotic resistance. Future Microbiology .; 7: 887-902.

3•Trevino, M.; Moldes L.; Martínez-Lamas L.; Varon C. and Regueiro B.(2009). Carbapenem resistant *Enterobacter cloacae* and the emergence of metallo-β-lactamase-producing strains in a third-level hospital .European Journal of Clinical Microbiology and Infectious Diseases.; 28(10):1253-8.

4•Antonio, C.; Maria L.; Antonino O.; Francesca D.; Giuseppina B.; Chiara I.; Stefania S. and Salvatore G.(2014). Extended-spectrum beta-lactamase-producing and carbapenemase- producing *Enterobacter cloacae* ventriculitis successfully treated with intraventricular colistin. International Journal of Infectious Diseases.; 20 : 66–67.

5 •Potron, A. ; Poire L. ; Rondinaud E.; and Nordmann P. (2013). Intercontinental spread of OXA-48 β-lactamase producing Enterobacteriaceae over a 11 year period 2001 to 2011. European surveillance. 18:2054.

6 •Akbari , M. ; Bita B. and Shahin N. (2016). Particular Distribution of *Enterobacter cloacae* Strains Isolated from Urinary Tract Infection within Clonal Complexes. Iranian Biomedical Journal.; 20(1): 49-55.

7 •CLSI, Clinical and Laboratory Standards Institute (2017). Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. M100. Wayne, PA .

8 •Harald Hoffmann and Andreas Roggenkamp(2003). Population Genetics of the Nomenspecies *Enterobacter cloacae*. Applied and environmental microbiology. ; 69(9): 5306–5318.

9 •Sambrook, J.; Fritsch E. and Maniatis T. (1989). Molecular Cloning: Cold Spring Harbor Laboratory Press Cold Spring Harbor, NY.

10 •Yahya, A. Abbas and Ghosoon(2016). Rapid Identification of *Enterobacter* spp. Isolated from hospital in Basrah province by Automated system (Vitek2 compact). International Journal of Micro Biology, Genetics and Monocular Biology Research .; 2(2) : 9-20.

11 •Andersen, J.; Asmar, B. I. and Dajani, A. S.(1994). Increasing *Enterobacter* bacteremia in pediatric patients. Journal of Pediatric Infectious Diseases .; 13:787-792.

12 •Melissa, S. ; Kate E. ; Sasha R. ; Matt M. and Luiz E. (2012). Beta-Lactam Antibiotic Resistance among *Enterobacter* spp. isolated from infection. Advances in Microbiology.; 2 : 129-137.

13 •Wisplinghoff, H.; Bischoff T.and Tallent S. (2004). Nosocomial bloodstream infections in us hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clinical Infections Disease.; 39: 309–317.

14 •Conceicao, T.; Faria , N.; Pimentel , M.; Soveral, G. and A. Duarte .(2004). New Chromosomal AmpC ß-Lactamase in *Enterobacter cloacae*. Antimicrobial Agents and Chemotherapy.; 48(4):1437 - 1445.

15 Salman, Hala Dawood (2006).Bacteriological study on local isolates of *Enterobacter cloacae* isolated from urine samples .M.Sc. thesis .Medicine collage .Babil university.

16 •Hoffmann, H.; Sturenburg E. ; Heesemann J. and A. Roggenkamp A.(2006). Prevalence of extended-spectrum b-lactamases in isolates of the *Enterobacter cloacae* complex from German hospitals. Clinical Microbiology and Infection.; 12: 322–**330.**

17 •Bush, K.; G. A. Jacoby and A. A. Medeiros(1995). A functional classification scheme for β -lactamases and its correlation with molecular structure. Antimicrobial Agents Chemotherapy.; 39: 1211-1233.

18 •Walsh, T. R. (2010). Emerging carbapenemases: a global perspective. International Journal of Antimicrobial Agents.; 36 (3) : 8-14.

19 •Noskin, G. A. (2005). Tigecycline: a new glycylcycline for treatment of serious infections. Clinical Infection Disease .; 41: 303-314.

20 •Kumari, S. and Sanjeev K.(2013).Isolation of microorganism from sample of diarrhea patient and effect of antibiotics and herbal extract. Journal of Microbiology.; 2 (2) : 24-32 .

21 •Lee, S. ; S. Kim ; Y. Cho; W. Shin; S. Lee ; C. Kim ; S. Hong ; B. Chung ; J. Kim and M. Yoon (2004). "A comparative multicentre study on the incidence of catheter-associated urinary tract infection between nitrofurazone-coated and silicone catheters." International Journal of Antimicrobial Agents.; 24 (1): 65-69.

22 •Nirbhavane, H. M. and Bagde U. S.(2017). Resistance by *Enterobacter* spp. towards several antimicrobial drugs and heavy metals. African Journal of Biotechnology.; 16(16): 826-841.

23 •Lee, C. T. (2007). Genetic methods for rapid detection of medically important nosocomial bacteria. M.SC. thesis . Department of Medicine. University of Sydney. Australia.

24 •Poirel, L.; Van De L.; Mammeri H.and Nordmann P.(2005). Association of plasmid mediated quinolone resistance with extended-spectrum β-lactamase VEB-1. Antimicrobial Chemotherapy.; 49:3091–3094.

25 •Armand, P.; C. Fluit ; J. Verhoef and Maurine A.(2006). Emergence of quinolone resistance gene in Dutch hospital. Emerging Infectious Diseases .; 12(5): 807-812.

26 •Walsh, C. (2003). Antibiotics: actions, origins, resistance. ASM Press, Washington, D.C, USA. pp3-143.

27 Brooks, G. F.; J.S. Butel and S.A. Morse (2001). Jawetz, Melnick and Adelberg's Medical Microbiology. 22nd ed. McGraw-Hill. U.S.A. pp: 197-202.

28 Moreira, C. G.; Palmer K.; Whiteley M.; Sircili M. P.; Trabulsi L.R.; Castro A. F. and Sperandio V. (2006). Bundle-forming pili and ESPA are involved in biofilm formation by enteropathogenic Escherichia coli. Journal of Bacteriology.; 188(11): 3952-3961.

29 •Gerk, P.M.; Kuhn R.J.; Desai N.S. and McNamara P.J. (2001) Active transport of nitrofurantoin into human milk. Pharmacotherapy.; 21: 669-675.

30 •David, L. and Derek F. (2001). Detection of -lactamase-mediated resistance. Journal of Antimicrobial Chemotherapy.; 48(1): 59–64.

31 •Tang, Y.; Ellis N.; Hopkins M.; Smith D.; Dodge D.and Persing D.(1998). Comparison of phenotypic and genotypic techniques for identification of unusual aerobic pathogenic gram-negative bacilli. Journal of clinical microbiology .; 36(12): 3674-3679.

32 •Teng, L. ; Hsueh p.; Tsai J. ; Chen P.; Hsu J.; Lai H.; Lee C. and Ho S. (2002). groESL sequence determination, phylogenetic analysis, and species differentiation for viridans group streptococci. Journal of Clinical Microbiology.;40:3172–3178.