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Research Article

Characterization of Some Multilocus Sequence Genes of *Serratia marcescens* in Nosocomial Infection

Alaa Salim Hamzah*, Basim raihan Sahar, Khalied Yassen zakair

Department of Anesthesia techniques, Middle Technical University, Baghdad, Iraq

ABSTRACT

This study aimed to detect characterization of some multilocus sequence genes of Serratia marcescens in nosocomial infection by use three important housekeeping genes were (*adk*, *gyrB*,*recA*) to help in new origin of epidimilogy nosocomial infection with *S. marcescens*. The study included collection two hundred fifty clinical samples from midstream urine and wound infection. After identification by morphological and biochemical tests and confirmation by VITEK- 2. the result showed found 43 isolates belong to Serratia marcescens (17.2%). DNA amplification of these genes that mention above carried out by specific primers. Sequencing of these genes of all bacterial isolates was sending to republic south korea (macrogen company). The results compared in NCBI by basic local alignment search tool and Analysis of sequencing of genes by using software program (Geneious version -9). In final conclusion molecular characterization of multilocos housekeeping genes (*adk*, *gyrB*, *recA*) in nosocomial Iraqi Serratia marcescens that documented in NCBI under accession numbers were (*adk* gene GenBank: LC647801.1 in LOCUS LC647801, GenBank: LC647802.1 in LOCUS LC647802. *GyrB* gene GenBank: LC647803.1 in LOCUS LC647803, GenBank: LC647804.1 in LOCUS LC647804. *recA* gene GenBank: LC647805.1 in LOCUS LC647805, GenBank: LC647806.1 in LOCUS LC647806) explained different levels of genetic relationship as anew alleles that similarity and dissimilarity in different levels with nearly sequencing of same these genes from another countries in the world were (USA, China, Finland, Canada, brazil, Taiwan and United kingdom) that documented in global data base in NCBI.

Keywords: Molecular characterization; MLST; Housekeeping genes; Epidemiology; Evolutionary

INTRODUCTION

S. marcescens is gram negative bacteria from group enterobacteriaceae. This opportunistic pathogen discovered in 1918 by Italian pharmacist call Bizio [1,2]. These bacteria live in different ecological system is a ubiquitous environmental microorganism like water [3,4]. *S. marcescens* is causal agent of different nosocomial infection like neonates infection, urinary tract infection, respiratory tract infection (pneumonia), skin infection (wound and burn infection), blood stream infection (bacteremia and septicemia), brain infection (meningititis), endocarditis and osteomylitis. Intensive care unit in hospitals contain many of epidemic sources of in infection with bacteria like equipment, lotions, antiseptics, medications, blood products in different ratio [5,6].

These bacteria able to occur mortality as a result of different nosocomial infection and risk factors which morality ratio is high in infection like bacteremia, septicemia, meningitis, endocarditis [6,7]. The major point of *S. marcescens* opportunistic pathogen is ability to spread in the hospital environment because it is have potential for expressing and disseminating antibiotic resistance, combining intrinsic mechanisms and acquired antimicrobial genes. *S. marcescens* produce different virulence factors that increase from pathogenicity like attachment factors, toxins and enzymes that able it from resistance antibiotics. Also it produce prodigiosin pigment that color pink to dark red and is consider main character in identification of these bacteria [5,7,8].

This study objected to detect characterization of Some multilocus sequence genes of *Serratia marcescens* in nosocomial infection by use three important housekeeping genes were (*adk*, *gyrB*,*recA*) to help in new origin of epidimilogy nosocomial infection with *S. marcescens*. All these three genes that mention above encode for essential protein like *recA* protein by *recA* gene that play multifunctional role in recombination and regulation process in these bacteria (ATP/GTP binding motif), *gyrB* gene encode for DNA gyrase subunit β, *adk* gene encode for adenylate kinase that regulate phosphorylation process in bacterial cell.

Correspondence to: Alaa Salim Hamzah, Department of Anesthesia techniques, Middle Technical University, Baghdad, Iraq; E-mail: Hamzah@fedpolel.ir

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MATERIALS AND METHODS

Experimental design

Nosocomial samples collection

This study included collection two hundred fifty clinical samples from midstream urine and wound infection through period from January into March 2021. The samples were distributed in different hospitals in Baghdad city from different age of patients.

Identification of pathogenic bacteria

The clinical specimens transported to laboratory by transport media. Then streaked on enriched and selective media were blood and MacConkey agar. Incubation period for 18-24 h at 37oC. Diagnosis of isolates according to morphological and biochemical test depend on MacFaddin, (2000) [9]. Certainly identification of *Serratia marcescens* Carried out by VITEK- 2 Compact system (Biomerieux/France) to use the identified bacteria in next experiment.

DNA extraction and gel electrophoresis

DNA extraction of *Serratia marcescens* isolates by (wizard® genomic DNA purification kit, Promega company, USA) according to manufacturer's instructions. Then amplification steps of three housekeeping genes (*adk*, *gyrB*,*recA*) by using specific primers and program condition as shown in table 1. Then gel electrophoresis of amplified genes against control DNA ladder marker (1500 bp) from promega company and recorded product size of amplicon as shown in figure 1.

Table 1: Details of primers of housekeeping genes (*adk* , *gyrB* , *recA*).

Name of Gene	Type of Primer	Sequences (5′ to 3′)	Annealing Temperature
adk gene	Forward primer	gggcgctggtaaaggtactc	60 °C
	Reverse primer	ctgccgcttctttgctgtag	
gyrB gene	Forward primer	gcggtaaattcgacgacaac	60 °C
	Reverse primer	tcatcgcggtacggaaac	
recA gene	Forward primer	gcgctggatcctatctatgc	55 °C
	Reverse primer	cttcgccgtacatgatttgg	

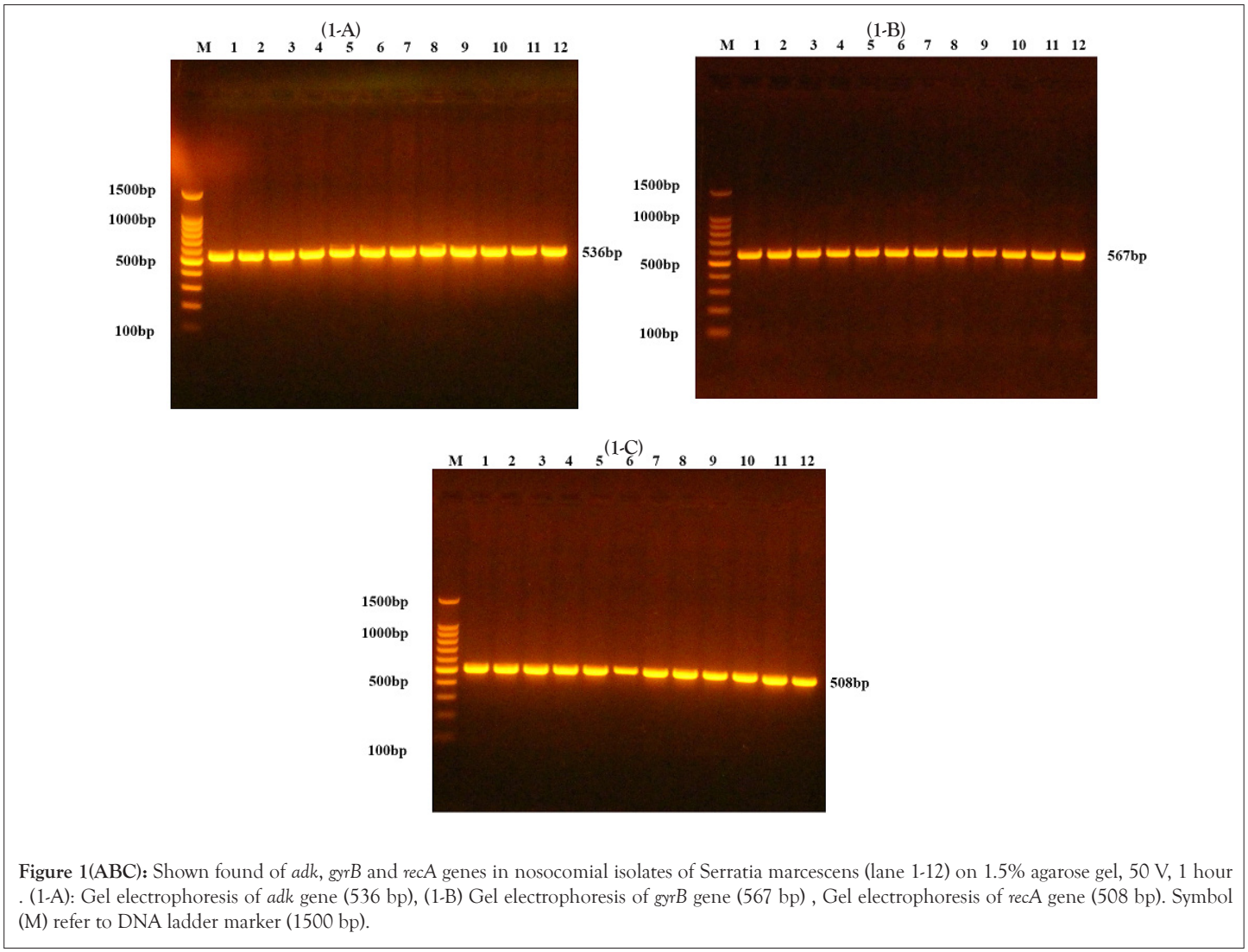

Figure 1(ABC): Shown found of *adk*, *gyrB* and *recA* genes in nosocomial isolates of Serratia marcescens (lane 1-12) on 1.5% agarose gel, 50 V, 1 hour . (1-A): Gel electrophoresis of *adk* gene (536 bp), (1-B) Gel electrophoresis of *gyrB* gene (567 bp) , Gel electrophoresis of *recA* gene (508 bp). Symbol (M) refer to DNA ladder marker (1500 bp).

Characterization of housekeeping genes (*adk, gyrB, recA*)

Molecular characterization of housekeeping genes was achieved by sent product of PCR of nosocomial *Serratia marcescens* isolates to macrogen company in republic south Korea to DNA sequencing of all target genes (*adk*, *gyrB, recA*) by ABI3730XL, automated DNA sequences. Then received results of sequencing by Email and compared in NCBI by basic local alignment search tool. Analysis of sequencing of genes that mention above carried out by using software program (Geneious version-9) to detect genetic relationship and origin of these nosocomial *Serratia marcescens* isolates as well as documented very important isolates that have new distinguishing matters in national center for biotechnology information.

RESULTS

Identification of *Serratia marcescens*

The results of bacterial identification in two hundred fifty nosocomial samples included (150 samples in midstream urine, 100 samples in wound infection) showed found 43 isolates belong to *Serratia marcescens* (17.2%).

Content of housekeeping genes (*adk, gyrB, recA*) in *Serratia marcescens*

DNA content of all isolates of *Serratia marcescens* was extracted and electrophoretically on 1.5% agarose gel. The results showed all isolates (100%) contain target genes that were *adk*, *gyrB*, *recA* in different product size compared with DNA ladder marker (1500 bp).

Interpretation of sequencing genes (*adk, gyrB, recA*)

Sequencing process of housekeeping genes (*adk, gyrB, recA*) was carried out of all nosocomial *Serratia marcescens* isolates to detect multilocus sequence of these genes that lead to attempt to distinguish origin of these bacteria (stream of evolutionary).

Documentation in national center for biotechnology information

Registration Iraqi nosocomial *Serratia marcescens* in NCBI achieved. Sequencing of six Iraqi strains to three housekeeping genes (*adk*, *gyrB, recA*) documented in NCBI. The documentation included two strain of *adk* gene GenBank: LC647801.1 in LOCUS LC647801, GenBank: LC647802.1 in LOCUS LC647802. Sequencing of *gyrB* gen registered under accession numbers GenBank: LC647803.1 in LOCUS LC647803, GenBank: LC647804.1 in LOCUS LC647804. Sequencing of *recA* gene documented in GenBank: LC647805.1 in LOCUS LC647805, GenBank: LC647806.1 in LOCUS LC647806.

DISCUSSION

Nosocomial infection by *Serratia marcescens* very important clinically because increase average of infection and epidemiology by ability of these bacteria to cause different diseases like urinary tract infection, pneumonia, meningititis, bacteremia, septicemia, skin infection and other nosocomial infection [10]. This study included collection two hundred fifty nosocomial specimens distributed into (150 samples from UTI, 100 samples from wound infection), after laboratory identification there are 43 isolates belong to *Serratia marcescens* (17.2%) distributed into 25 isolates from urine (16.66%), 18 isolates from wound infection (18%).

Detection origin of epidemiology and evolutionary of these nosocomial *Serratia marcescens* infection focused on molecular characterization of Some multilocus sequence genes of these bacteria in nosocomial infection by using three very important housekeeping genes were (*adk*, *gyrB*,*recA*) to help in new origin of epidimilogy these bacterial isolated from Iraq. The result showed that all *Serratia marcescens* nosocomial isolates contain these genes that mention above in percentage (100%) in different product size of PCR (amplicon) were (*adk* 536 bp), (*gyrB* 567 bp), (*recA* 508 bp) that refer to important of these genes to the continuously epidemiology of these bacteria in nosocomial infection.

Housekeeping gene (*adk*) encode for adenylate kinase that regulate phosphorylation process in bacterial cell by catalyzes the reversible ATP dependent phosphorylation of AMP to ADP and dAMP to dADP as well as catalyze the conversion of nucleoside diphosphates to the corresponding triphosphates. There are found that ability of T4 bacteriophage to produce ndk with mutation in E.coli and act implying that adenylate kinase can meet a demand for deoxyribonucleoside triphosphates that increases by up to 10-fold as a result of T4 infection, also original *adk* gene can complement a site specific disturbance of ndk.

Another housekeeping gene (*gyrB*) encode for DNA gyrase subunit β. Types of DNA gyrase subunit A and B play important role in bacteria DNA metabolism. Also they are introduces negative superhelical twists into bacterial chromosomes and maintains a particular level of supercoiling. These proteins consider have major principle function in DNA replication and play role in bacterial conjugation process that include horizontal transport of genes from species to another.

Gene recA is also typically constitutive gene carried out in this study that play multifunctional roles in recombination and regulation process in ATP/GTP binding motif. Also this enzyme play role in the homologous pairing and exchange of DNA, ATP and DNA-dependent co-proteolytic processing of effector proteins, interaction with mutagenic protein factors to facilitate error-prone DNA synthesis past DNA lesions. Another function of *recA* protein help in general genetic recombination, regulation of the co-ordinated expression of many unlinked genes in response to DNA damage called SOS response, the mistake-prone replicative by pass of DNA lesions lead to mutagenic DNA repair system.

Molecular characterization of housekeeping genes (*adk*, *gyrB, recA*) after detection sequencing of these genes of nosocomial *Serratia marcescens* isolates and alignment by basic local alignment search tool in NCBI. Analysis of phylogenetic tree of these genes compared with another nearly sequencing in different countries by software program (Geneious version -9). The results showed found genetic relationship in different levels between genes of Iraqi nosocomial *Serratia marcescens* and strains in these countries.

The results of evolutionary phylogenetic tree of sequence *adk* gene in *Serratia marcescens* in the Iraqi strains that documented in NCBI under accession numbers were (LC647801, LC 647802) have different sequence of *adk* gene compare with comparison with nearly sequence of *adk* gene in countries (USA, China, Finland). Result of evolutionary phylogenetic tree of sequence *gyrB* gene in *Serratia marcescens* in the Iraqi strain under global accession number (LC647803) have similarity with sequencing of same gene in strain found in china, but another Iraqi strain under global accession number (LC647804) have similarity in sequence *gyrB* gene comparison with nearly sequencing in Canada and china in

two separated groups.

Evolutionary phylogenetic tree of sequence *recA* gene in *Serratia marcescens* in Iraqi strain under accession number (LC647805) that documented in NCBI high degree of similarity comparison with nearly global strains in China, Brazil and Taiwand), but another Iraqi strain under accession number (LC647806) in NCBI exhibited similarity of nucleotide sequencing *recA* gene comparison with strain from United kingdom. All these genetic relationship and allele variation in sequencing housekeeping genes that mention above may be belong to effect of mutation, difference of isolation clinical sources and ecology effect in all these countries that lead to difference of MLST of these genes in all these countries comparison with MLST of Iraqi strains that documented in NCBI.

MLST consider very important molecular method that lead to detect origin, evolutionary and epidemiology of *Serratia marcescens* and anotherbacteria through studying multilocos sequencing of housekeeping gene of these bacteria. There are variation in sequence of housekeeping gene led to variation in number of alleles, for example average of thirty alleles per locus allow about many genotypes (about twenty billion) may be resolved that due to recombination replacement.

CONCLUSION

Molecular characterization of multilocos housekeeping genes (*adk*, *gyrB*, *recA*) in nosocomial Iraqi *Serratia marcescens* that documented in NCBI under accession numbers were (*adk* gene GenBank: LC647801.1 in LOCUS LC647801, GenBank: LC647802.1 in LOCUS LC647802. *gyrB* gene GenBank: LC647803.1 in LOCUS LC647803, GenBank: LC647804.1 in LOCUS LC647804. *recA* gene GenBank: LC647805.1 in LOCUS LC647805, GenBank: LC647806.1 in LOCUS LC647806) explained different levels of genetic relationship as anew alleles that similarity and dissimilarity in different levels with nearly sequencing of same these genes from another countries in the world were (USA, China, Finland, Canada, brazil, Taiwan and United kingdom) that documented in global data base in NCBI.

REFERENCES

1. Jang TN, Fung CP, Yang TL, Shen SH, Huang CS, Lee SH. Use of Pulsed-field Gel Electrophoresis Toinvestigate an Outbreak of *Serratia marcescens* Infection in a Neonatal Intensive Care Unit. J Hosp Infect. 2001; 48(1):13-9.
2. Montagnani C, Cocchi P. *Serratia marcescens* Outbreak in a Neonatal Intensive Care Unit: Crucial Role of Implementing Hand Hygiene among External Consultants. BMC Infect Dis. 2015; 15(11): 1-5.
3. Zingg W, Soulake I, Baud D, Huttner B, Pfister R, Renzi G, et al. Management and Investigation of a *Serratia marcescens* Outbreak in a Neonatal Unit in Switzerland–the Role of Hand Hygiene and Whole Genome Sequencing. Antimicrob Resist Infect Control. 2017;6(1):1-6.
4. Åttman E, Korhonen P, Tammela O, Vuento R, Aittoniemi J, Syrjänen J, et al. A *Serratia marcescens* Outbreak in a Neonatal Intensive Care Unit Was Successfully Managed by Rapid Hospital Hygiene Interventions and Screening. Acta Paediatr. 2018;107(3):425-429.
5. Redondo-Bravo L, Gutiérrez-González E, San Juan-Sanz I, Fernández-Jiménez I. *Serratia Marcescens* Outbreak in a Neonatology Unit of a Spanish Tertiary Hospital: Risk Factors and Control Measures. Am J Infect Control. 2019;47(3):271-9.
6. Rossen JW, Dombrecht J, Vanfleteren D, De Bruyne K, van Belkum A, Rosema S, Lokate M, et al. Epidemiological Typing of *Serratia Marcescens* Isolates by whole-Genome Multilocus Sequence Typing. J Clin Microbiol. 2019;57(4):e01652-18.
7. Yeo KT, Octavia S, Lim K, Lin C, Lin R, Thoon KC, Tee NW, Yung CF. *Serratia marcescens* in the Neonatal Intensive Care Unit: A Cluster Investigation Using Molecular Methods. J Infect Public Health. 2020;13(7):1006-11.
8. Ferreira RL. Characterization of Kpc-producing *Serratia Marcescens* in an Intensive Care Unit of a Brazilian Tertiary Hospital. Front Microbiol. 2020;13(7):950-956.
9. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG. Primer3-new Capabilities and Interfaces. Nucleic Acids Res. 2012;40(15):e115.
10. Casolari C, Pecorari M, Fabio G. A Simultaneous Outbreak of *Serratia marcescens* and Klebsiella pneumoniae in a Neonatal Intensive Care Unit. J Hosp Infect. 2005;61(4):312-20.