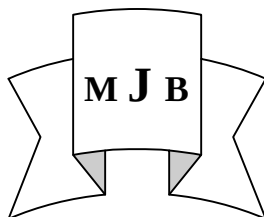


Molecular Investigation of Phylogeny Groups of *Morganella morganii* Isolated from Catheter Associated UTI

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Abstract

This study included, 100 urine samples obtained from patients suffering from Catheter-associated Urinary Tract Infection (CAUTI) and admitted to Hilla Teaching Hospital for Surgery for the period from October 2010 to February 2011 in Hilla city. Out of 78 positive cultures, only three isolates showed positive for *Morganella morganii* (3.8 %), one from male and two from females.

Phylogeny markers used in detection of *Morganella* phylogeny were *E.coli* phylogeny markers. These include *yjaA*, *chuA* and *TspE4C2*. The results showed that one isolate was positive (amplicon) for *yjaA*, and two isolates for *TspE4C2* but none of them was positive for *chuA*. Since the isolates are positive to *yjaA* and *TspE4C2*. So, they belong to phylogenetic group A and B1 respectively (intestinal group). This may conclude that the *Morganella* source (in UTI) is intestinal.

الخلاصة

في هذه لدراسة تم الحصول على مائة عينة إدرار من المصابين بالتهاب المجاري البولية المصاحب للقسطرة البولية من المراجعين والراقدين في مستشفى الحلة الجراحي للفترة من تشرين الثاني ٢٠١٠ إلى شباط ٢٠١١ من مجموع ٧٨ عينة زرع موجبة وجد ان ثلاثة عزلات كانت عائدة للنوع *Morganella morganii* (٣,٨%)، واحدة من الذكور واثنان من الإناث . تم استخدام بعض المؤشرات الخاصة بدراسة العلاقات التطورية للتحري عن مصدر *Morganella morganii* في إصابات الجهاز البولي وقد أظهرت النتائج بان العزلات الثلاثة تعود إلى المجاميع التطورية (A و B1) وهذا يؤكد بان مصدر الإصابة هي من الجهاز الهضمي وليس من خارج الجهاز الهضمي .

Introduction

M*organella morganii* is a gram negative rod commonly found in the environment and in the intestinal tracts of humans, mammals, and reptiles as normal flora. Despite its wide distribution, it is an uncommon cause of community - acquired infection and is most often

encountered in postoperative, catheter - associated bacteriuria and other nosocomial settings [1, 2]. The genus *Morganella* belongs to the tribe Proteae of the family Enterobacteriaceae. Currently, *Morganella* contains only a single species, *Morganella morganii*, with two subspecies, *morganii* and *sibonii*. *Morganella morganii* was

previously classified under the genus *Proteus* as *Proteus morganii* [3, 4].

Phylogeny of *Morganella morganii* is yet not well – understood. However, molecular phylogeny has revealed that horizontal gene transfer plays an important and unexpected role in evolution. For *Enterobacteriaceae* such as *E.coli yja A* and *TspE4C2* are widely used for identification of bacterial phylogeny although the function of them are unclear. However, *chuA*, encoding a hem transport protein in *E.coli* O157:H7 [5]. According to these markers, it was shown that bacteria isolated from human sources are classified phylogenetically in to four groups: A, B1, B2, and D [6]. These phylogeny groups were defined by different combinations of these genes : i.e. group A : *yja A* positive , group B1 : *TspE4C2* positive ; group B2 : *chuA* + *yja A* positive or *chuA* + *yja A* + *TspE4C2* (all positive) ; group D: *chuA* + *TspE4C2* (positive) [7].

The aim of this study is to investigate the phylogeny of *Morganella* isolated from catheter associated urinary tract infection.

Patients and Methods

One hundred urine samples were collected from patients suffering from catheter associated UTI (CAUTI). The urine samples cultured on blood agar and MacConkey agar using calibrated standard loop and by biochemical tests. Isolates from cases with significant bacteriuria ($>10^5$ colony / ml) were identified using APi 20 E (Biomérieux, France).

The identification of these isolates depend on the main characteristics of this bacteria according to [8].

DNA extraction from Gram negative bacteria

Chromosomal and plasmid DNA were made according to the genomic and plasmid DNA purification kits supplemented by manufacturer company (Promega, USA).

Phylogenetic group determination

The phylogenetic group of each isolate was determined according to [5] by PCR of the genes *chuA* and *yjaA* and anonymous DNA fragment *TspE4.C2*. Each 25 μ l of PCR reaction mixture for PCR contained 2.5 μ l of upstream primer, 2.5 μ l of downstream primer, 2.5 μ l of nuclease free water, 5 μ l of DNA extraction and 12.5 μ l of master mix. Thermal cycler conditions are shown in the table (1). The primers used were *chuA*, *yjaA* and *TspE4C2* which generated 279, 211 and 152 bp fragments respectively. The amplification products were separated in 1% agarose gels containing ethidium bromide. After electrophoresis, the gel was photographed under UV light, and the isolates were assigned to the phylogenetic groups A (*chuA*-, *TspE4C2*-, *yjaA*+), B1 (*chuA*-, *TspE4C2*+, *yjaA*-), B2 (*chuA*+, *yjaA*+, *TspE4C2*+ or-) and D (*chuA*+, *yjaA*-, *TspE4C2*+) [7].

Table 1 Primers sequences and thermal cyclers conditions.

Markers	Oligonucleotide Sequences 5'- 3'	Size of amplicon (pb)	Thermal cyclers conditions
<i>yjaA</i> F <i>yjaA</i> R	TGAAGTGTCTCAGGAGACGCTG ATGGAGAATGCGTTCCTCAAC	211	95°C for 4 min 94°C for 30sec 59°C for 30sec 30 cycles 72°C for 30sec 72°C for 5min
<i>TspE4C2</i> F <i>TspE4C2</i> R	GAGTAATGTCTCGGGGCATTCA CGCGCCAACAAAGTATTACG	152	95°C for 4 min 94°C for 30sec 57°C for 30sec 30 cycles 72°C for 30sec 72°C for 5min
<i>chuA</i> F <i>chuA</i> R	GACGAACCAACGGTCAGGAT TGCCGCCAGTACCAAAGACA	279	95°C for 4 min 94°C for 30sec 57°C for 30sec 30 cycles 72°C for 30sec 72°C for 5min

Results and Discussion

Out of 78 positive cultures, only three showed positive for *Morganella morganii* (3.8 %), one from male and two from females.

The isolates were motile, non encapsulated, and cause β -hemolysis on blood agar media and non lactose fermented on MacConkey agar. However, the isolates were shown to be catalase positive, oxidase negative, urease positive and negative to gelatinase. Also, the isolates were found to be unable to ferment the sugars: Lactose, Mannitol, Sorbitol, Sucrose, Arabinose , Inositol, Rhamnose , Melibiose, but they were able to ferment Glucose only with gas. API 20E was used to confirm the results of identification and the results of API 20E come with those obtained

in traditional tests. It should be noted that although *Morganella morganii* was urease and indole positive, it does not swarm and was negative for most of the biochemical reactions related to the *Proteus* spp.

The low prevalence of this bacteria when compared to other bacteria causing UTI such as *P. mirabilis* is mainly due to the slow growth rate of *M. morganii* in urine compared to *P. mirabilis* and the non inducible nature of its urease may delay the ability of bacteria to grow [9], however, this study was not in agreement with local study which indicated that the rate of isolation was approximately 10% [10].

Molecular detection of *Morganella* phylogeny

Phylogeny markers used in detection of *Morganella* phylogeny were *E.coli* phylogeny markers. The results showed that one isolate was positive (amplicon) for *yjaA*, and two isolates for *TspE4C2* but none of them was positive for *chuA* (figure - 1). According to these results *Morganella* isolates belong to phylogenetic groups A and B1 respectively which are intestinal group. This may conclude that the *Morganella* source was intestinal.

There are no previous studies done in Iraq for determination of the source of *Morganella* in human diseases. However, molecular markers are used for detection the

source of this bacteria which isolated from catheter associated UTI. The markers *yjaA*, *TspE4.C2*, and *chuA* are the common once used in identification of Enterobacteraceae at phylogenetic levels.

It was seen that *Morganella* isolates were belonging to the phylogeny groups A and B1. The results of PCR showed that *yjaA* was detected in the first isolate but *TspE4.C2* and *chuA* were not, so according to phylogeny classification [7], the isolate no.1 was belonged to phylogeny group A. On the other hand, *TspE4.C2* marker was detected in the two

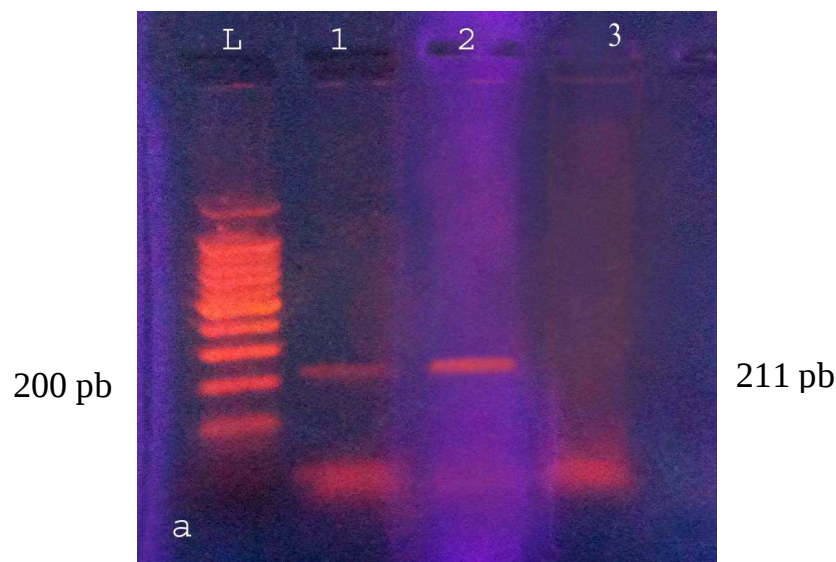


Figure 1 (a) Gel electrophoresis of PCR product of *yjaA* marker.

L, allelic leader; lane 1, *E.coli* as positive control; lane 2, chromosomal DNA of isolate no.1(+ve); lane 3, chromosomal DNA of isolate no2(-ve).

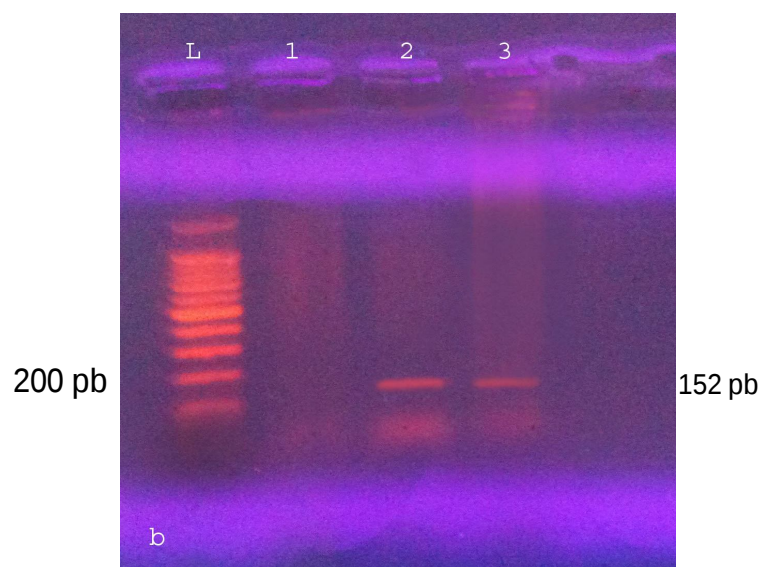


Figure 1 (b) Gel electrophoresis of PCR product of *TspE4C2* marker: L, allelic leader; lane 1, chromosomal DNA of isolate no.1(-ve); lane 2, chromosomal DNA of isolate no.2(+ve); lane 3, chromosomal DNA of isolate no. 3(+ve).

Other isolated bacteria but *yjaA* and *chuA* were not detected, so, the

isolates no.2 and 3 belong to the phylogeny group B1 (table -2).

Table 2 phylogeny groups of *Morganella*

Isolate No	Phylogenetic Markers			Phylogeny Group
	<i>ChuA</i>	<i>yjaA</i>	<i>TspE4C2</i>	
1	-	+	-	Group A (Intestinal source)
2	-	-	+	Group B1 (Intestinal source)
3	-	-	+	Group B1 (Intestinal source)

Since *Morganella* isolates belong to the phylogeny group A and B1, the

source of these isolates is often from the intestine. This will confirm that

Morganella isolates are present normally in the intestine as a normal flora and may cause UTI in human incidentally. Also, this study confirmed that the genes involved in determination of the phylogeny may indicate that *Morganella* was close to *E.coli* phylogenetically.

Other studies showed that phylogenetic trees showing the evolutionary related of Enterobacteraceae based upon many genetic markers. One of these markers is *yjaA*, *TspE4.C2* and also *chuA* [11,12].

Actually, phylogenetic groups can be determined by multilocus enzyme electrophoresis or ribotyping, both of which are complex and time consuming techniques [13].

A simple and rapid phylogenetic grouping technique based on PCR described by [5]. This method, uses a combination of two genes (*chuA* and *yjaA*) and an anonymous DNA fragment, showing excellent correlation with reference methods and obtained an accuracy of more than 99% compared to the reference method.

Phylogenetic analyses have shown that *E.coli* strains fall into four main phylogenetic groups (A, B1, B2, and D) and that virulent extra-intestinal strains belong mainly to group B2 and, to a lesser extent, to group D, whereas most commensal strains belong to group A. These methods have also given us a better understanding of how pathogenic strains acquire virulence genes [14,15,16].

According to the data obtained in this study, it was concluded that *E.coli* and *Morganella* phylogeny

were related each to other and this may confirm that *Morganella* is positioned close to *E.coli* and may be for somewhat to *Proteus* group and *Morganella morganii* isolates were belonging to phylogeny group A and B1.

Besides, phylogeny markers determine the source of *Morganella* infection in CAUTI was confirmed to be intestinal source.

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