Isolation and Characterization of *Morganella morgani* from Alkaline Urine.

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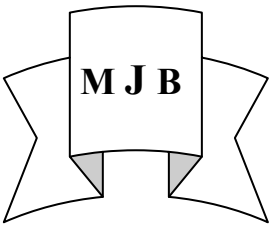

Abstract

A total of 75 alkaline urine samples obtained from patients with urinary tract infections, only six isolates of *Morganella morgani* were isolated. All these isolates were urease producers, which was produced constitutively & was responsible for changing the pH of urine into alkaline.

Also, some isolates were able to produce haemolysin (two isolates), uronic acid (one isolates) and morganocin (one isolates).

Furthermore, Antimicrobiol sensitivity tests were performed on *Morganella* isolates & they showed high resistance rates against the commonly used antimicrobial agents: ampicilline, amoxicilline, trimethoprim, nalidixic acid & cephalexin, where as all isolates were sensitive at high rates to ciprofloxacin & cefotaxim which nowadays used successfully in treatment of UTI. This work was the first one performed one *Morganella morgani* in Hilla province Iraq.

الخلاصة

تم جمع ٧٥ عينه إدرار قاعدية من مرضى يعانون من اصابات المجاري البولية وقد عزلت (٦) سلالات من بكتريا الموركانيلا وقد وجد ان جميع السلالات منتجة لانزيم اليوريز بشكل طبيعي والذي يلعب دورا" مباشرا" في تحويل باهاء الإدرار الى الباهاء القاعدي . وقد اظهرت النتائج قابلية عز نتيجة من أنتاج الهيمولايسين ، وعزل واحدة في انتاج كا من حامض اليورونيك والموركانوسين إضافة لذلك لوحظ ان السلالات كانت مقارنة بدرجة كبيرة لكل من المضاد الحيوي للسيروفلوكساسين والسيفوتاكسيم اللذان يستعملان بنجاح في معالجة الإصابات البولية . هذا البحث يعد هو الأول خول بكتريا الموركانيلا في مدينة الحلة .

Introduction

Morganella morgani is gram negative bacteria, closely related to proteus [1] . This bacteria is found in natural flora of gasterointestinal system and it is a rare cause of infection without predisponding factors [2].

Morganella morgani is frequently associated with urinary tract

infection, pnemonia , bacteriaemia and peritonitis . The infection causes by this bacteria is generally a slowly progressive ongoing process cause with remissions and attacks [3].

Clinical isolates of *morginella morgani* are usually resistant to multiple antibiotics particularly to B-lactam antibiotics [4]

Morganella morgani isolation and it's virulence factors have not been previously studied in any region in Iraq , so , the aim of this study is to isolate and identify *Morganella morgani* from patients with UTI and study some virumlence associated factors and also to show the effect of some antibiotics on bacterial isolates .

Materials of methods

Seventy-five urine samples were collected from patients suffering from UTI. The pH of all the samples was above 7. Mid. stream urine samples cultured on blood agar and maconkey agar using a calibrated standard loop. Isolates from cases with significant bacteriuria ($>10^5$ colony /ml) were identified using APi 20 (bio merieux, France) General urine examination was also performed to study the cytology of the samples.

Antibiotics susceptibility patterns were determined by the stokes disc diffusion method [5] performed on diagnostic sensitivity test agar plates.The isolates were tested against ampicillin, cephatexin, gentamicin, ciprofloxacin, amikacin, nalidixic acid, amoxicilline, trimethoprim & cefotaxim.

Urease activity was detected by using urea broth with or without addition urea.

Haemolysin activity was tested by using human blood agar.Bacteriocin morganocin production &detection was performed according to the method described by Shannon &Graham[6].

Uronic acid synthesis was carried out on minimal media (Mq)&the acid was estimated in the supernatants by using the colometric method [7] standard curve was performed by using various consentrations of galacturonic acid &absorbance was read at 530nm.

Result and Discussion

Six isolates of *Morganella Morgani* were recovered from 75 mid-stream urine samples obtained from patients with urinary tract infections. The pH of urine samples is alkaline, ranging from 7.8-8.3 . So any urine samples with pH less than or equal to 7 are discarded in this study.

General urine examination was performed for each sample and it was

seen that most urine samples contained pus cells, crystals and epithelial cells .

Urease production

Urease was produced by all *Morganella* isolates and the supernatants obtained from the culture media contained high urease activity without adding urine as inducer .This means that urease production was constitutive and not inducible, like urease produced by proteus Spp.[8] Urease produced by *Morganella* is very important virulence factor and there is an evidence that this enzyme was responsible for chemical changes in urine Which resulted in formation of strovite stones .

Morganocin synthesis

Furthermore ,the isolates of *Morganella* were subjected for synthesis the bacteriocin, (morganocin) and the results were showed that only one isolate was able to produce this agent and the rest failed in the production of it extracellularly. However , the synthesis of bacteriocin was not inducible & only two isolates of *Morganella* were sensitive to it .

The synthesis of morganocin was investigated by some researchers and they stated that this product was used in typing of *Moganella* strains [9] .

Morganocin was considered as spreading factor which can contribute in facilitating the spread of this bacteria in it's environment and also it's synthesis as pharmaceutical agent may play a role in restriction and in treatment of *Moganella* infections [10] .

Haemolysin activity

Two isolates of Morganella were showed Haemolysin activity (ß- haemolysis) on human blood agar .this result was correlated with those results obtained by senior and Hughes [11] who indicated that some *Moganella morgani* strains had the ability to produce Haemolysin in the blood agar.

Haemolysin was considered as an important factor for uptaking iron from environment and it was found that 4 isolates had no ability to produce this protein ,it means that there are another mechanism to obtain iron such as siderophores production which are produced by many species related to Entero bacteriaceae.

Uronic acid production

Only one isolate of *Moganella* was found to have the ability to produce uronic acid in the culture media .Uronic acid was detected by the colorometric method. This acid was very important virulence factor for the encapsulated bacteria and this capsular

acid play arole in both pathogenecity of bacteria and in it's adherence [12]. Negative stain was performed for this the producer of uronic acid and it was observed under microscopic examination that this isolate contains a capsule around it. The presence of capsule also plays arole as antiphagocytic agent [7].

The results obtained about the virulence factor are summarized in table 1

Table 1 Summary for some virulence associated factors of Morganella

Characteristics	No. of isolates (producers)	notes
urease	All isolates	The enzyme was produced constitutivly without addition urea
Morganocin (bacteriocin)	One isolates	Isolates no.2 produces this pacteriocin without induction and the two isolates (3 and 4) are sensitive to it.
haemolysin	Two isolates	The activity of haemolysin was shown on human blood agar, by only the isolate no. (1 and 5)
Uronic acid	One isolates	Uronic acid produced by isolate no.4 which was associated with presence of capsule.

Antimicrobial activity

Nine types of antibiotics were used to show their effect on Morganella isolates. (Table 2).

This study showed a high prevalence of antibiotic resistance among *Moganella* isolates .For instance, the percentage of the isolates to ampicilline, amoxicilline and caphalexin was 100%where as to getamicin , trimethoprim and nalidixic acid was 83.4% . in contract to the relatively low rate of resistance for ciprofloxacin and cefotaxime (16.6% for each). On the other hand some isolates showed multiple resistance to more than one antibiotics (ranging from5-8 antibiotics). This high percent of multiple drug resistance observed in this study causes speculation that it may be determined by a common transferable factor which spreads among enterobaeteriacea family [13].

The wide use of antibiotics due to high incidence of infecious diseases may play a role in revealing high level of

resistant to antibiotics by *Moganella* strains .However it was seen that amikacin usually had an effect on *Morganella* isolates (50%),although recent data had shown high levels of resistance to amikacin [14].

Resistance to commonly prescribed antibiotics is an expanding global problem and has been observed in both developed and developing countries .

We recommended that physicians seek updated knowledge of the common antibiotic sensitively patterns when starting empirical antibiotic therapy in Iraqi patients with.

Table 2 effect of some antibiotics on *Morganella* isolates.

Isolate no.	AP	Amx	Tm	Cp	CFT	GN	NA	CL	AMK
1	+	+	+	-	-	+	+	+	+
2	+	+	-	-	-	+	+	+	-
3	+	+	+	-	-	-	+	+	-
4	+	+	+	-	+	+	+	+	+
5	+	+	+	+	-	+	+	+	-
6	+	+	+	-	-	+	-	+	+
Resistant %	100	100	83.4	16.6	16.6	83.4	83.4	100%	50%

+ resistant - sensetive

Ap: Ampicillin ; (25µg); Amo: Amoxicilline (10µg), Tm: Trimethoprim (5µg) .

CP: Ciprofloxacin (5µg), CFT: Cefotaxime (30µg: Nalidixic acid (30µg); CL: Cepholexin (30µg); AMK: Amikacin (30µg).

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