Bacteriological and genetic study on Gram negative bacteria isolated from urinary tract infection of diabetic women in Hilla

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Abstract:

Nineteen isolates of Gram negative bacteria were isolated and identified out of 43 urine samples of diabetic women. The isolates showed a high resistance to antibiotic ampicillin, amoxicillin and carpinicillin, and intermediate resistance to gentamycin, kanamycin, chloramphnicol and naldixic acid, while show low resistance to ciproflaxin, and doxycyclin. Also they showed no ability to produce bacteriocin except only one isolate of *Klebsiella pneumonia* which had the ability to produce bacteriocin. The plasmid profile of the isolates showed that 10 isolates have mega plasmid but only one isolate have small plasmid.

دراسة بكتريولوجية و وراثية للبكتيريا السالبة لصبغة غرام المعزولة من اخماج السبيل البولي للنساء المصابات بداء السكري في الحلة

الخلاصة:

تم عزل وتشخيص ١٩ عزلة عائدة لمجموعة البكتريا السالبة لصبغة غرام من اصل ٤٣ عينة ادرار لنساء مريضات بالسكري و مصابات بالتهابات السبيل البولي. اظهرت العزلات الكتيرية مقاومة عالية لمضادات الامبسلين والاموكسلين والكاربنسلين ومقاومة متوسطة لمضادات الجنتامايسين والكانامايسين و الكلورامفنيكول و حامض النالديسك، في حين اظهرت مقاومة واطئة لمضادات السبروفلاكسين والدوكسي سايكلين . اظهرت العديد من العزلات القدرة على انتاج عوامل الاستيطان او الاستعمار من النوعين الاول والثالث وانتاج الهيمولايسين والسايدوفور في حين لم تتمكن أي من العزلات باستثناء عزلة واحدة تابعة لبكتريا الكلبسيلا الرئوية من انتاج البكتريوسين. اظهرت نتائج الترحيل الكهربائي للدنا البكتيري امتلاك ١٠ سلالات منها على بلازميد مشترك كبير واحتواء سلالة واحدة فقط على بلازميد صغير.

Introduction:

Urinary tract infection (UTI) is the most common of all bacterial infections seems to affect persons during their life time, starting with an incidence of 1% in the neonatal age group. This increases to its peak during the reproductive age group. Females are more likely to be affected than males, except in the neonates, where the trend is reversed (Sen *et al*. ,2006). Many different microorganisms can infect the urinary tract, but by far the most common agents are gram negative bacilli, *Escherichia coli* causes approximately 80% of the cases, other gram negative rods including *Proteus*, *Klebsiella, Enterobacter* and *Pseudomonas* account for a smaller proportion of uncomplicated infection, Gram positive cocci play lesser role in urinary tract infection.(Waler and Marvin, 1980).Diabetes mellitus (DM) has reached epidemic proportions world wide. Many chronic complications of DM, including neuropathy, retinopathy and nephropathy, have been well studied and although urologic complications have been recognized since 1935, little is known about DM as a pathophysiological risk factor for development of lower urinary tract symptoms in women (Hill *et al*., 2008). Urinary tract infections are more frequent in diabetic patients than in non-diabetics, or take a

more severe course. The difference is more pronounced in women both in symptomatic infections and asymptomatic bacteriuria. The spectrum of pathogens is similar to that of non-diabetic patients (Ludwig, 2008). Patients with diabetes mellitus have a higher prevalence of asymptomatic bacteriuria and incidence of urinary tract infections compared with patients without DM. It has been suggested that the presence of glucosuria can explain this increased incidence, but this has never been scientifically confirmed. Furthermore, UTIs in diabetic patients are mostly considered as complicated UTIs and therefore experts recommend treating them for longer than UTIs in non-diabetic patients (Geerlings, 2008).

Materials and methods:

A. Samples collection and bacterial diagnostic:

Thirty four urine samples were collected from diabetic women in Hilla surgical hospital during the period October until December 2008, and cultured on MacConkey 's agar. Bacteril diagnostic was studied as described in (Holt *et al*.1994 and Macfaddin, 2000).

B. Detection of virulence factors:

1.Capsule was studies by negative staining method described by Stukus (1997).

2. Hemolysin production was studied by culturing the isolates on blood agar and incubation for 24 hour at 37C and noticing the type of hemolysis.

3. Sidrophore production was studied by culturing the isolates M9 agar containing 0.2 mili molar 2,2-dipyidyl and incubation for 24 hour at 37C and noticing the isolates ability to grow (Nassif and Sansonetti, 1987).

4. Colonization factors I and III were studied by hemagglutenation test in the presence of D-Mannose and Tannic acid as described in Symth (1982).

5. Bacteriocin production of all the isolates was studied by Abbot and Shannon method modifided by Abbot and Graham, with the use of *E. coli*, *Staphylococcus auerus* and *Klebseilla pneumonia* as a test strains.(Abbot and Graham, 1961)

6. Antibiotic resistance was studied by Kirby-Bauer method described in Stukus (1997).

C. Genetic content study

Total DNA of all the bacterial isolates were extracted by salting out method described by Pospiech and Neuman (1995), the chemical reagent used in the study as described by (Sambrook and Russell, 2001). Electrophoresis for the extracted DNA was done in agarose electrophoresis unit from (Labnet international corporation, Korea) under 60 V, 20 mA at room temperature for 3 hours with agarose concentration 1%, with. Then agarose gel was stained with ethidium bromide and placed in UV light transllluminator, submitted to 256 nm wavelength and photographed by 7.2 Mega pixel digital camera (Sony-Japan).

Results and discussion:

Nineteen isolates of Gram negative bacteria were identified and diagnostic of 43 urine samples, these isolates were identified according to their morphological, cultural and biochemical properties. The results revealed that 7 isolates belong to *Klebsiella pneumonia*, 5 isolates to *Escherichia coli*, 4 isolates to *Pseudomonas aeroginosa* , 2 to *Proteus spp* and only one isolate of *Enterobacter spp*.The isolation rate of the Gram negative bacteria was 44.186 %, distributed as 36.84% of *K. pneumonia*, 26.31% of *E.*

coli, 21.05% of *Pseudomona*s, 10.526% of *Proteus* and 5.263% of *Enterobacte*r , these rates showed an increase in the incidence of *K. pneumonia* compare to Al-Rubaiy (1994) who found that *E. coli* represent 82.8% , *K. pneumonia* 8.1% , *Proteus spp* and *Pseudomonas* 0.9% from pregnant UTI patients and Bonadio *et al*.(1999) who found that *E. coli* represent 56.1%, *Proteus spp* 7.9% *Pseudomonas* 6.7% of the bacterial causal of UTI in diabetic patients , Jasim (2006) who found that *E. coli* represent 29.87%, *Klebsiella spp* 28.57%, *Enterobacter spp*12.98% of the of UTI in menopausal women.The ability of bacterial isolates to produce virulence factors was investigated and the results are shown in Table (1), the isolates were tested for their abilities to produce capsule, and it was found that 100% of both *K. pneumonia* and *Enterobacter*, 80% of *E. coli*, 50% of *Pseudomonas* contain a polysaccharide capsule, that is known to mediate specific or non specific adherence of bacteria to particular surfaces and also protect bacteria from engulfment by predatory phagocytes and from attack by antimicrobial agents (Todar, 2008). Also, it was found that 50% of *Pseudomonas* ,42.86% of *K. pneumonia* and 20% of *E. coli* have the ability to produce hemolysin, which contribute to invasion through its cytotoxic effect on the eukaryotic cells (Peterson, 2009), and 100% of the *Pseudomonas* and *Enterobacter* isolates, 80% of *E. coli* , 57.14% of *K. pneumonia* and 50% of *Proteu*s produce sidrophores, which are low molecular weight iron chelators produced by bacteria to capture iron bound to the host proteins from the host (Podschun and Ullmann, 1998). Other searchers found that 30% of *Pseudomonas* and 0% of *Klebsiella* isolated from ostitis media patients produce hemolysin and 100% of both of them produce sidrophores (Al-Waeli *et al*., 2009).The ability of the isolates to produce colonization factor (CF) I and III as a major virulence factor that help in the bacterial adhesion and colonization to the uroepithial cells were investigated, and it was found that 100% of the *Enterobacter* , 80% of *E. coli* , 75% *of Pseudomonas*, 71.43% of *Klebsiella* and 50% of the *Proteus* isolates were able to produce CF I ; and 100% of the *Enterobacter* , 80% of *E. coli* , 75% of *Pseudomonas* and 71.43% of *Klebsiella* isolates were able to produce CF III . These result are close to the results of Bunyan (2006) who found that 100% of uropathogenic *E. coli* produce both of colonization factor I and III , and Al-Waeli *et al*. (2009) who found that 50% of *Pseudomonas* and 75% of *Klebsiella* isolated from ostitis media patients produce colonization factors. Johnson (1991) mention that virulence factors occur more frequently in uropathogenic *E. coli* than enteropathogenic isolates. *Proteus mirabilis* expresses different types of fimbriae have been shown to be associated with bacterial colonization of the lower urinary tract (bladder) and kidney (Zunino *et al*.,2003).

Table (1) Virulence factors detected in gram negative bacteria isolated from UTI of diabetic women

	Capsule	Hemolysin	Sidrophore	CFI	CFIII	Bacteriocin
K. pneumonia1	+	+	-	+	-	-
K. pneumonia2	+	-	-	+	+	-
K. pneumonia3	+	-	+	-	+	-
K. pneumonia4	+	+	+	+	-	-
K. pneumonia5	+	-	-	+	+	-
K. pneumonia6	+	-	+	-	+	-
K. pneumonia7	+	+	+	+	+	+
E. coli1	+	-	+	+	+	-
E. coli2	+	+	+	+	+	-
E. coli3	+	-	+	+	-	-
E. coli4	-	-	-	+	+	-
E. coli5	+	-	+	-	+	-

Pseudomonas1	-	+	+	+	+	-
Pseudomonas2	+	+	+	-	+	-
Pseudomonas3	-	-	+	+	+	-
Pseudomonas4	+	-	+	+	-	-
Proteus1	-	-	-	+	-	-
Proteus2	-	-	+	-	-	-
Enterobacter	+	-	+	+	+	-

The ability of the isolates of bacteriocin production was investigated and the results revealed that only one isolate of *Klebseilla* (*K. pneumonia 7*) was able to produce bacteriocin that inhibit the growth the *E. coli* test strain. While non of the *E. coli* , *Pseudomonas*, *Proteus* and *Enterobacter* isolates were able to produce bacteriocin that effect the test strains or may produce bacteriocin that effect other strains than the test strains. The antibiotic sensitivity test of the isolates Tables (2) and (3) revealed that the isolates were 100% resistant to ampicillin and amoxicillin, 84.21% resistant to carpenicillin, 63.13% resistant to cefataxim, 42.1% resistant to gentamycin kanamycin and chloramphenicol, 36.84% resistant to naldixic acid, 26.31% resistant to both tetracyclin and streptomycin, 10.52% resistant to deoxycyclin and 5.26% resistant to ciproflaxin. It also revealed that both *Klebseilla* and *Pseudomonas* isolates were more resistant to the tested antibiotic than other Gram negative bacteria.

Table (2) Antibiotic resistance Gram negative bacteria isolated from UTI of diabetic women

	Do	AX	AM	S	C	K	NA	TE	CIP	PY	CN	CTX
K. pneumonia1	-	+	+	-	-	-	-	+	-	-	-	-
K. pneumonia2	-	+	+	-	+	+	+	-	-	+	+	+
K. pneumonia3	-	+	+	+	-	-	-	-	-	+	-	+
K. pneumonia4	-	+	+	+	-	-	-	-	-	-	-	-
K. pneumonia5	+	+	+	-	-	-	-	+	-	+	-	+
K. pneumonia6	-	+	+	+	+	+	+	-	-	+	-	+
K. pneumonia7	-	+	+	-	-	-	-	-	-	+	-	-
E. coli1	-	+	+	-	-	-	-	-	-	+	-	-
E. coli2	-	+	+	-	-	-	-	-	-	+	+	-
E. coli3	-	+	+	-	+	+	+	-	-	+	+	+
E. coli4	-	+	+	-	+	-	-	-	-	+	-	-
E. coli5	+	+	+	-	-	-	-	+	-	+	-	+
Pseudomonas1	-	+	+	+	+	+	+	-	+	+	+	+
Pseudomonas2	-	+	+	+	-	-	-	+	-	+	+	+
Pseudomonas3	-	+	+	-	+	+	+	+	-	+	+	+
Pseudomonas4	-	+	+	-	+	+	+	-	-	+	+	+
Proteus1	-	+	+	-	-	+	+	-	-	+	-	+
Proteus2	-	+	+	-	-	-	-	-	-	-	+	+
Enterobacter	-	+	+	-	+	+	-	-	-	+	-	-

Do =deoxycyclin , AX= amoxicillin, AM= ampicillin, S= streptomycin, C= chloramphenicol, K= kanamycin, NA= naldixic acid, TE=tetracycline, CIP=ciproflaxin, PY= carpenicillin, CN= gentamycin, CTX= cefataxim

Table (3) The percentage of antibiotic resistance of the Gram negative bacteria isolated from UTI of diabetic women

	Do	**AX**	**AM**	**S**	**C**	**K**	**NA**	**TE**	**CIP**	**PY**	**CN**	**CTX**
K. pneumonia	**14.29**	**100**	**100**	**42.86**	**28.57**	**28.57**	**28.57**	**28.57**	**0**	**71.43**	**14.29**	**57.14**

E. coli	**20**	**100**	**100**	**0**	**40**	**20**	**20**	**20**	**0**	**100**	**40**	**40**
Pseudomonas	**0**	**100**	**100**	**50**	**75**	**75**	**75**	**50**	**25**	**100**	**100**	**100**
Proteus	**0**	**100**	**100**	**0**	**0**	**50**	**50**	**0**	**0**	**50**	**50**	**100**
Enterobacter	**0**	**100**	**100**	**0**	**100**	**100**	**0**	**0**	**0**	**100**	**0**	**0**
	10.52	**100**	**100**	**26.31**	**42.10**	**42.10**	**36.84**	**26.31**	**5.26**	**84.21**	**42.10**	**0**

The plasmid profile of the isolates Figure (1) showed that 9 isolates contain mega plasmid these isolates were *Klebseilla 3,4,6,7* , *E. coli 3,4,5* and *Pseudomonas 1,2,3* ; and that only 1 isolate (*Pseudomonas 4*) contain both small and mega plasmid. Hull *et al.* (1981) found that the expression of CFI in uropathogenic *E. coli* was carried by plasmids. Saunders *et al.*(1992) discovered the presence of a commom large plasmid about 90 kb in enterohemorrhagic *E. coli* which have similar properties and share a 23 kb DNA fragment with Inc-IIA plasmid R1 of the enteropathogenic *E. coli* . Also Johnson (1991) mentioned that the aerobactin system (type of sidrophores) in uropathogenic *E. coli* is encoded by a large conjugative colicin V plasmid. .Also there were increase in the incidence of B-lactamase and plasmid mediated AmpC enzyme (Robberts *et al*., 2009).

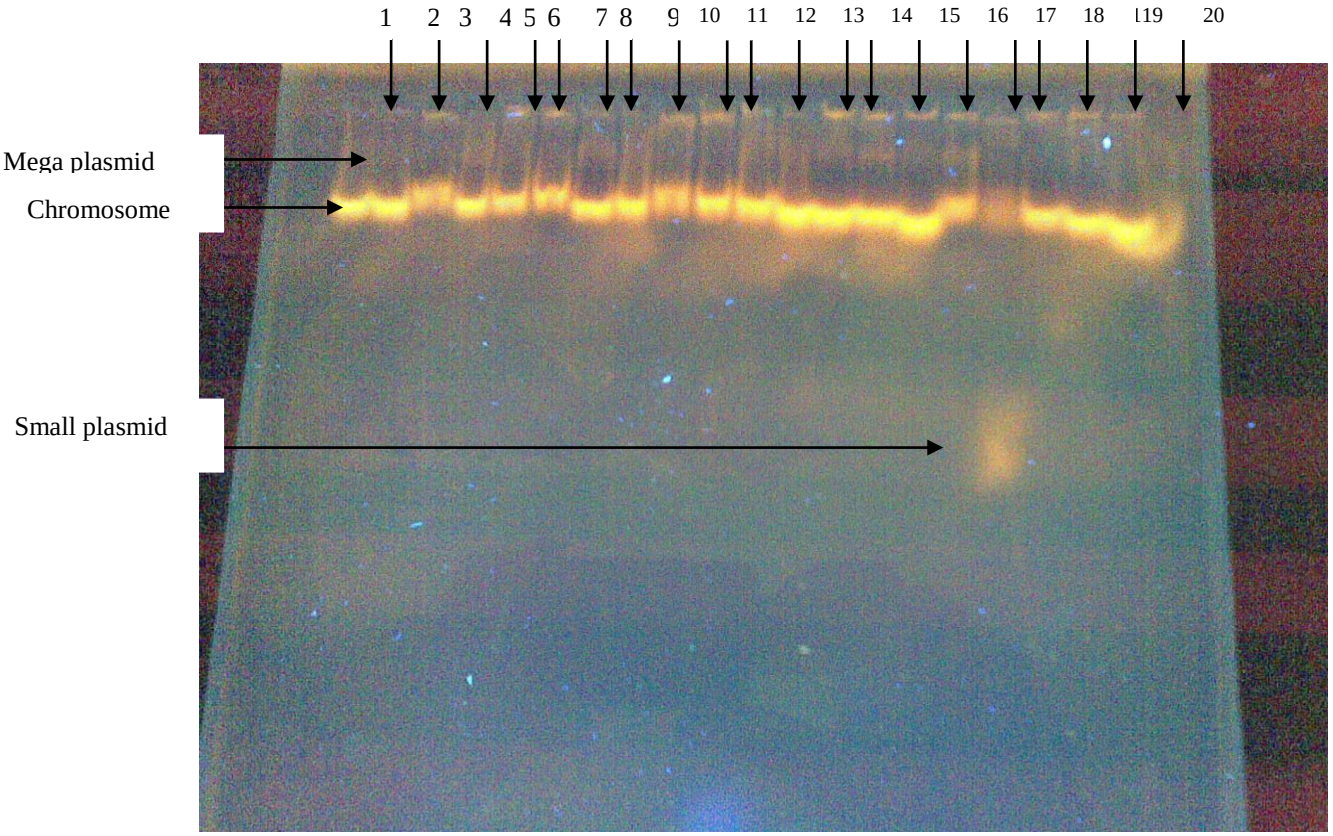

Figure (1) DNA content of Gram negative bacteria isolated from UTI of diabetic women (Agarose gel electrophoresis perfored with 1% agarose under 60V/20mA for 3

h)

Lane 1 *K. pneumonia1* **Lane 2 *K. pneumonia2***
Lane 3 *K. pneumonia3* **Lane 4 *K. pneumonia4***
Lane 5 *K. pneumonia5* **Lane 6 *K. pneumonia6***
Lane 7 *K. pneumonia7* **Lane 8 *E.coli1***
Lane 9 *E.coli2* **Lane 10 *E.coli3***
Lane11 *E.coli4* **Lane 12 *E.coli5***
Lane 13 *Pseudomonas1* **Lane 14 *Pseudomonas2***
Lane 15 *Pseudomonas3* **Lane 16 *Pseudomonas4***
Lane 17 *Proteus1* **Lane 18 *Proteus2***
Lane 19 *Enterobacter spp* **Lane 20 *E.coli* MM294**

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