Original Article

Detection of Escherichia Coli Strains among Bacteriuria of Diabetic and Non-Diabetic patients, With Their Antibiotic Resistant and Hematuria Containing Enterotoxin (Sea) Gene

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ABSTRACT

Objective: Detection of Escherichia coli Strains among bacteriuria of diabetic and non-diabetic patients
Study Design: Descriptive (cross-sectional) study
Place and Duration of Study: This study was conducted at the Department of Biology, College of Science, University of Basrah, Basrah, Iraq from 2nd March 2024 to 3rd April 2025.
Methods: Out of 42 Gram-positive and Gram-negative bacterial isolates from diabetic and non-diabetic patients, across 26 antibiotics, 89 pattern results were observed versus 83. Three Escherichia coli isolates that shared identical biochemical profiles and antibiotic resistance patterns were found to be genetically different strains using random amplified polymorphic DNA – polymerase chain reaction.
Results: The 22 bacteria isolated from diabetic patients showed that 17 (77%) had the Seagene, with 17 (100%) hematuria samples, compared to 13 (65%) with the Sea gene, with 8 (61%) hematuria among 20 bacteriuria samples from non-diabetic patients.
Conclusion: The bacteriuria from diabetic patients exhibited high resistance for Ticarcillin, Piperacillin and Ciprofloxacin antibiotics revealing a significant difference from that bacteriuria of non-diabetics' patients. There is a relationship between the presence of enterotoxin and hematuria.

Key Words: Urinary tract infection, diabetes, Sea gene, Hematuria, Antibiotic resistance

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INTRODUCTION

Urinary tract infections (UTIs) are more frequent and severe in diabetic patients due to impaired immunity, poor glycemic control, and bladder dysfunction. E.coli is the predominant pathogen, thriving in glucose-rich urine and causing recurrent infections, particularly in females.[1] Other uropathogens, including Klebsiella, Proteus, and Pseudomonas, are also common and often exhibit increased antimicrobial resistance.[2]

Automated identification and antimicrobial susceptibility testing using the VITEK 2 Compact system enables rapid and accurate detection through metabolic analysis with Gram-specific AST cards.[3]

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UTIs in diabetic patients pose a clinical challenge because common pathogens such as E. coli and Klebsiella pneumoniae frequently show resistance to widely used antibiotics, including third-generation cephalosporins and fluoroquinolones. Compared with non-diabetics, diabetic patients harbor more diverse microbial flora, including Proteus, Pseudomonas, and Enterococcus species, driven by glycosuria, immune dysfunction, and repeated antibiotic exposure.[4]

Random Amplified Polymorphic DNA analysis is widely used for strain differentiation, epidemiological tracing, and susceptibility pattern discrimination, enabling detection of genetic variation without prior genomic knowledge.[5] In addition, toxin production—particularly exotoxins and enterotoxins - plays a key role in bacteriuria pathogenesis in diabetic patients by damaging host tissues and disrupting immune responses. Common uropathogens, including E. coli and Enterobacter spp, may harbor genes such as the Seagene, encoding staphylococcal enterotoxins. PCR-based detection of these toxin genes provides valuable diagnostic and epidemiological insights.[6]

METHODS

This descriptive (cross-sectional) study was conducted at College of Science, University of Basrah from 2nd March 2024 to 3rd April 2025 vide letter. 3/6/95 dated 5th October 2022. Forty-two bacteriuria isolates as Gram-positive and Gram-negative were identified by 16S rRNA gene sequencing The data were analyzed for comparing among isolates from diabetic and non-diabetic patients enabling the detection of biochemical pattern and specific antibiotic resistance trends in each group.

Three E. coli isolates (No. 26, 38 and 42) that showed the identical biochemical patterns and antibiotic resistance were further examined to understand their genetic relationship. RAPD technique was used. The primers, PCR reagents and conditions for amplification followed the method described by Zare et al.[7] The RAPD-PCR reaction was prepared in a total volume of 25 µl consisting of 12 µl Go Taq green master mix (Promega, USA), 4 µl DNA template, 2 µl RAPD primer (Bioneer, Korea) and 7 µl nuclease-free water (Bioneer, Korea). The PCR program included the following thermal cycling conditions: an initial denaturation at 94°C for 5 minutes (1 cycle), followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 42°C for 1 minute and extension at 72°C for 2 minutes. A final extension was performed at 72°C for 5 minutes (1 cycle). The primer used for RAPD was 5'-AGAGGGCACA-3' (Macrogen, Southkorea). Detection of RAPD products using agarose gel electrophoresis was performed using 2% agarose in 100 ml of TBE buffer (Bioneer, Korea), with the addition of 0.5 µl of ethidium bromide (Biobasic, Canada). A 100 bp DNA ladder (Promega, USA) was used as a molecular marker to detect the banding patterns of E. coli strains under UV illumination using a transilluminator (Wisd, Korea). The distances between the RAPD bands of all isolates were calculated based on the DNA ladder using Microsoft Word. The data were then analyzed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to construct a dendrogram representing the genetic relationships among isolates.[8]

RESULTS

The isolates were further categorized into resistant (R) and sensitive (S). The Antibiotic resistance in Gr- ve bacteria showed the ability of 50 isolates resistant to 14 different antibiotics while 118 isolates were sensitive to them in diabetics group, while 64 versus 118 in non-diabetics, respectively, with significant differences ($P \leq 0.05$). In Gr+ ve bacteria 39 isolates were resistant to 12 different antibiotics versus 93 sensitive in diabetic, while in non- diabetic, 53 versus 19 respectively, with significant differences ($P \leq 0.05$). Generally, the study found that both Gr+ ve and Gr- ve bacterial isolates from diabetic showed resistance in 89 isolates to 26 different types of antibiotics versus 211 isolates were sensitive.

Table No. 1: The antibiotic susceptibility of different bacterial species in diabetic and non-diabetic cases

Type of bacteria	Antibiotic	Diabetic		Non-diabetic	
		S	R	S	R
Gram negative bacteria (n=25)					
Escherichia coli (n=8)	TIC	1	11	-	13
Escherichia fergusonii (n=4)	TIC/CLA	8	4	10	3
Klebsiella pneumoniae (n=4)	PIP	2	10	-	13
Citrobacter (n=1)	PIP/TAZ	10	2	10	3
Enterobacter hormaechei (n=2)	CAZ	6	6	8	5
Enterobacter bugandensis(n=1)	FEP	11	1	11	2
Raoultella ornithinelytica (n=2)	IPM	11	1	13	-
	MEM	11	1	13	-
Pluralibacterge	AMK	12	-	13	-
Enterobacter cloacae (n=1)	GEN	10	2	8	5
	TOB	10	2	8	5
	CIP	9	3	6	7
	COL	8	4	13	-
	SXT	9	3	5	8
Total (24)		**118***	**50**	**118**	**64**
Gram positive bacteria (n=17)					
Pseudomonas fragi (n=1)	BenzPen	5	6	2	4
Staphylococcus aureus (n=2)	OXA	6	5	2	4
Staphylococcus hominis (n=3)	MOX	8	3	5	1
Bacillus safensis (n=2)	ERY	8	3	4	2
	CLI	10	1	6	0
Bacillus velezensis (n=1)	LNZ	9	2	5	1
Coryne-bacterium aurimucosum (n=2)	TEC	9	2	5	1
Kocuria Rhizophila (n=1)	VAN	9	2	5	1
Staphylococcus saprophyticus	TET	7	4	5	1
Staphylococcus epidermidis (n=1)	TGC	9	2	5	1
Lachnospiraceae bacterium (n=1)	FUS	4	7	4	2
Candidatus Erwinia (n=1)**	RIF	9	2	5	1
Total (10)		**93***	**39**	**53***	**19**
Total (42)		**211***	**89**	**171***	**83**

*$P \leq 0.05$

**Candidatus Erwinia was recorded as Gr+ve of antibiotic susceptibility test only

Table No. 2: Comparison of biochemical test for E. coli isolates

Test	No. Escherichia coli isolates							
	Healthy					Diabetics		
	6	15	26	33	38	41	42	51
ADO	-	-	-	-	-	⊕	-	-
IARL	-	-	-	-	-	-	-	-
dCEL	-	-	-	⊕	-	⊕	-	-
BGAL	+	⊖	+	-	+	+	+	+
H2S	-	-	-	-	-	-	-	-
AGLU	-	-	-	-	-	-	-	-
BGLU	-	-	-	⊕	-	⊕	-	-
dMAL	+	⊖	+	-	+	+	+	+
dMAN	+	-	+	+	+	+	+	+
dMNE	+	-	+	⊕	+	+	+	+
BXYL	-	-	-	-	-	-	-	-
LIP	-	-	-	-	-	-	-	-
PLE	-	-	-	-	-	⊕	-	-
URE	-	-	-	-	-	-	-	-
dSOR	⊖	⊖	+	+	+	+	+	+
SAC	+	-	+	+	+	+	+	+
dTAG	-	-	-	-	-	-	-	-
dTRE	+	⊖	+	+	+	+	+	+
MNT	⊖	-	-	-	-	-	-	-
ILATK	+	⊕	-	-	-	⊕	-	⊕
AGAL	+	-	+	⊖	+	+	+	+
Total	8 (38%)	1 (4.7%)	8 (38%)	7 (33.3%)	8 (38%)	13(61.9%)	8 (38%)	9(42.8%)

Table No. 3: Distance matrix for bacterial strains (26, 38 and 42) based on RAPD analysis

Strains	26	42	38	1C*
26	-	138.448	102.711	0.000
42		-	103.450	138.448
38			-	102.711
1*				-

*Out group

Table No. 4: Frequency of Sea gene among different bacterial species

Bacteria species	Total	Diabetics (n = 22)			Non-diabetics (n=20)		
		No.	Sea	Haematuria	No.	Sea	Haematuria
Escherichia coli	8	3	3 (100%)	3 (100%)	5	3 (60%)	2 (40%)
Klebsiella pneumoniae	4	2	1 (50%)	2 (100%)	2	1 (50%)	-
Escherichia fergusonii	4	2	2 (100%)	2 (100%)	2	2 (100%)	1 (50%)
Staphylococcus hominis	3	1	1 (100%)	1 (100%)	2	1 (50%)	2 (100%)
Enterobacter hormaechei	2	1	1 (100%)	-	1	1 (100%)	1 (100%)
Raoultella ornithinolytica	2	1	1 (100%)	-	1	-	-
Corynebacterium aurimucosum	2	-	-	-	2	2 (100%)	1 (50%)
Bacillus safensis	2	2	1 (50%)	1 (50%)	-	-	-
Staphylococcus aureus	2	1	1 (50%)	1 (100%)	1	-	-
Kocuria rhizophila	1	1	1 (100%)	-	0	-	-
Bacillus velezensis	1	-	-	-	1	1 (100%)	1 (100%)
Enterobacter cloacae	1	-	-	-	1	1 (100%)	-
Mammaliicoccus vitulinus	1	1	1 (100%)	1 (100%)	-	-	-
Candidatus Erwinia	1	-	-	-	1	-	-
Enterobacter bugandensis	1	-	-	1 (100%)	1	1 (100%)	1 (100%)
Staphylococcus saprophyticus	1	1	-	1 (100%)	-	-	-
Lachnospiraceae bacterium	1	1	-	1 (100%)	-	-	-
Staphylococcus epidermidis	1	1	-	1 (100%)	-	-	-
Citrobacter amalonaticus	1	1	1 (100%)	1 (100%)	-	-	-
Micrococcus luteus	1	1	1 (100%)	1 (100%)	-	-	-
Pluralibacter gergoviae	1	1	1 (100%)	1 (100%)	-	-	-
Pseudomonas fragi	1	1	1 (100%)	-	--	-	-
Total	**42**	**22**	**17 (77%)**	**17 (100%)***	**20**	**13 (65%)**	**8 (61%)***

*P≤0.05

In contrast, non-diabetic showed 83 versus 171 respectively, with significant differences (P≤0.05) [Table 1].

Table 2 showed the results of biochemical production for all 8 Escherichia coli isolates, it was observed that only three isolates (No.26,38 and 42) showed consistent patterns across all biochemical production, these isolates were selected to test by RAPD analysis for further explore their genetic similarities to detect the strains level (Figs. 1,2, Table 3). By added an outgroup data (1C) to the profile sharing in the phylogenetic tree distance, the tree showed all the 3 isolates were from different strains.

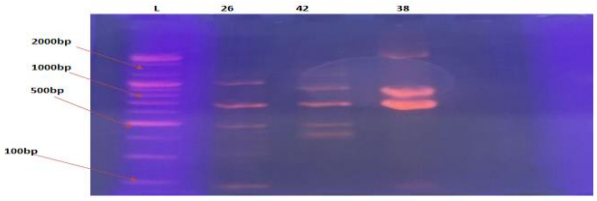

Figure No. 1: Agarose gel electrophoresis (2%) showing RAPD pattern of Escherichia coli Lane L: 100 bp Marker, Lane 26, 42 and 38: RAPD gene bands of Escherichia coli

Figure No. 2: Dendrogram of 3 bacterial strains (26, 38 and 42) constructed by a set of RAPD bands using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm. Boots trap values after 100 repetitions were indicated. Strain 26 and the out group (1C) were identical with a branch length of strain 26. No strains were identical with other

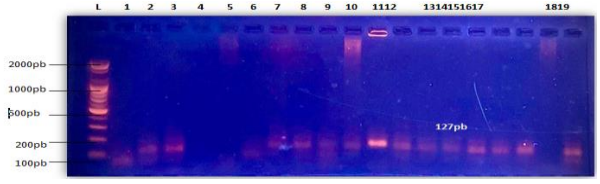

Figure No. 3: Agarose gel electrophoresis (2%) showing amplified Sea gene (127 bp)

Figure No. 4: Urine sample A shows visible hematuria with presence of blood in the urine, while sample B appears clear and free of any visible blood representing a normal urine sample.

The results of the Sea gene analysis showed its presence in 17 (77%) bacteriuria isolates out of 22 from diabetic and 13 (65%) isolates of 20 from non-diabetic cases with no significant differences (P≤0.05) [Fig. 3, Table 4]. Hematuria was observed in all the 17 isolates (100%) possessing the Sea gene from diabetic patients, suggesting an association between the presence of this gene and urinary tract damage in this group. In contrast, hematuria was present in only 8 (61%) of 13 (65%) of Sae positive in non-diabetic patients. There is a distinct difference between the two groups of patients in hematuria (P≤0.05) [Fig. 4].

DISCUSSION

Gram-positive and Gram-negative bacteria isolated from glucose-rich urine showed higher antibiotic sensitivity than resistance regardless of diabetic status, indicating that sugar fermentation alone does not explain antimicrobial response patterns. Gram-negative isolates such as Escherichia coli and Klebsiella pneumoniae demonstrated increased susceptibility, consistent with evidence that glucose enhances metabolic activity and vulnerability to antimicrobials.[9] This may result from altered transporter expression, stress-response pathways, and oxidative stress under fermentable glucose conditions. Similarly, Gram-positive isolates including Enterococcus faecalis and Planomicrobium showed elevated sensitivity, supporting previous findings of notable susceptibility patterns.[10] These observations indicate that antimicrobial responsiveness is not solely determined by cell wall structure but by interactions among metabolic pathways, host immunity, and antimicrobial exposure.[11]

Glucose-rich environments may enhance antibiotic uptake or modify resistance mechanisms[12], and may reduce biofilm-related virulence gene expression, as observed in Pseudomonas aeruginosa.[13] Overall, antibiotic responsiveness in UTI pathogens is multifactorial and influenced by metabolic stress, environmental composition, and antimicrobial pressure.[14] Although E. coli isolates (26, 38, and 42) shared biochemical and antimicrobial profiles, RAPD-PCR revealed distinct banding patterns, confirming genetic diversity. This demonstrates that biochemical similarity does not necessarily reflect genetic identity under diverse selective pressures.[10] Elevated urinary glucose, particularly in isolate 42 from a diabetic patient, may contribute to oxidative stress and increased mutation rates, promoting genomic variability and rearrangements.[15] Such environmental stressors and selective pressures can generate minor but significant genetic divergence among strains.[1]

Hematuria was more frequent in diabetic patients and was strongly associated with uropathogens such as E. coli and Klebsiella pneumoniae, likely due to glucose-enhanced bacterial growth and tissue damage leading to bleeding within the urinary tract.[4] Visible hematuria was observed in these infections, while other cases

were detectable only by urine tests. Hematuria in diabetics was also associated with Staphylococcus aureus, S. saprophyticus, and S. epidermidis, which produce hemolysins capable of lysing erythrocytes and irritating urinary tissues.[16] Enterobacter hormaechei was isolated from both patient groups, suggesting roles for host immune responses or bacterial genetic factors in tissue damage across different hosts. Conversely, glycosuria in diabetics may sometimes reduce tissue destruction and hematuria occurrence.[17]

The detection of Enterobacter bugandensis, a recently described pathogen, supports its role in urothelial adhesion and vascular permeability leading to hematuria. Staphylococcus hominis, detected in both groups, likely causes only mild irritation due to its lower virulence. The absence of significant differences between groups suggests that host immunity and bacterial behavior influence clinical severity.[18] These findings highlight the importance of identifying hematuria-associated uropathogens, particularly in diabetic patients.[19] Enterotoxin genes, particularly the Seagene, play a major role in UTI pathogenicity. The Seagene encodes staphylococcal enterotoxin A, a potent toxin capable of epithelial damage, necrosis, and inflammation.[6] In this study, the Seagene was detected in most isolates, except Candidatus Erwinia, Staphylococcus saprophyticus, Lachnospiraceae bacterium, and Staphylococcus epidermidis, consistent with variability in enterotoxin gene distribution. The presence of hematuria in several samples correlated with Seagene detection, suggesting toxin-mediated mucosal damage.[20]

CONCLUSION

The bacteriuria from diabetic patients exhibited high resistance for Ticarcillin, Piperacillin and Ciprofloxacin antibiotics revealing a significant difference from that bacteriuria of non-diabetics' patients. All the predominant E. coli were of different strains depending on genetical analysis. Importantly, the present of Seagene reveals to play arule in hematuria samples.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Zainab S. Baqer, Munaff J. Abd Al-Abbas
Drafting or Revising Critically:	Zainab S. Baqer, Munaff J. Abd Al-Abbas
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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