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INVESTIGATING THE RELATIONSHIP BETWEEN SOME VIRULENCE AND ANTIBIOTICS RESISTANCE GENES OF SOME LOCAL PATHOGENIC BACTERIA IN IRAQ

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ABSTRACT

Since the potential link between virulence genes and bacterial antibiotic resistance is of great importance in medical aspects, the properties of such a link were thoroughly investigated in this work. Sixty bacterial isolates were collected from patients with urinary tract infection (UTI) who visited the medical lab at the College of Science/the University of Al-Kufa. Preservation and sub-culture of these isolates were performed according to the guidelines of microorganism's care and the Research Committee of Al-Qasim Green University. Several phenotypic tests were performed involving hemolysis, haemagglutination, serum resistance, and biofilm formation. Genotypic analysis of virulence genes (biofilm and *Iss*) and antibiotics resistance genes (bla_{AmpC}, bla_{TEM}, $bla_{SHV\text{-}5}$, and $bla_{CTX\text{-}M}$) were measured using the polymerase chain reaction (PCR) technique. All isolates of *P. mirabillis* were extracted as positive for the serum resistance test with 100%, while a percentage of than less 14% was observed with isolates of *Pseudomonas aeruginosa*. From the acquired results of the biofilm formation test, it was revealed that all bacterial isolates exhibited negative results except in isolates of *E. coli*, *Proteus mirabillis*, and *Enterococcus faecalis*. As far as the genetic study is concerned, the biofilm gene was abundant and was also found in 95% of bacterial isolates followed by an increase in the serum survival (*Iss*) gene by 86.66%. Interestingly, the genotype study of antibiotic resistance genes showed that both bla_{AmpC} and bla_{TEM} had significantly greater prevalence in all isolates compared to $blaSHV_{\text{-}5}$ and $bla_{CTX\text{-}M}$ at $P \leq 0.05$. In addition, the percentages of the prevalence of bla_{AmpC} and bla_{TEM} were significantly higher (93.33 and 71.66), respectively, compared to the respective values found in blaSHV$_{\text{-}5}$ and $bla_{CTX\text{-}M}$ (20%, 46.66%). From the correlation findings, a significant constructive association between virulence and resistance to antibiotic profile was found (r=0.957, P=0.000). In conclusion, our work provides valuable insights for better understanding the potential link between genotypic (virulence genes) and phenotypic traits (antibiotic resistance). Future research holds the potential to deepen our comprehension of the intricate relationship between virulence genes and antibiotic resistance, thereby making substantial contributions to the development of more efficacious approaches for the management of bacterial infections. This recommendation extends to fellow researchers in the field.

Keywords: biofilm, beta-lactamase, *Iss* gene

INTRODUCTION

The microbial infection is usually measured using two routes. More specifically, the first detection could be performed from its virulence while the other could be traced back due to its resistance to antibiotics **(Uddin et al., 2021).** At the current period, the remarkable number of appointments yields an inconsistency of infections formed by microbes that can be retained even in the non-operative Lag phase. It is well-established that the increased resistance of antibiotics is possibly associated with the pathogenicity of bacteria with both G^{-ve} and G^{+ve}, which could subsequently increase or decrease virulence. Additionally, a specific species' resistance and virulence could also be traced back to the same genetic trait and transformed into the next generation (**Uddin et al., 2021**).
Microbial pathogenicity has been well described as all potential biochemical tools that could be used by a microbe can induce an infection (**Fierer et al., 2010**). This pathogenicity is usually controlled by several factors that are shared between the host and microbe, such as the host's immunity, virulence traits, microbial load, and microbial capacity against the host's protection mechanisms **(Leitão, 2020)**. The term virulence is utilized to express the scale of pathogenicity among diverse isolates that are related to similar types and is known as the cell dose required to cause a pathological reaction in the patient. Consequently, isolates with dissimilar scales of virulence may occur in similar types. Comparatively, it has been also suggested that a few virulence elements could be located in chromosomal areas, which are called pathogenicity islands (PAIs) and are known to be unstable though (**Desvaux et al., 2020**). In addition, these virulence elements could be also located in plasmids, which allow them to be transformed into other strains or even different species (**Given et al., 2022; Tian et al., 2021**). Consequently, one of the problems that the scientific community is currently facing is the bacterial resistance against antibiotics, which could be due to the variation of bacterial phenotypes (**Chinemerem Nwobodo et al., 2022; Tamhankar & Stålsby Lundborg, 2019**). Resistance, as well as virulence, are related in almost of the factors, which can be conveyed among microbes by horizontal gene transmission and transmission of DNA. The latter is possibly the best vital device for the distribution and assists in the selection of virulence and resistance characteristics (**Deng et al., 2019**).
Antibacterial resistance can be measured as a virulence feature in several pathogenic microorganisms by detecting the rising rates of antimicrobial resistance. On top of that, the existence of recognized virulence factors and the relation of the two characters might probably induce global rising virulence in these microbes. Along these lines, in this work, the possible genes that are driving the microbial virulence and antibiotic resistance of pathogenic bacteria were systematically investigated from several sources in order to evaluate the relationship among these genes.

MATERIAL AND METHODS

Bacterial isolates

Sixty isolates from pathogenic bacteria were isolated from patients with UTI. These isolates encompassed 16 isolates of *E. coli*, nine isolates of *Pseudomonas aeruginosa* and *Salmonella typhi*, five isolates of *Proteus mirabillis*, four isolates of *Klebsiella pneumonia*, *Shigella dysenteriae*, and *Serratia marcescens*, as well as three isolates of *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus pyogenes*. Preservation and sub-culture of these isolates were performed according to the guidelines of microorganism's care and the Research Committee of Al-Qasim Green University (ethics committee approval code: 533FD2).
To maintain the isolated bacteria for enough period for dropping their genomic features, a plate of nutrient agar (Himedia/ India) was mixed with one colony of the bacterium at 37 ºC. Then, this mixture was incubated with five microliters of nutrient broth and BHI (Himedia/ India) enriched with sterilized 15% glycerol (GCC/ Singapore) was inoculated with a single colony. Finally, the slants were stored at -20 ˚C for 6-8 months (**Ermenlieva et al., 2021**).

Table 1 The primers used in this study (BIONEER/ South Korea).

TARGET GENE	PRIMER SEQUENCE (5'- 3')	PRODUCT SIZE (BP)	PURPOSE	REFERECE
ISS	F GGCAATGCTTATTACAGGATGTGC R GAGCAATATACCCGGGCTTCC	260 bp	Detection of serum increase survival gene	Gharrah *et al*., 2017
BIOFILM	F GATTCAATTTTGGCGATTCCTG R TAATGAAGTCATTCAGACTCATCC	225 bp	Detection of biofilm gene	Gharrah *et al*., 2017
$BLA_{CTX\text{-}M}$	F AGCGATAACGTGGCGATGAA R TCATCCATGTCACCAGCTGC	247bp	Detection of Cefotaximase gene	Eftekhar *et al*., 2012
$BLA_{SHV\text{-}5}$	F CCGCCATTACCATGAGCGAT R AATCACCACAATGCGCTCTG	410bp	Detection of sulfhydryl variable gene	Eftekhar *et al*., 2012
BLA_{TEM}	F GGTGCACGAGTGGGTTACAT R TGCAACTTTATCCGCCTCCA	531bp	Detection of temoneira gene	Eftekhar *et al*., 2012
BLA_{AMPC}	F AAACGACGCTCTGCACCTTA R TGTACTGCCTTACCTTCGCG	670bp	Detection of ampicillin gene	Eftekhar *et al*., 2012

Phenotypic Detection of biofilm and *Iss* genes

The microtiter-plate test was carried out to detect the biofilm formation for all bacterial isolates as follows (**Stepanović et al., 2007**). Twenty µmicroliters of overnight isolated cultures were added to 180 µl of TSB (Oxoid/ England) and all inoculated in 96-microtitier wells plate (Immunochemistry Technologies, CA, USA), while the wells with negative control had broth only. Afterward, the growth was detached and the wells were further washed away with TSB and left for about fifteen minutes at room temperature. Additional 200 microliters of crystal violet (Thermo Scientific Chemicals/ UK) (1%) were added to each well for about twenty minutes. The produced biofilms were rewashed three times through PBS (Leinco Technologies/ USA) (pH 7.2), and left at 25 ºC for fifteen minutes. The optical density (OD) of the wells was measured at 630 nm to get more accurate readings. All samples were measured twice and the mean of the absorbance was calculated for all studied samples. A total of three standards of deviations (SD) over the mean of OD of the -ve control was used as a cut-off rate (ODc). The OD of an isolate was calculated by subtracting the OD of an isolate and ODc. The final results of biofilms isolates were categorized based on the following conditions:

- When (OD ≤ ODc), it implies that no-biofilm is produced.
- When (ODc < OD ≤ 2 x ODc), it implies that weak-biofilm is produced.
- When (2 x ODc < OD ≤ 4 x ODc), it implies that Moderate biofilm is produced.
- When (4 x ODc < OD), it implies that strong biofilm is produced.

The serum resistance was measured using a turbid metric method as follows (**Munkhdelger et al., 2017**): A total of fifty microliters of suspension of bacteria were added to the 150 collected microliters of serum and mixed in 96 well microplates. The bacteria were then examined in replica and also measured for -ve controls (0.9% NaCl as an alternative of serum) at 630 nm. The outcomes were compared with another one that was measured three hours later, the ultimate optical density was calculated as the mean of the two repeats, and the ratio residual optical density was extracted from the first optical density. If the residual absorbance was 100%, the isolates were considered serum resistant or sensitive if it was lower than 100% (**Kumar M et al., 2022**).

Genotypic detection of biofilm and *Iss* genes

All bacterial isolates were sub-cultured on nutrient agar and cultivated intended for 18-24 hours at 37 ºC. Five ml of nutrient broth in 60 sterile screwcaps (Scientific/ USA) (five ml for each screwcap) were prepared and autoclaved (Hiclave-Hirayama/ Japan) for 15 min. Next, they were inoculated by a single colony of each bacterial isolates that were previously prepared and then incubated intended for 18-24 hours at 37 ºC (**Mackie & McCartney, 1999**). The genomic DNA of isolates was taken out via chromosomal DNA Mini Kit following the instructions of the manufacturer (iNtron company/ Korea). The extracted genomic DNA was visualized by red safe (Chembio, UK) staining after gel electrophoresis on 1.3% of agarose (Bio basic/ USA) according to **(Sun et al., 2020)**. In addition, the concentration of isolated DNA was measured using nanodrop (Thermo Scientific/ UK). The mixture of PCR for virulence genes was made by using iNtron Kit/ Korea as follows Table 2**:**

Table 2 The component of Master mix in PCR technique

PCR MASTER MIX REACTION COMPONENTS		VOLUME
DNA TEMPLATE		2 µL (30 NG/UL)
PRIMERS	**F. primer**	1 µL (10 PMOL/UL)
	R. primer	1 µL (10 PMOL/UL)
PCR WATER		16 µL
TOTAL VOLUME		20 µL

while, the following PCR conditions were used for virulence genes that were made via conventional PCR thermocycler (Labnet/ USA) scheme as follows Table 3:

Table 3 The PCR conditions of biofilm and *Iss* gene for all bacterial isolates

BIOFILM GENE			
PCR CYCLE	repeat	Temp.	TIME
INITIAL DENATURATION	1	94C	2 MIN
DENATURATION	35	94C	40 SEC.
ANNEALING		48C	1 MIN
EXTENSION		72C	1 MIN
FINAL EXTENSION	1	72C	5 MIN
HOLD	-	4C	-
ISS GENES			
PCR CYCLE	repeat	Temp.	TIME
INITIAL DENATURATION	1	94C	2 MIN
DENATURATION	40	94C	40 SEC.
ANNEALING		50C	1 MIN
EXTENSION		72C	1 MIN
FINAL EXTENSION	1	72C	5 MIN
HOLD		4C	-

Finally, the PCR products of virulence genes were performed by loading in 1.3% agarose as mentioned by (**Sambrook & Russell, 2001**).

Genotypic detection of antibiotics resistance genes

The PCR master mix was prepared as described in the genotyping study of virulence genes table (2), while, the PCR thermocycler conditions for all antibiotics resistance genes were completed via a conventional PCR thermocycler scheme as follows table (4):

Table 4 The PCR conditions of antibiotics resistance genes of all bacterial isolates *BLA_{CTX-M}, BLA_{SHV-5}, BLA_{TEM} AND BLA_{AMPC}*

PCR CYCLE	repeat	Temp.	TIME
INITIAL DENATURATION	1	94C	5 MIN
DENATURATION	30	95C	30 SEC.
ANNEALING		58C	30 SEC.
EXTENSION		72C	1 MIN
FINAL EXTENSION	1	72C	5 MIN
HOLD	-	4C	-

The PCR products of all antibiotic resistance genes were evaluated via loading in 1.3% agarose, as was previously described in the detection of virulence genes according to (**Sambrook & Russell, 2001**).

Statistical Analysis

Statistical analysis was performed by using SPSS software package version 20 (the data were stated as standard in of frequencies and relative frequencies (percentages). The least significant difference (LSD) and Pearson correlation were also calculated at $p \leq 0.05$ and $p \leq 0.01$, respectively.

RESULTS

Phenotype results of virulence genes

The results of the biofilm formation test classified the bacterial isolates into three groups, strong biofilm, weak biofilm, and negative biofilm. Strong biofilm was observed in 83.33% of *E. coli* isolates, 50% of *Shigella dysenteriae*, *Streptococcus pyogenes*, and *Enterococcus faecalis*, whereas 40% of *Klebsiella pneumonia*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. The weak biofilm results were observed in 100% of *Proteus mirabilis*, 60% of *Salmonella typhi*, 50% of *Serratia marcescens* and *Enterococcus faecalis*, 40% of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, while 37.5%, 25%, and 16.67% were detected in *Shigella dysentery*, *Streptococcus pyogenes*, and *E. coli*, respectively. The negative biofilm results were perceived in all bacterial isolates except in *E. coli*, *Proteus mirabillis*, *Salmonella typhi*, and *Enterococcus faecalis*, as shown in Figure (1).
The bacterial isolates were also tested for serum resistance at an absorbance of 630 nm. From the acquired results, it was demonstrated that 100% of *Proteus mirabilis* isolates and 57% of *E. coli* isolates were positive, while 50% of *Shigella dysenteriae*, *Streptococcus pyogenes*, and *Enterococcus faecalis* isolates were positive. The other positive percentages were 33%, 25%, 20%, 17%, and 14% for *Staphylococcus aureus*, *Serratia marcescens*, *Klebsiella pneumonia*, *Salmonella typhi*, and *Pseudomonas aeruginosa*, respectively, as can be observed in Figure (2).

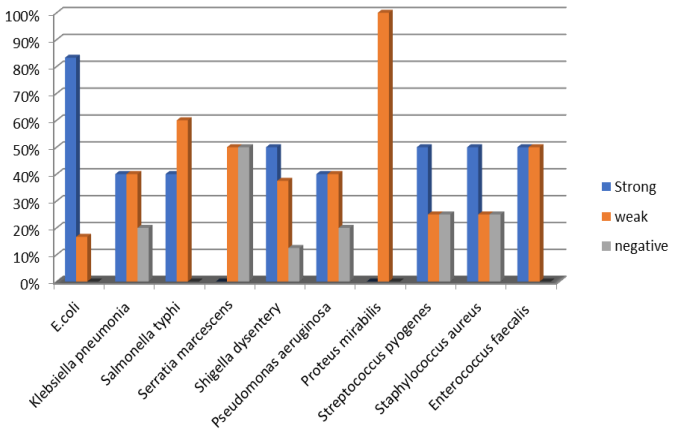

Figure 1 The percentage of biofilm formation test of bacterial isolates

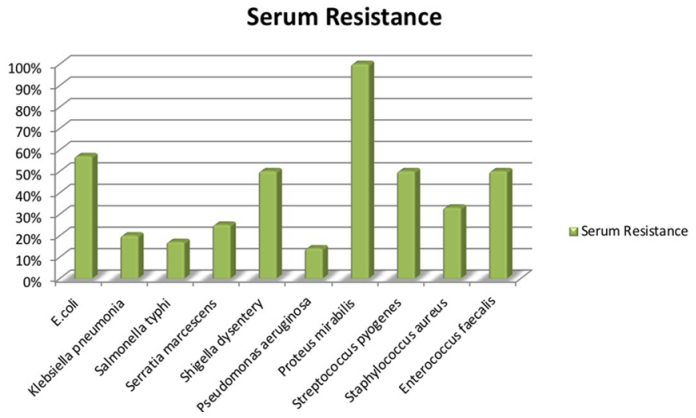

Figure 2 The percentages of serum resistance test of bacterial isolates.

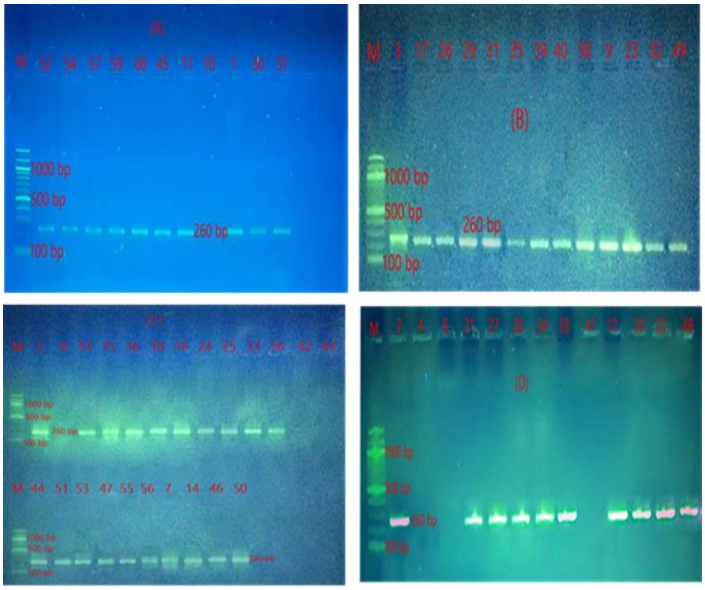

Figure 3 Products of polymerase chain reaction achieved through biofilm gene of bacterial isolates. M: 100 bp DNA ladder. *E. coli* (5, 6, 13, 15, 16, 18, 19, 24, 25, 33, 36, 42, 43, 44, 51, 53); *P. aeruginosa* (3, 17, 26, 29, 31, 35, 39, 40, 58); *S. typhi* (2, 4, 8, 21, 27, 28, 34, 38, 41); *Sh. Dysenteriae* (12, 20, 22, 48); *K. pneumoniae* (9, 23, 32, 49)*; S. aureus* (10, 11, 45), *E. faecalis* (47, 55, 56); *S. pyogenes* (1, 30, 37); *S. marcescens* (7, 14, 46, 50); *P. mirabillis* (52, 54, 57, 59, 60). The concentration of gel was 1.3% and the DNA dye was RedSafe. Electrophoresis conditions, V: 90, Time: 40 minutes.

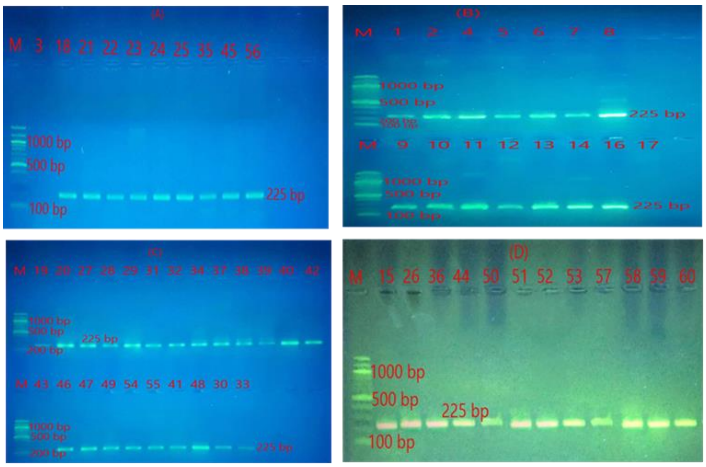

Figure 4 Products of polymerase chain reaction achieved through *Iss* gene of bacterial isolates. M: 100 bp DNA ladder. (A): *P. mirabillis* (52, 54, 57, 59, 60); *S. aureus* (10, 11, 45)*; S. pyogenes* (1, 30, 37). (B): *P. aeruginosa* (3, 17, 26, 29, 31, 35, 39, 40, 58)*; K. pneumoniae* (9, 23, 32, 49). (C): *E. coli* (5, 6, 13, 15, 16, 18, 19, 24, 25, 33, 36, 42, 43, 44, 51, 53); *E. faecalis* (47, 55, 56); *S. marcescens* (7, 14, 46, 50). (D): *S. typhi* (2, 4, 8, 21, 27, 28, 34, 38, 41); *Sh. Dysenteriae* (12, 20, 22, 48). The concentration of gel was 1.3% and the DNA dye was RedSafe. Electrophoresis conditions, V: 90, Time: 40 minutes.

Genotype results of virulence genes

The results of biofilm detection by PCR technique revealed that all isolates of *Proteus mirabilis*, *Klebsiella pneumonia*, *Enterococcus faecalis*, *Shigella dysenteriae*, *Salmonella typhi*, *Serratia marcescens*, *Staphylococcus aureus*, *and Streptococcus pyogenes* were positive in 100%, while 93.75% and 77.77% of *E. coli* and *Pseudomonas aeruginosa* were positive for the gene of interest respectively table (5). Moreover, all bacterial isolates yielded the same band size (225 bp), which was the product size of primers used for identification, as shown in Figure (3).

The increase in the serum survival (*Iss*) gene by PCR could explain the fact that the gene of interest was observed with 100% in all bacterial isolates of *Proteus mirabilis*, *Klebsiella pneumonia*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Shigella dysenteriae*, and *Streptococcus pyogenes*. Additionally, 81.25% of *E. coli*, 66.66% of *Staphylococcus aureus*, and 55.55% of *Salmonella typhi* indicated the presence of *Iss* gene table (5), where all these isolates provided the same band size (260 bp), as shown in Figure (4).

Table 5 The occurrence percentage of virulence genetic factor in bacterial isolates

BACTERIA	VIRULENCE GENES					
	Biofilm			*Iss*		
	Total No.	Positive	%	Total No.	Positive	%
E. COLI	16	15	93.75	16	13	81.25
S. AERUGINOSA	9	7	77.77	9	9	100
S. TYPHI	9	9	100	9	5	55.55
P. MIRABILLIS	5	5	100	5	5	100
K. PNEUMONIAE	4	4	100	4	4	100
SH. DYSENTERIAE	4	4	100	4	4	100
S. MARCESCENS	4	4	100	4	4	100
E. FAECALIS	3	3	100	3	3	100
S. AUREUS	3	3	100	3	2	66.66
S. PYOGENES	3	3	100	3	3	100
TOTAL	60	57	95	60	52	86.66

Genes of antibiotics resistance

The recognition of some antibiotic resistance genetic factors (β-lactamase genes) was also carried out using the multiplex PCR technique. The outcomes of the existing study revealed that bla_{AmpC} and bla_{TEM} were the most prevalent genes among all bacterial isolates, while $bla_{SHV\text{-}5}$ and $bla_{CTX\text{-}M}$ were the less prevalent genes at P≤ 0.05, as can be observed in table (6). In addition, the results showed that the percentages of β-lactamase genes varied in all bacterial isolates and the highest percentages were observed in bla_{AmpC} and bla_{TEM} in comparison with $bla_{SHV\text{-}5}$ and $bla_{CTX\text{-}M}$ table (7). The positive results of electrophoresis multiplex PCR product of β-lactamase genes appeared with 670 bp, 531 bp, 410 bp, and 247 bp for bla_{AmpC}, bla_{TEM}, $bla_{SHV\text{-}5}$, and $bla_{CTX\text{-}M}$, respectively, as shown the Figure (5).

Table 6 Statistical examination of the occurrence of β-lactamase genes among bacterial isolates

Type of gene		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
bla_{AmpC}	*blaTEM*	1.20000	1.01708	.2461	-.8627-	3.2627
	blaSHV-5	4.10000*	1.01708	.000	2.0373	6.1627
	blaCTX-M	2.50000*	1.01708	.019	.4373	4.5627
bla_{TEM}	bla_{AmpC}	-1.20000-	1.01708	.246	-3.2627-	.8627
	$bla_{SHV\text{-}5}$	2.90000*	1.01708	.007	.8373	4.9627
	$bla_{CTX\text{-}M}$	1.30000	1.01708	.209	-.7627-	3.3627
$bla_{SHV\text{-}5}$	bla_{AmpC}	-4.10000-*	1.01708	.000	-6.1627-	-2.0373-
	bla_{TEM}	-2.90000-*	1.01708	.007	-4.9627-	-.8373-
	$bla_{CTX\text{-}M}$	-1.60000-	1.01708	.124	-3.6627-	.4627
$bla_{CTX\text{-}M}$	bla_{AmpC}	-2.50000-*	1.01708	.019	-4.5627-	-.4373-
	bla_{TEM}	-1.30000-	1.01708	.209	-3.3627-	.7627
	$bla_{SHV\text{-}5}$	1.60000	1.01708	.124	-.4627-	3.6627

*. The mean alteration was significant at the 0.05 level

Table 7 The occurrence percentage of β-lactamase genes in bacterial isolates

BACTERIA	B-LACTAMASE GENES											
	bla_{AmpC}			bla_{TEM}			$bla_{SHV\text{-}5}$			$bla_{CTX\text{-}M}$		
	Total No.	positive	%	Total No.	positive	%	Total No.	positive	%	Total No.	positive	%
E. COLI	16	15	93.75	16	11	68.75	16	1	6.25	16	4	18.75
S. AERUGINOSA	9	9	100	9	6	66.66	9	4	44.44	9	6	66.66
S. TYPHI	9	8	88.88	9	4	44.44	9	1	11.11	9	6	66.66
P. MIRABILLIS	5	5	100	5	5	100	5	2	40	5	3	60
K. PNEUMONIAE	4	3	75	4	3	75	4	1	25	4	0	0
SH. DYSENTERIAE	4	3	75	4	3	75	4	1	25	4	1	25
S. MARCESCENS	4	4	100	4	4	100	4	0	0	4	2	50
E. FAECALIS	3	3	100	3	3	100	3	1	33.33	3	2	66.66
S. AUREUS	3	3	100	3	1	33.33	3	0	0	3	3	100
S. PYOGENES	3	3	100	3	3	100	3	1	33.33	3	1	33.33
TOTAL	60	56	93.33	60	43	71.66	60	12	20	60	28	46.66

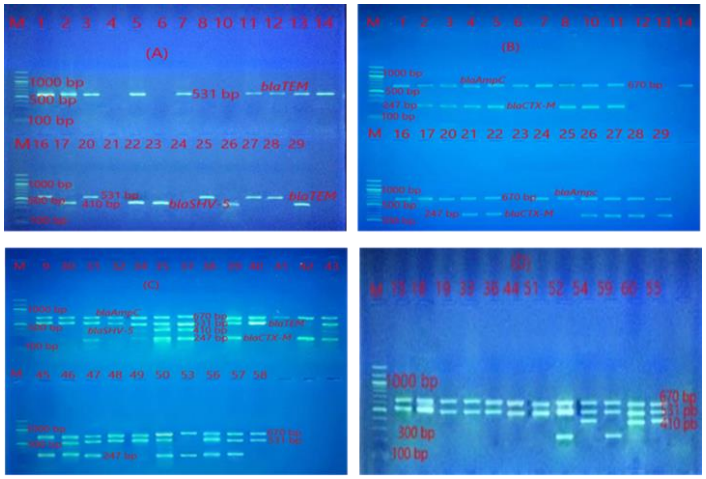

Figure 5 Products of polymerase chain reaction achieved through *β*-lactamase genes of bacterial isolates. M: 100 bp DNA ladder. *P. mirabillis* (52, 54, 57, 59, 60); *S. aureus* (10, 11, 45)*; S. pyogenes* (1, 30, 37); *P. aeruginosa* (3, 17, 26, 29, 31, 35, 39, 40, 58)*; K. pneumoniae* (9, 23, 32, 49); *E. coli* (5, 6, 13, 15, 16, 18, 19, 24, 25, 33, 36, 42, 43, 44, 51, 53); *E. faecalis* (47, 55, 56); *S. marcescens* (7, 14, 46, 50). (D): *S. typhi* (2, 4, 8, 21, 27, 28, 34, 38, 41); *Sh. Dysenteriae* (12, 20, 22, 48). The concentration of gel was 1.3% and the DNA dye was RedSafe. Electrophoresis conditions, V: 90, Time: 40 minutes.

The results in table (8) revealed the profile of virulence genetic factors and antibiotics resistance profile of all bacterial isolates. The statistical analysis of the results displayed a strong significant association between virulence and antibiotics determinants, where the value of Person correlation was 0.957 at P≤ 0.01, as revealed in table (9).

Table 8 The separate profiles of virulence genetic factors and antibiotic resistance profiles of bacterial isolates

ISOLATE NO.	NAME OF ISOLATES	VIRULENCE GENES		ANTIBIOTICS RESISTANCE GENES			
		Biofilm	*ISS*	*blaAmpC*	*blaTEM*	*blaSHV-5*	*blaCTX-M*
5	*E. coli*	+	+	+	+		+
6	*E. coli*	+		+			
13	*E. coli*	+	+		+		
15	*E. coli*	+	+	+	+		
16	*E. coli*	+	+	+	+		
18	*E. coli*	+	+	+	+		
19	*E. coli*	+	+	+	+		
24	*E. coli*	+	+	+			
25	*E. coli*	+	+	+	+		
33	*E. coli*	+	+	+	+		
36	*E. coli*	+	+	+			+
42	*E. coli*	+		+	+		
43	*E. coli*			+		+	+
44	*E. coli*	+	+	+	+		
51	*E. coli*	+	+	+	+		
53	*E. coli*	+	+	+			+
3	*P. aeruginosa*		+	+	+		+
17	*P. aeruginosa*		+	+		+	
26	*P. aeruginosa*	+	+	+		+	+
29	*P. aeruginosa*	+	+	+		+	+
31	*P. aeruginosa*	+	+	+	+		+
35	*P. aeruginosa*	+	+	+	+	+	+
40	*P. aeruginosa*	+	+	+	+		
58	*P. aeruginosa*	+	+	+	+		
39	*P. aeruginosa*	+	+	+	+		+
2	*S. typhi*	+		+	+		+
4	*S. typhi*	+		+			+
8	*S. typhi*	+		+			+
21	*S. typhi*	+	+	+			+
27	*S. typhi*	+	+	+	+		+
28	*S. typhi*	+	+	+	+		+
34	*S. typhi*	+	+	+	+	+	+
38	*S. typhi*	+	+				
41	*S. typhi*	+		+			
52	*P. mirabillis*	+	+	+	+		+
54	*P. mirabillis*	+	+	+	+	+	
57	*P. mirabillis*	+	+	+	+		+
59	*P. mirabillis*	+	+	+	+		+
60	*P. mirabillis*	+	+	+	+	+	
9	*K. pneumoniae*	+	+	+	+		
23	*K. pneumoniae*	+	+			+	
32	*K. pneumoniae*	+	+	+	+		
49	*K. pneumoniae*	+	+	+	+		
12	*Sh. dysenteriae*	+	+		+		
20	*Sh. dysenteriae*	+	+	+	+		
22	*Sh. dysenteriae*	+	+	+		+	+
48	*Sh. dysenteriae*	+	+	+	+		
7	*S. marcescens*	+	+	+	+		
14	*S. marcescens*	+	+	+	+	+	

Table 8 - continue

46	*S. marcescens*	+	+	+	+		+
50	*S. marcescens*	+	+	+	+		+
47	*E. faecalis*	+	+	+	+		+
55	*E. faecalis*	+	+	+	+	+	
56	*E. faecalis*	+	+	+	+		+
10	*S. aureus*	+		+			+
11	*S. aureus*	+	+	+	+		+
45	*S. aureus*	+	+	+			+
1	*S. pyogenes*	+	+	+	+		
30	*S. pyogenes*	+	+	+	+		
37	*S. pyogenes*	+	+	+	+	+	+

Table 9 The correlation between virulence and antibiotics determinants of bacterial isolates

Correlations			
		Virulence	Antibiotics
Virulence	Pearson Correlation	1	.957**
	Sig. (2-tailed)		.000
	N	10	10
Antibiotics	Pearson Correlation	.957**	1
	Sig. (2-tailed)	.000	
	N	10	10

**. Correlation was significant at the 0.01 level (2-tailed).

DISCUSSION

Phenotype and genotype study

Bacterial virulence not only indicates the ability to induce infection but also refers to its capacity in penetrating the lost. At the finish of the 1980s and the start of the 1990s, virulence was suggested to be a genetic cornerstone of pathogenicity and this nation was first posted at the end of 19 century (**Brown et al., 2012**). Particularly, it has been suggested that Genetic virulence could drive most phenotypic traits, such as flagella, curli, fimbriae, adhesions, biofilm, enzymes, and toxins (**Vega-Hernández et al., 2021**).

In this work, it was experimentally verified that biofilm formation was the most virulence determinant that appeared in all bacterial isolates followed by *Iss* and *FimH*, whereas these results were similar to the results obtained from many works in the literature (**Ballén et al., 2022**). However, the occurrence of biofilm in our work was higher than reported in other investigations, in which there were 17% to fecal isolates, 43% to isolates collected from patients suffering from cystitis, 40% to pyelonephritis and 42% to bacteraemia *E. coli* isolates (**Ballén et al., 2022**). Furthermore, it was also found that the 63% occurrence of biofilm produced among isolates was taken from patients with prostatitis.

Biofilms are aggregations of adhesive microbes on a target surface. Produced Biofilm could serve as a shield for the bacteria from being influenced by the hydrodynamic stream (**Singh et al., 2021**). More than half of bacterial infections have been proven that can be combined with biofilm production (**Vestby et al., 2020**).

The prevalence of *Iss* among bacteria was the second stage after biofilm and this result clearly indicated the importance of the *Iss* gene in the pathogenicity of isolated bacteria (**Sarowska et al., 2019**). In spite of the fact that human serum taken from healthy people has bactericidal activity against many pathogenic bacteria, these bacteria could counteract this bactericidal activity due to the complement cascade. Endosymbiotic bacteria are usually susceptible to this bactericidal activity while nosocomial bacteria are not influenced by such type of activity (**Alhumaid et al., 2021**). The results of the current work were partially matched with the results of similar works in the literature, which were performed in Iraq **(Al-Janabi et al., 2018**). Our work provided a close link between bacterial resistance to serum bacterial activity and bacterial pathogenicity (**Łapińska et al., 2022**). What's more, the results of the current study possessed differences between the phenotype and genotype with respect to individual isolates. The detection rates were also higher for all tests when genotype assays were used. This effect could be attributed to the DNA amplification by the PCR technique that rarely yields false negative results since this technique is precise and more accurate than the phenotype technique (**Maheaswari et al., 2016**).

From the acquired results of antibiotics resistance determinants, it was revealed that both bla_{AmpC} and bla_{TEM} were the most occurrence genes in bacterial isolates, which are in direct agreement with many previously reported outcomes in the literature **(Shahid et al., 2009; Shahid et al., 2012**).

The occurrence and kind of *ESBLs* might diverge from one topographical area to another. For example, in China *TEM* kind they were found to be the greatest predominant *ESBLs* between the *E. coli* (ESBL-making isolates) shadowed by *SHV* and finally *CTX*-M kind enzymes (**Bastidas-Caldes et al., 2022)**. In another work from Canada, it was shown that *SHV* was a foremost set of *ESBLs* in *E. coli* and 6% of *ESBL* makers were present to carry *blaTEM* and *blaCTX*-M (**Mulvey et al., 2004**). A set of two *CTX*-M enzymes were also found to be the greatest predominant *CTX*-M enzymes in South America (**Bonnet, 2004**), while set 9 enzymes were present to be predominant in Spain **(Hernández et al., 2005**).

Bacterial virulence and its resistance against antibiotics

In this work, concrete pieces of evidence for the strong relationship between virulence and antibiotic resistance determinants were provided. Our results were also comparable to the respective outcomes in the literature (C**epas & Soto, 2020; Shah et al., 2019; Yazdanpour et al., 2020**).

In general, a considerable association between resistance and virulence of bacteria exists. It has been suggested that some of bacterial virulence trails, such as biofilms, outer membrane lipoproteins, and capsular polysaccharides were mainly involved in helping the bacteria with antibacterial resistance **(Uddin et al., 2021**). Biofilms, capsular polysaccharides, and external membrane lipoproteins have a decomposable impact on antibacterial. The beta lactamase enzymes were excreted and preserved their action intrinsically of the biofilm matrix and degraded beta lactam antibiotics prior to their arrival to the bacterial cells (**Uddin et al., 2021**). Antibacterial diffusion of biofilms, capsular polysaccharides, and outer membrane might be congested by another element. For instance, the existence of surfaces through negatively charged, mainly for big polar particles (i.e. aminoglycosides) with positively charged. In addition, the low rates of metabolism combined with the depleted oxygen could also boost the bacterial resistance against cephalosporins, aminoglycosides, β-lactamase, and fluoroquinolones (**Bernal-Bayard et al., 202**3).

CONCLUSION

In conclusion, the association between resistance and virulence seems to be multifactorial and many other factors could be involved, such as antibacterial genes of resistance, virulence elements, microbial species, and also the type of patients. Therefore, as far as the integration among these factors is concerned, special attention should be given to medical diagnosis, cure, and prognostic prediction of reinfection especially with UTI infections.

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