How to Cite:
Mahdi, J. R., Makhrmash, J. H., & Jawad, T. M. (2022). Prevalence of some virulence factor genes in bacterial acquired hospital infections isolated from hospitals of Wasit province. *International Journal of Health Sciences*, *6*(S6), 9202–9215. https://doi.org/10.53730/ijhs.v6nS6.12424

Prevalence of some virulence factor genes in bacterial acquired hospital infections isolated from hospitals of Wasit province

Jasim R. Mahdi
Department of Microbiology, College of Medicine, Wasit University, Iraq
*Corresponding author email: Naseer.iaq@gmail.com

Jasim H. Makhrmash
Department of Microbiology, College of Medicine, Wasit University, Iraq
Email: jmkharmish@uowasit.edu.iq

Tuqa M. Jawad
Department of Family and Community Medicine, College of Medicine, Wasit University, Iraq
Email: Naseer.sabah2@gmail.com

***Abstract*---**Background: Antimicrobial resistance genes are an acquired phenomenon in nosocomial pathogens as results to uncontrolled and randomly used of antibiotics. Methods: A total of 308 samples were collected from different places and surfaces for bacterial contamination in three main hospitals in Wasit province. Bacteria were isolated and identified using biochemical test, then PCR was used in order to analysis he 16S rRNA gene in bacterial isolates and virulence genes using specific primers. Results: The results of bacterial cultures indicated the presence of four bacterial isolates included A. baumannii, S. marcescens, E. cloacae and S. haemolyticus, PCR product analysis of the 16S rRNA gene in bacterial isolates showed that all bacteria 100% were positive for 16S rRNA gene. PCR analysis of virulence factor AdeC gene in A. baumannii showed that 7(70%) of bacteria were positive, while 7(70%) of bacteria were positive for CsuE gene, S. Marcescens, bsmA gene were positive in 5(100%) and fimA were 0(0%) of bacteria isolates, regarding E. cloacae, fimA and CsgA genes were positive in bacterial isolates in a percentage of 5(55.5%) and 9(100%) respectively, finally in S. haemolyticus it was indicated that AtlE and Fbe genes were positive in 7(70%) and 6(50%) of bacteria isolates. Conclusion: There was a high prevalence of virulence genes among bacterial isolates included in the current study especially, in S. Marcescens100%, bsmA and E. cloacae 100%CsgA, while S. marcescens fimA 0%.

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.
Manuscript submitted: 9 April 2022, Manuscript revised: 18 June 2022, Accepted for publication: 27 July 2022

Keywords---bacterial acquired, hospital infections, virulence factor sgenes.

Introduction

The term “nosocomial” applies to any disease contracted by a patient under medical care. Nosocomial infections (NIs), also known as healthcare-associated infections (HAIs), are illnesses contracted while receiving medical care but which did not exist at the time of admission. They may occur in a variety of healthcare supply unit locations, including hospitals, long-term care homes, and ambulatory settings. They may also manifest themselves upon exoneration. Infections connected to the workplace that might impact personnel are also included in HAIs. The source or kind of infection and the causative pathogen, which may be bacterial, viral, or fungal, determine the etiology of HAIs (1, 2, 3).

A variety of bacteria may be found in hospitals, which is a reservoir A number of pathogenic bacterial strains have frequently been observed on commonly touched objects and areas close to patients, including bed rails, tray tables, working hands, bedside tables, patient chairs, nurse call buttons, doorknobs, push plates, bed rails, faucet handles, and poles. transmission methods Different transmission pathways may exist for infections connected to HAIs (4). Touch is the most frequent method of transmission, when the organisms are disseminated through either direct or indirect contact. The most typical microorganisms (M.O) that can spread by touch include multidrug-resistant bacteria i.e. *Staphylococcus* spp., *Pseudomonas* spp., *Acenitobacter* spp., *Enterobacter* spp., and *Klebseilla pneumoniae*. As well as, the most common Gram-negative organisms include *Enterobacteriaceae* species family e.g. *Klebsiella pneumonia, Klebsiella oxytoca*, *Escherichia coli*, *Proteus mirabilis*, *Enterobacter* spp., *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and *Serratia marcescens* (5). *A. baumanii* is linked to a high mortality rate in the critical care environment because of its innate multi-drug resistance characteristics (6).

Diverse types of HAI are a representation of the responsible pathogens, which come from a range of different sources. The kinds of HAI are broadly categorized by “Centers for Disease Control” (CDC) and Prevention as follows: Surgical site infections (SSIs), catheter-associated urinary tract infections (CAUTIs), central line-associated bloodstream infections (CLABSI), and ventilator-associated pneumonia (VAP) (7). Pollution of hospital surfaces by multi-drug resistant (MDR) bacteria is real threat for public health. Moreover, the idea of environmental bacterial reservoir is a truth that needs strict compliance with current strategies and recommendations for hand hygiene, cleaning, and disinfection of surfaces in hospitals (8).

Materials and Methods

Study design

The specimens were collected during the period 9st November, 2021 to 15th February, 2022. At least 308 samples of pathogenic bacteria spread on the surfaces of different departments in three hospitals including Al-Zahra Teaching Hospital, Al-Karma Hospital and Al-Kut hospital (Burns, Operating theaters, Birth operations room, Intensive care units (ICUs), preterm, catheter). All samples were taken using sterile disposable cotton and transport swabs. Then samples cultured onto (MacConkey agar, Chocolate agar, and Blood agar), after incubation samples were identified based on microscopic, colony morphology, Gram stain, and biochemical tests. In addition it has been used PCR technique in order to analysis he 16S ribosomal RNA gene in bacterial isolates, and to determine virulence genes using specific primers (9).

Primers

Polymerase chain reactions (PCR) primers were designed in the current study using NCBI Genbank sequence database design online software and these primers were synthesized by (Alpha DNA, Canada), as summarized in the Table 1.

Table 1
The specific primers and their sequence used in current study

Bacteria	Primer Name	Sequences 5'-3', 3'-5'	Size (bp)	Accession No.
E. cloacae	*16S rRNA*	TCTTGCCATCAGATGTGCCC	250	KC763625.1
		TAACCACAACACCTTCCTCCCC		
	CsgA	CTCAACCCTGAGCATTTAC C	142	EF490314.1
		AGAACTGTCATCAGAGCC C		
	fimA	ACCACCTCCAATATTCCCTTC	246	CP009854.1
		GTTAGTCCCTTCAATCAGGTTC		
A. baumannii	*16S rRNA*	GTTACTCGCAGAATAAGCACC	191	LN611374.1
		CTACCATCCTCTCCCATACTC		
	CsuE	TTTATCGAGCCGACTCACC	280	EU478794.1
		TTTACCGAACAACGTATTCCC		
	AdeC	AAAAATAGCCAGCTCCCAAC	121	MG242456.1
		CATAGGCAGTCATTCCCAAG		
S. marcescens	*16S rRNA*	TACTCGCAGAAGAAGCACC	218	HG423362.1
		CTACGCATTTCACCGCTAC		
	bsmA	TTACGCTGGATCTCCGCTTC	196	QYSA01000006.1
		CACTCATCATCCTGACGCTG		
	FimA	TGGAGAAATGTGAAGCGGG	184	AY730610.2
		GCAGCAACAGTAACGGTAG		
St. haemolyticu	*16S rRNA*	GCTAACGCATTAAG CACTCC	232	MF578766.1
		ACCCAACATCTCA CGACAC		
	Atl	ACGCTGATTATGCTGCAAC	230	FJ472949.1
		CCAAGGTGCTACTTGCTTC		
	Fbe	TGGATCGCTTTTATGA TGCAC	225	FJ472950.1

		ACGCTATAACAGAATC GTCAC		

bp= product size

Results

Molecular analysis

16S ribosomal RNA for *Acinetobacter. Baumannii*

Diagnosis using PCR recorded *A. baumannii* as 10(100%), as shown in the Figure 1.

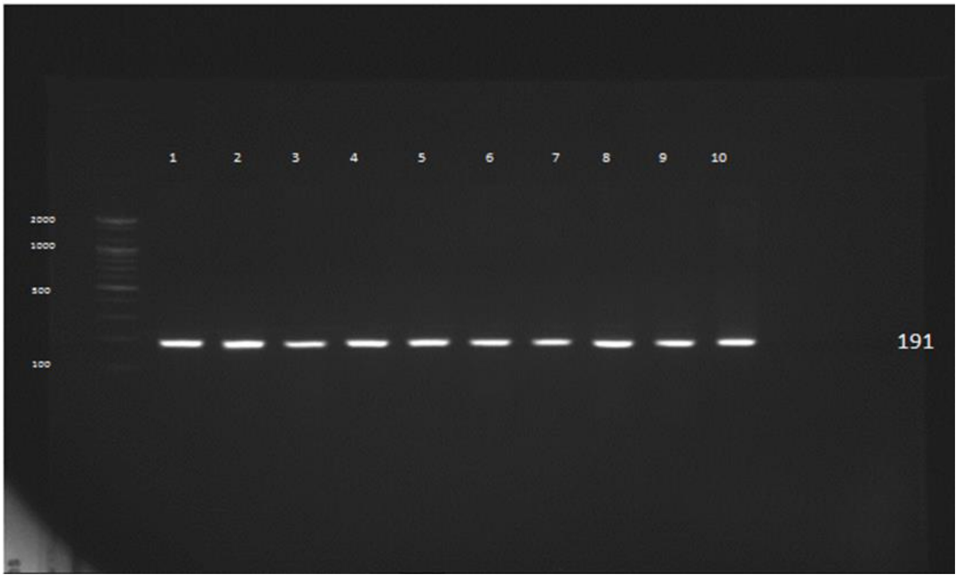

Figure 1. Agarose-gel-electrophoresis images that demonstrated the PCR product. Investigation of *16S rRNA* gene in *A. baumannii* isolates. Lane (M): marker ladder (100- 2000bp), lane (1-10): *16S rRNA* gene positive at 191bp PCR product size

AdeC gene of A. baumannii

Agarose gel electrophoresis that displayed a product of PCR analysis of virulence agent accumulation-associated protein *AdeC* gene in isolates of *A. baumannii* were showed that 7(70%) of bacteria were positive for this gene, as shown in the Figure 2.

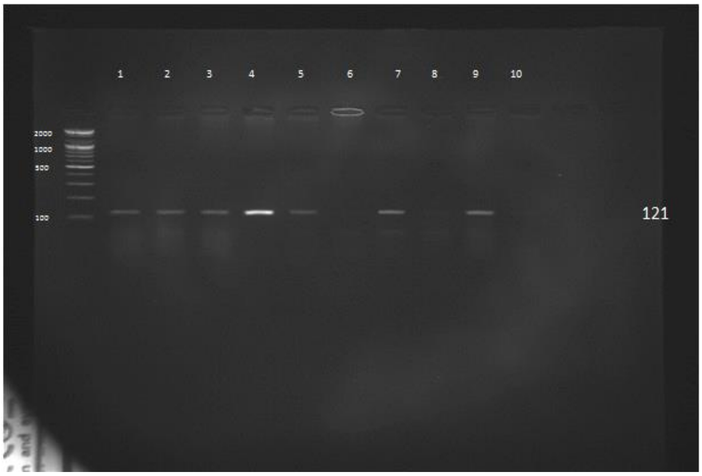

Figure 2. Agarose gel electrophoresis images that displayed a product of PCR analysis of virulence factor *AdeC* gene in isolates of *A. baumannii*. Marker ladder, lane (M) (100-2000bp), lane (1-10): showed positive *AdeC* gene at 121 bp PCR product size

CsuE gene of A. baumannii

Agarose gel electrophoresis that displayed a product of PCR analysis of virulence agent accumulation-associated protein *CsuE* gene in isolates of *A. baumannii* showed that 7(70%) of bacteria were positive for this gene, as shown in the Figure 3.

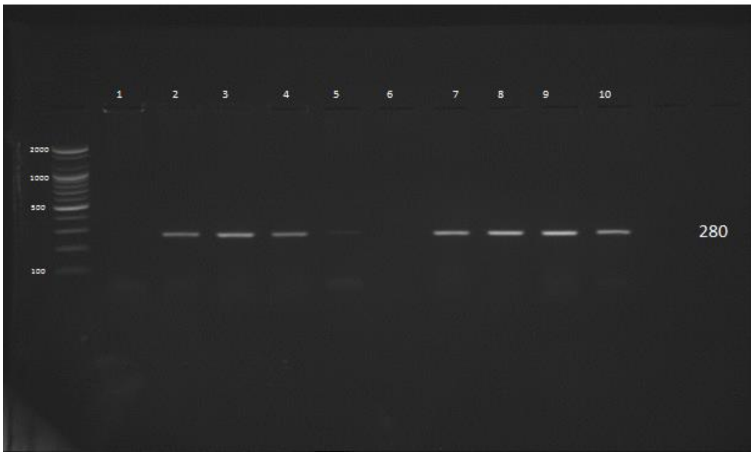

Figure 3. Agarose gel electrophoresis images that displayed a product of PCR analysis of virulence factor *CsuE* gene in isolates of *A. baumannii*. Marker ladder, lane (M) (100-2000bp), lane (1-10): showed positive *CsuE* gene at 280 bp PCR product size

16S ribosomal RNA for *Serratia. marcescens*

Diagnosis using PCR recorded *S. marcescens* 5(100%), as shown in the Figure 4.

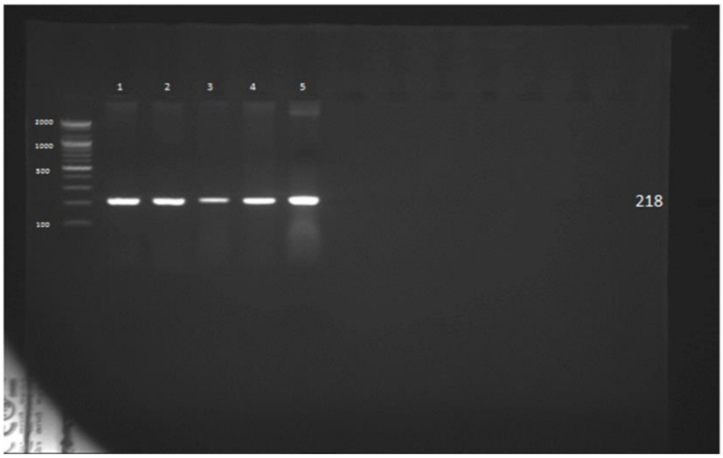

Figure 4. Agarose-gel-electrophoresis images that demonstrated the PCR product. investigation of *16S rRNA* gene in *S. marcescens* isolates. Lane (M): Marker ladder (100–2000 bp), Lanes (1–5): Positive *16S rRNA* gene at 218 bp PCR product size

bsmA gene of S. marcescens

Agarose gel electrophoresis that displayed a product of PCR analysis of virulence agent accumulation-associated protein *bsmA* gene in isolates of *S. Marcescens* showed that all bacteria isolates 5(100%) were positive for this gene, as shown in the Figure 5.

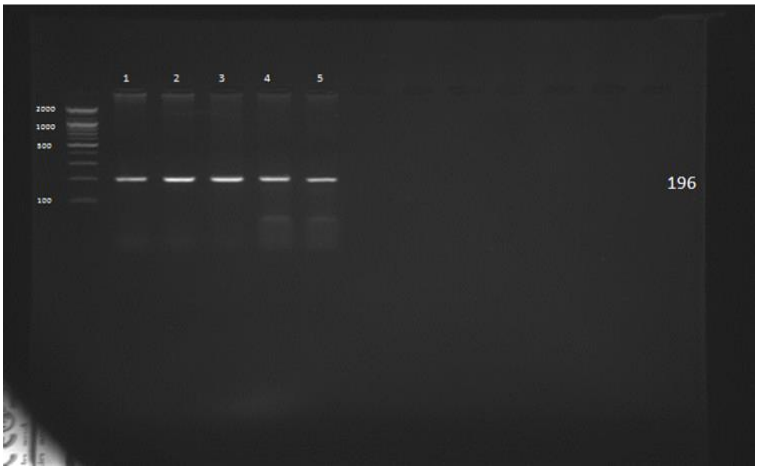

Figure 5. Agarose gel electrophoresis images that displayed a product of PCR analysis of virulence factor *bsmA* gene in isolates of *S. marcescens*. Marker ladder, lane (M) (100-2000bp), lane (1-5): showed positive *bsmA* gene at 196 bp PCR product size

FimA gene of S. marcescens

Agarose-gel-electrophoresis that displayed a product of PCR analysis of virulence agent *FimA* gene in isolates of *S. marcescens* showed that all bacteria isolates 5(100%) were negative for this gene, as shown in the Figure 6.

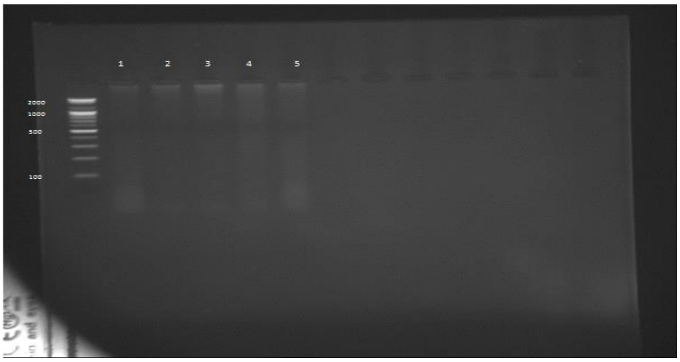

Figure 6. Agarose gel electrophoresis images that displayed a product of PCR analysis of virulence factor *FimA* gene in isolates of *S. marcescens*. Marker ladder, lane (M) (100-2000bp), lane (1-5): showed positive *FimA* gene at 184 bp PCR product size

16S ribosomal RNA for Enterobacter. Cloacae

Diagnosis using PCR recorded *E. cloacae* 9(100%), as shown in the Figure 7.

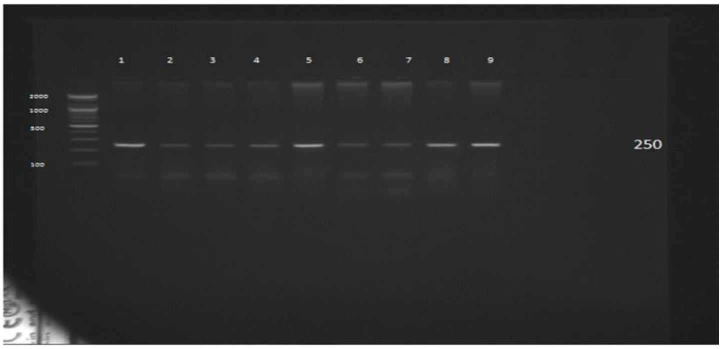

Figure 7. Agarose-gel-electrophoresis images that demonstrated the PCR product. Investigation of *16S rRNA* gene in *E. cloacae*. Lane (M): marker ladder (100-2000bp), lane (1-9): *16S rRNA* gene positive at 250 bp PCR product size

fimA gene of Enterobacter. Cloacae

Agarose gel electrophoresis that displayed a product of PCR analysis of virulence agent accumulation-associated protein *fimA* gene in isolates of *E. cloacae* showed that 5(55.5%) of bacteria isolates were positive for this gene, as shown in the Figure 8.

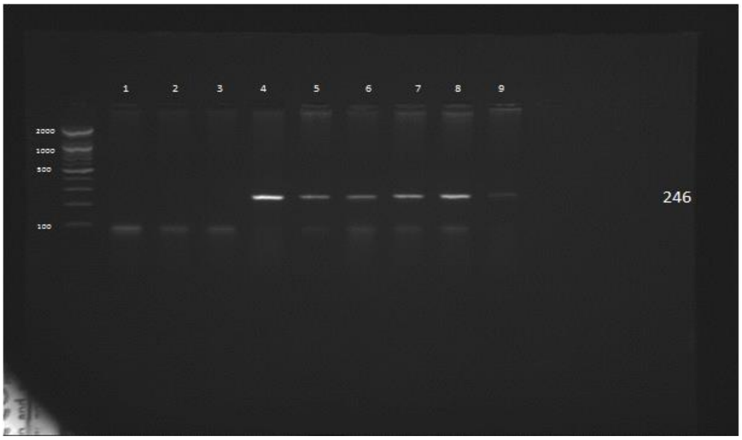

Figure 8. Agarose-gel-electrophoresis images that displayed a product of PCR analysis of virulence factor fimA gene in isolates of E. cloacae. Marker ladder, lane (M) (100-2000bp), lane (1-9): showed positive fimA gene at 246 bp PCR product size

CsgA gene of Enterobacter. Cloacae

Agarose gel electrophoresis that displayed a product of PCR analysis of virulence agent accumulation-associated protein *CsgA* gene in isolates of *E. cloacae* showed that all bacteria isolates 9(100%) were positive for this gene, as shown in the Figure 9.

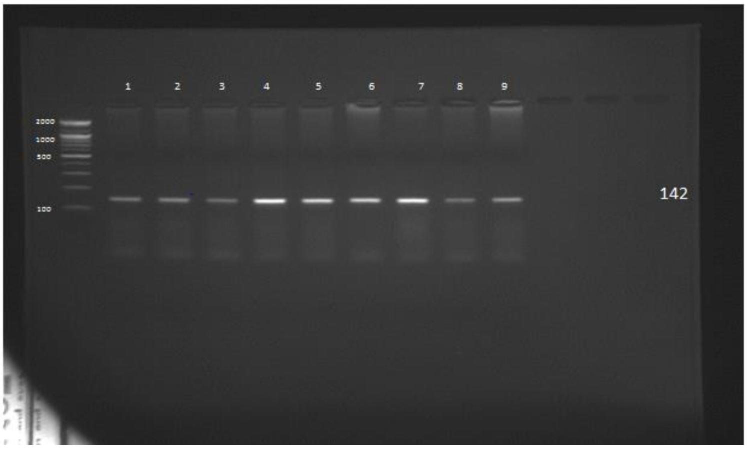

Figure 9. Agarose gel electrophoresis images that displayed a product of PCR analysis of virulence factor *CsgA* gene in isolates of *E. cloacae*. Marker ladder, lane (M) (100-2000bp), lane (1-9): showed positive *CsgA* gene at 673bp PCR product size

16S ribosomal RNA for Staph. Haemolyticus

Diagnosis using PCR recorded *S. haemolyticus* 10(100%), as shown in the Figure 10.

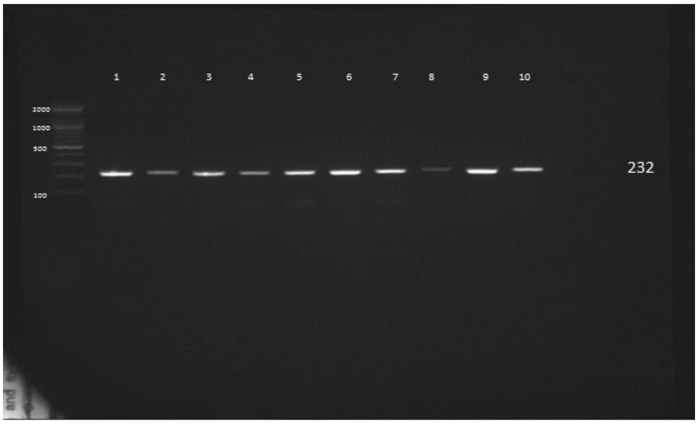

Figure 10. Agarose-gel-electrophoresis images that demonstrated the PCR product. investigation of *16S rRNA* gene in *S. haemolyticus* isolates. Lane (M): marker ladder (100- 2000bp), lane (1-10): *16S rRNA* gene positive at 232 bp PCR product size

AtlE gene of Staph. Haemolyticus

Agarose gel electrophoresis that displayed a product of PCR analysis of virulence agent accumulation-associated protein *AtlE* gene in isolates of *S. haemolyticus* showed that 7(70%) of bacteria isolates were positive for this gene, as shown in the Figure 11.

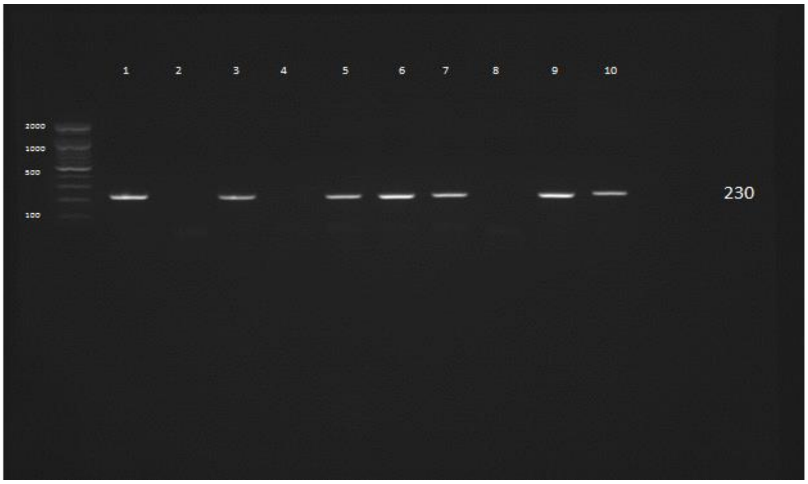

Figure 12. Agarose gel electrophoresis images that displayed a product of PCR analysis of virulence factor *AtlE* gene in isolates of *S. haemolyticus*. Marker ladder, lane (M) (100-2000bp), lane (1-10): showed positive *AtlE* gene at 230 bp PCR product size

Fbe gene of Staph. haemolyticus

Agarose gel electrophoresis that displayed a product of PCR analysis of virulence agent accumulation-associated protein (*Fbe*) gene in isolates of *S. haemolyticus* showed that 6(50%) of bacteria isolates were positive for this gene, as shown in the Figure 12.

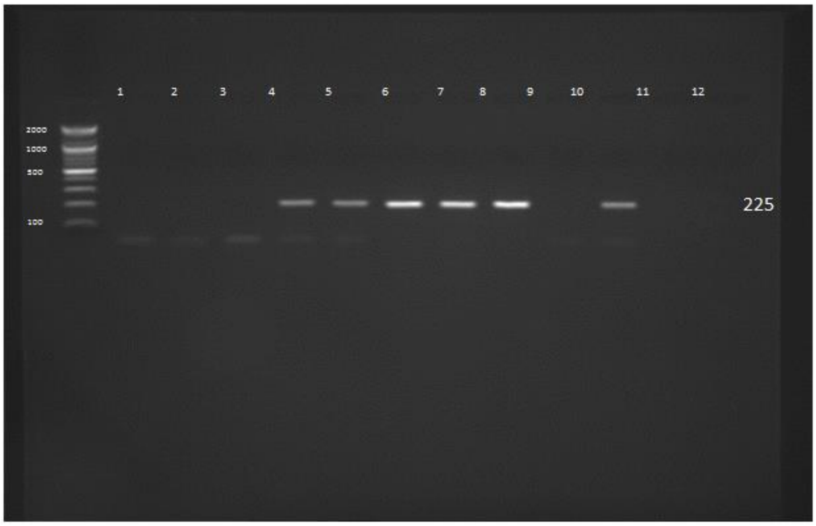

Figure 12. Agarose gel electrophoresis images that displayed a product of PCR analysis of virulence factor *Fbe* gene in isolates of *S. haemolyticus*. Marker ladder, lane (M) (100-2000bp), lane (1-10): showed positive *Fbe* gene at 225 bp PCR product size

Discussion

The first efflux pump of *A. baumannii, AdeABC*, regulated by *AdeRS*, was found in multidrug-resistant *A. baumannii*, the study of the efflux pump system in *Acinetobacter* subsequently developed. *AdeDE* and *adeXYZ* were detected in *Acinetobacter* genomic DNA group 3 (GDG3) in 2004 and 2006, respectively (10). A product of PCR analysis of virulence factor accumulation-associated protein *AdeC* gene in isolates of *A. baumannii* showed that 7(70%) of bacteria were positive for this gene in this study. Using a multiplex PCR test, the existence of the *adeA, adeB*, and *adeC* genes was also determined. The high frequency of the *AdeABC* efflux system genes may contribute to *A. baumannii* clinical isolates' resistance to IMI, particularly (11). Another study showed that the presence of *AdeC* in *A. baumannii* was 85% (12).

In the present study, the results were in accordance with study showed that 100% of *A. baumannii* isolates from immune-compromised patients in ICU were investigated to biofilm formation, the presence of biofilm associated genes (*bap, ompA, csuE, fimH, epsA,* bla_{PER-1}*, bfmS, ptk, pgaB, csgA, kpsMII*), integron character and molecular typing based on REP-PCR, study revealed the high frequency of biofilm forming *A. baumannii* in ICU patients, with a high incidence 70% of biofilm linked genes of *csuE* (13). It seems that the appropriate surveillance and control measures are essential to prevent the emergence and transmission of MDR *A. baumannii*, other study showed the same outcomes (14). While the frequency of *csuE* gene in *A. baumannii* was 32.7 in another study (15). Also, 98% to 100% in other studies (16, 17).

The results in the present study were compatible study revealed that *bsmA* and *bsmB* responsible for biofilm formation of *S. marcescens* (18). And associated in the adherence to the abiotic surfaces and their presence was 100% in all bacterial isolates (19). The results in the present study were agreed study showed that *fimA* gene was present in a percentages of 100% (20). This finding demonstrated a greater frequency of *fim* genes. In general, adhesions usually are exposed to the bacterial surface or they carried by filamentous structures fimbriae or pili and most of UPECs contain type 1 fimbriae encoded by *fim* operon (*fimB*, *E*, *A*, *I*, *C*, *D*, *F*, *G*, *H*) which are existed in the chromosome of most bacterial isolates (21). Other studies recorded that the reduction of up to 43% in *fimA* machinery was related to the measurement of the expression of *fimI*. It is a pilus anchor termination subunit (22). The differences in these ratios for other studies may be due to non-sampling regularly.

In the present study, *E. cloacae* were showed that 5(55.5%) of bacteria isolates were positive for this *fimA* gene, while all bacteria isolates 9(100%) were positive for this *CsgA* gene. These data were in accordance with studies showed that *E. cloacae*, a member of the *Enterobacteriaceae* family of bacteria, is responsible for community-associated infections as well as HAIs linked to genes, particularly the *fimA* and *CsgA* genes, whose percentages for these genes are 60% and 99%, respectively (23, 24). Since they are the second or third most prevalent *Enterobacteriaceae* that produce the carbapenemase during the past few decades, species of the *E. cloacae* have given rise to increased worry (25, 26). While one study indicated that both genes *fimA* and *CsgA* were present in a percentage of 100% (27).

In the present study, *S. haemolyticus* were showed that 7(70%) of bacteria isolates were positive for *AtlE* gene, while all bacteria isolates 6(50%) were positive for *Fbe* gene. These data were compatible to Soumya *et al.* (2017) as *S. haemolyticus* was found to have *fbe* and *atlE* genes, in a percentage of 90% and 50 % respectively. Among CNS isolates, 42 isolates 16.5% were positive for *atlE* gene and was mostly detected in *S. haemolyticus* 73.3% (28). Moreover, other study reported that 16.7% of CONS had embp gene, close to the results in the current study results (29). The first stage of biofilm formation takes place via proteins expressed on the bacterial cell wall, including autolysin *Atl*E, and fibrinogen binding protein *Fbp* (30).

On the other hand, it was mentioned low frequency for *fbe* 25% and *atlE* 30% genes (31). Moreover, one study that a collection of 226 CNS (168 *S. epidermidis* and 58 *S. haemolyticus*) recovered from BSIs 100 and PDAIs 126 from different inpatients, was tested for biofilm formation, antimicrobial susceptibility, *mecA*, ica operon, adhesin (*aap, bap, fnbA, atlE, fbe*) and toxin (tst, sea, sec) genes carriage (32). The selected CNS were classified into pulsotypes by PFGE and assigned to sequence types by MLST. In total, 106 from 226 isolates 46.9% produced biofilm, whereas 150 (66.4%) carried ica operon. Most isolates carried *mecA* and were multidrug resistant 90.7%.

Conclusions

There was a high prevalence of virulence genes among bacterial isolates included in the current study especially, in *S. Marcescens, bsmA* 100% and *E. cloacae CsgA* 100%, while *S. marcescens fimA* 0%. The differences in these ratios for other studies may be due to non-sampling regularly.

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