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%.
Keywords—bacterial acquired, hospital infections, virulence factor genes.

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., Acinetobacter spp., Enterobacter spp., and Klebsiella 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 multidrug 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 a 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 9th 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.

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<tr>
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Results

Molecular analysis
16S ribosomal RNA for *Acinetobacter. Baumannii*

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

![Agarose gel electrophoresis images that demonstrated the PCR product.](image)

**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.

![Agarose gel electrophoresis images](image)

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*, *blaPER-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 fimbrae or pili and most of UPECs contain type 1 fimbrae 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 AtlE, 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 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.

References


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