Molecular Detection of *Van A* and *bap* Genes in *Staphylococcus* Species Isolated from Hemodialysis Patients with Blood Stream Infections

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Abstract

Background: Bloodstream infections (BSIs) are a frequent consequence of hemodialysis caused by bacterial pathogens associated with *Staphylococcus* spp. especially *Staphylococcus aureus* followed by coagulase-negative Staphylococci, which can produce biofilms, and also show its ability to increase resistance to vancomycin. **Objectives:** Estimate the percentage of *Staphylococcus* spp. associated with BSIs in hemodialysis (HD) patients. Study the antibiogram pattern of isolates and detection of the resistance gene *van A* and virulence *bap* gene. **Materials and Methods:** A total of 120 blood samples, 60 from patients on hemodialysis and 60 patients suspected of bacteremia, were collected. The isolation of bacteria was performed by blood samples inoculated in BacT/ALERT bottles and then subcultured on blood agar. Identification and antibiotic sensitivity testing detection were performed by the Vitek-2 system; then a conventional polymerase chain reaction for *van A* and *bap* genes was done. **Results:** The percentage of *S. aureus* was 64.7% within the group of HD patients followed by *Staphylococcus lentus* 11.8%, *Staphylococcus hemolyticus* 8.8%, and *Staphylococcus hominis* 5.9%, whereas in non-HD patients' group, *S. aureus* was 50.0% and *S. lentus* 25.0%. All *Staphylococcus* spp. was completely resistant to vancomycin in HD and non-HD patients. The percentage of *van A* gene was 91.2% and 83.3% within HD and non-HD patients, respectively. The percentage of *bap* gene was 55.9% and 58.3% within HD and non-HD patients, respectively. **Conclusion:** *Staphylococcus aureus* is the most common bacterium that causes bacteremia in dialysis patients, while other types contribute with a lower frequency.

Keywords: Bacteremia, *bap* gene, *van A*

Introduction

Dialysis patients with end-stage renal disease are more susceptible to bloodstream infections (BSIs). The infectious complications in hemodialysis patients (HD) are still the main reasons for increased morbidity and mortality; this could be caused by deficiencies in the host's protective mechanisms, comorbid conditions, invasive procedures, and virulence factors of the invading organisms.[1] Bacteremia is 26 times higher in comparison to the general population, with Gram-positive bacteria serving as the main infectious agent.[2] Particularly, *Staphylococcus aureus* was the most common pathogen encountered in HD patients, followed by coagulase-negative staphylococci.[3] A major problem and danger to public health is antibiotic resistance.[4] Vancomycin-resistant enterococci (VRE), methicillin-resistant Gram-negative bacilli, and methicillin-resistant *Staphylococcus aureus* (MRSA) were shown by Calfee[5] to be the most prevalent pathogens.[5,6] The five vancomycin-resistance phenotypes that have been discovered are *van A*, *van B*, *van C*, *van D*, and *van E*. Two of these, *van A* and *van B*, are mediated by recent gene cluster acquisition.[7] The acquisition of the *van A* gene from VRE resulted in the first report of completely vancomycin-resistant

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Staphylococcus aureus strains in the United States in 2002.[8] Tn1546, a member of the Tn3 family of transposons, has been designated on both conjugative and nonconjugative plasmids and is the site where the *van A* gene cluster is typically found.[9] Many virulence factors, including biofilm development, have been linked to the capacity of *Staphylococcus* spp. to infect humans and cause illness. *Staphylococcus aureus* and coagulase-negative staphylococci have been characterized as having this capability.[10] The ubiquitous ability of microorganisms to adhere to surfaces and create extracellular polysaccharides leads to the development of biofilms. Because of the susceptibility of organisms associated with biofilms to antimicrobial drugs, biofilms constitute a severe threat to public health. Some *Staphylococcus* species depend on the biofilm-associated protein (Bap), a surface protein expressed by the *bap* gene, to form biofilms. There is a mobile staphylococcal pathogenicity island that contains the *bap* gene.[11] This study aimed to estimate the percentage of bacteria associated with BSIs in HDs and patients without hemodialysis admitted to the hospital and suspected bacteremia and detect the resistance genes *van A* and virulence *bap* genes in isolated multidrug resistance (MDR) and extensively drug resistance (XDR) *Staphylococcus* species.

Materials and Methods

A total of 120 blood samples, 60 from patients on hemodialysis and 60 from patients without hemodialysis admitted to the hospital and suspected bacteremia, were collected in a period from the first of August 2022 to the end of October 2022 from Al-Imamain Al-kadhimain Medical City. This study was approved by the ethical committee (Institutional Review Board) of the College of Medicine-Al-Nahrain University and conducted in the Microbiology Department of this college. The patient's age was limited from 18 to 70 years old. The isolation of bacteria was performed by culturing on blood samples and collected in bottles that have been incubated in BacT/ALERT 3D Microbial Identification System for 5–7 days at 37°C, and then subcultured on Blood Agar (HIMEDIA/India) for 24 h at 37°C; the second part of the blood sample was stored at 4°C for DNA extraction. The identification of bacteria was done by the Vitek-2 system.

Antimicrobial susceptibility tests

Resistance patterns of *Staphylococcus* spp. isolates were detected by Vitek-2 System (BioMerieux/France) to 21 different antibiotics (amikacin 1 µg/mL, amoxicillin/clavulanic acid 1 µg/mL, benzylpenicillin 1 µg/mL, cefoxitin 6 µg/mL, ciprofloxacin 10 µg/mL, clindamycin 1 µg/mL, erythromycin 2 µg/mL, gentamicin 8 µg/mL, imipenem 10 µg/mL, levofloxacin 2 µg/mL, linezolid 1 µg/mL, meropenem 10 µg/mL, moxifloxacin 2 µg/mL, nitrofurantoin 16 µg/mL, norfloxacin 8 µg/mL, oxacillin 1 µg/mL, rifampicin 0.5 µg/mL, tetracyclin 1 µg/mL, tiecoplanin 4 µg/mL, trimethoprim/sulfamethoxazole 8/152 µg/mL, vancomycin 1, 2 µg/mL) according to Clinical and Laboratory Standards Institute (CLSI) 2016.[12]

Detection of biofilm formation

Quantitative microtiter plate assays for biofilm formation were performed as described by Fields *et al*.[13] Briefly, *Staphylococcus* spp. isolates were subcultured in brain–heart infusion broth for 24 h at 37°C. One hundred microliters of *Staphylococcus* broth turbidity equal to 0.5 McFarland and an equal volume of Luria–Bertani (LB) broth (Oxoid, UK) supplemented with 20% glucose (Gibco life Tech., USA) were added to each well in 96-well polystyrene microtiter plates. The plates were incubated overnight at 37°C. The cultures were softly removed. The wells were washed three times with phosphate-buffered saline to remove nonadherent cells. The adherent cells were fixed with (200 µL) absolute methanol 99% for 10 min; methanol was removed and the plate was left dried in an upside-down position at room temperature, and then stained with (200 µL) 0.4% crystal violet for 15 min, washed three times with sterile distilled water, and then allowed for air-dried. A 250-µL glacial acetic acid (Scharlau, Spain) was added to one well and was considered a negative control, and 250 µL of bacterial broth was added to another well and considered a positive control. All other wells were filled with 250 µL of 33% glacial acetic acid and waited for 15 min at room temperature. Finally, the reading of optical density (OD) at 595 nm absorbance was determined using an ELISA plate reader (BioTek, USA).

Molecular methods

DNA extraction

DNA was extracted according to the manufacturer's instructions by using a Genomic DNA extraction kit from Geneaid (blood/cultured cell).

Conventional polymerase chain reaction

PCR was used to detect resistance gene *van A* and virulence *bap* gene in *Staphylococcus* spp. Sequences of primers had been designed and synthesized in Alpha-DNA (USA) as shown in Table 1 in a lyophilized form, which was prepared according to the manufacturer's recommendations.

A total of 20 µL of the PCR mixtures were spun down with a microcentrifuge, concerning *van A*[14] and *bap*[15]. The PCR program was pre-installed in the PCR machine. The Fisher Scientific Thermal Cyclers (USA) was used for all PCR amplification reactions as illustrated in Tables 2 and 3, respectively.

Agarose gel electrophoresis for detection of PCR products

After PCR amplification, according to the method of Sambrook *et al.*,[16] 1.5% and 1% agarose gel were used

Table 1: Sequences and product size of *van A* resistance gene *and bap* virulence genes

Genes	Sequence 5′ → 3′	Product size (bp)	Ref.
van A	F: GGCAAGTCAGGTGAAGATG R: ATCAAGCGG TCAATCAGTTC	763	Maharjan *et al.*[14]
Bap	F: CCCTATATCGAAGGTGTAGAATTGCAC R: GCTGTTGAAGTTAATACTGTACCTGC	971	Ballah *et al.*[15]

Table 2: PCR program for amplification of *van A* resistance gene by thermal cycler

No.	Steps	Temperature (°C)	Time (min)	No. of cycles
1	Initial denaturation	95	2	1
2	Denaturation	95	1	35
3	Annealing	54	1	
4	Elongation	72	1	
5	Final elongation	72	10	1

Table 3: PCR program for amplification of *bap* virulence factor by thermal cycler

No.	Steps	Temperature (°C)	Time (min)	No. of cycles
1	Initial denaturation	94	2 min	1
2	Denaturation	94	20 s	40
3	Annealing	42	20 s	
4	Elongation	72	50 s	
5	Final elongation	72	5 min	1

to confirm the presence of amplified bands of *van A* gene and *bap* gene, respectively. Eight microliters of each product were loaded into the wells. For 5 µl of 100 bp, the DNA ladder was inoculated to a single well. Electrical power was turned on at 7 V/cm for 90 min and ethidium bromide-stained bands in gel were displayed by UV transilluminator and then photographed by mobile device camera (Samsung).

Statistical methods

The statistical analysis system program includes chi-square, *t*-test, and ANOVA tests that were used to analyze the data of this study. Entry of data into Excel systems and exact tests were achieved by Statistical Package for Social Sciences (SPSS) version 20.0 (2020) (SPSS Inc., Chicago, IL, USA, 2016). *P* values ≤0.005 were considered statistically significant.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol, the subject information, and the consent form were reviewed and approved by a local ethics committee according to the document number 07177 (including the number and the date in 9/5/2022) to get this approval.

Table 4: The frequency of sex distribution in HD patients and non-HD

HD	Male	26	43.3
	Females	34	56.7
	Total	60	100.0
Non-HD	Male	39	65.0
	Female	21	35.0
	Total	60	100.0

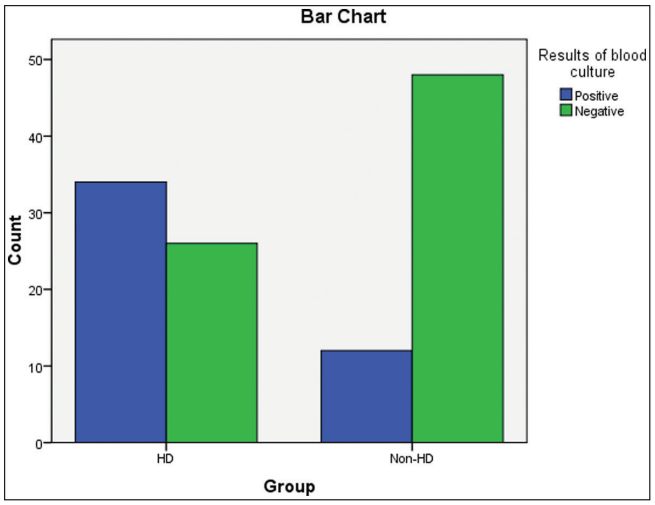

Figure 1: Distribution of results of blood culture in groups

Results

The patient's age ranged from 18 to 70 years. The mean age of patients with HD was 52 years, while the mean age of patients with non-HD was 51 years; the current study found that females undergoing hemodialysis are more exposed to bacteremia than males, while in non-HD males are more exposed to bacteremia than females as shown in Table 4.

Regarding blood culturing via BacT/ALERT culture bottles, it was found that 46 (38.3%) out of 120 were positive. The positive blood culture within a group of HD patients was 34 (56.7%) and within a group of non-HD patients were 12 (20.0%) positive isolates, as shown in Figure 1.

Regarding subculture from BacT/ALERT-positive bottles in blood agar and mannitol salt agar, the results are interpreted as follows: in HD patients, *S. aureus* was 22 (64.7% within group, 78.6% within species of bacteria), *Staphylococcus lentus* 4 (11.8% within group, 57.1% within species of bacteria), *Staphylococcus hemolyticus* 3 (8.8% within group, 100% within species of bacteria), *Staphylococcus hominis* 2 (5.9% within group, 100% within species of bacteria), *Kocuria kristinae* 3 (8.8%

within group, 50% within species of bacteria). In non-HD patients' group, *S. aureus* 6 (50.0% within group, 21.4% within species of bacteria), *S. lentus* 3 (25.0% within group, 42.9% within species of bacteria), *K. kristinae* 3 (25.0% within group, 50% within species of bacteria) as shown in Figures 2 and 3.

Antibiotic resistance

Vitek-2 system showed that all *Staphylococcus* spp. isolates were completely resistant (100%) to vancomycin, cefoxitin screen, and benzylpenicillin in HD and non-HD patients; the resistance within a group to oxacillin was 94.1% in HD, whereas all isolates were completely resistant (100%) in non-HD to oxacillin. In HD patients, all isolates were resistant to teicoplanin (100%) followed by erythromycin, rifampicin, tetracycline cefotaxime, amikacin, amoxicillin/clavulanic acid, ceftriaxone, nitrofurantoin, gentamicin, and trimethoprim/sulfamethoxazole. Resistance was moderate to low for norfloxacin, imipenem, ciprofloxacin meropenem, levofloxacin, and linezolid. In non-HD patients, highly resistant to tetracycline and rifampicin followed by amikacin, ceftriaxone, cefotaxime, and levofloxacin gentamicin, imipenem, trimethoprim/sulfamethoxazole, amoxicillin/clavulanic acid, norfloxacin, teicoplanin. Resistance was moderate to low in meropenem and ciprofloxacin, linezolid, and nitrofurantoin as shown in Table 5.

Molecular methods

Sequence amplification of *Staphylococcus* spp. resistance and virulence genes were done by PCR with a product size of 763 bp and 971 bp of *van A* and *bap* gene, respectively, as shown in Figures 4 and 5.

The current study revealed that the percentage of *van A* from bacteria within HD patients was 91.2% (31 isolates), whereas the percentage of the gene using DNA extracted from blood was 58.8% (20 isolates). In comparison with non-HD, the percentage of *van A* from bacteria by conventional PCR was 83.3% (10 isolates), whereas the percentage of the gene from blood was 66.7% (8 isolates) as shown in Tables 6 and 7.

The percentage of *bap* gene in bacteria isolated from HD patients was 55.9% (19 isolates), whereas the percentage of the gene from blood was 23.5% (8 isolates). In comparison to the non-HD patient group, the percentage of *bap* gene from bacteria by conventional PCR was 58.3% (7 isolates), whereas the percentage of the gene from blood was 41.7% (5 isolates) as shown in Tables 8 and 9.

In HD patients, all isolates were positive for biofilm formation, but according to the sex, females were more frequent to exposure to biofilm-producer bacteria than

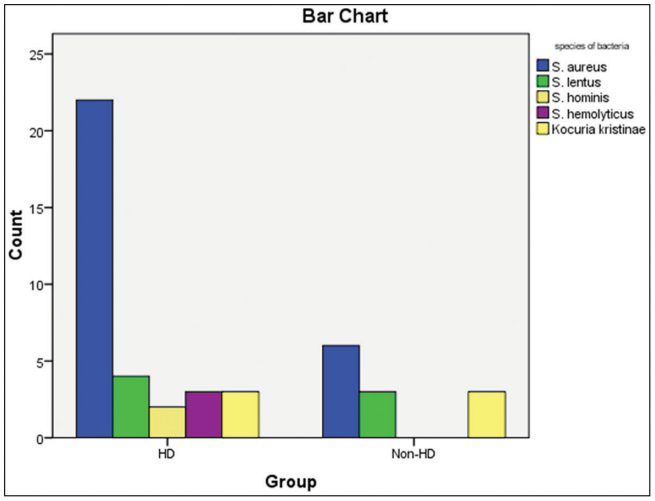

Figure 3: Distribution groups and species of bacteria

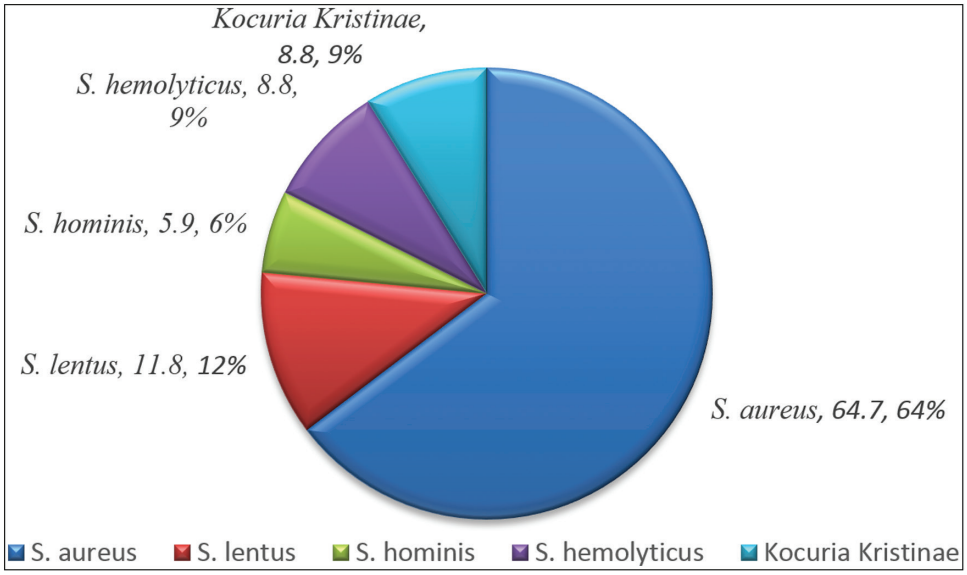

Figure 2: The percentage of *Staphylococcus* spp. in HD patients

males. The results of this study also revealed that the resistance genes in isolates from growth culture were more frequent in biofilm-producer bacteria than genes of isolates from blood as shown in Table 10.

In non-HD patients, 11 isolates were positive for biofilm formation, while only one isolate was negative. According to the sex, males were more frequent exposure to biofilm-formation bacteria than females. The results of this study also showed that the resistance genes of isolates from growth culture were more frequent in biofilm-formation bacteria than genes in isolates from blood as shown in Table 11.

Table 5: Antibiotic resistance in HD and non-HD patients

Antibiotic	Resistance (%) HD	Resistance (%) Non-HD
Oxacillin	94.12	100.00
Vancomycin	100.00	100.00
Cefoxitin screen	100.00	100.00
Gentamicin	73.53	66.67
Amikacin	85.29	75.00
Imipenem	61.76	66.67
Meropenem	52.94	58.33
Trimethoprim/Sulfamethoxazole	70.59	66.67
Amoxicillin/Clavulanic acid	85.29	66.67
Ciprofloxacin	58.82	58.33
Norfloxacin	64.71	66.67
Ceftriaxone	79.41	75.00
Cefotaxime	88.24	75.00
Levofloxacin	23.53	75.00
Linezolid	5.88	25.00
Tetracycline	88.24	83.33
Erythromycin	97.06	100.00
Teicoplanin	100.00	66.67
Nitrofurantoin	79.41	16.67
Rifampicin	91.18	83.33
Benzylpenicillin	100.00	100.00

Discussion

This study aimed to estimate the percentage of *Staphylococcus* spp. associated with BSIs in HD and non-HD patients. Regarding sex distribution, this study revealed that the percentage of females was 56.7% and males were 43.3% within the HD patients' group, whereas the percentage of females was 35% and males was 65% within the non-HD patients' group. These results agreed with a study done in Greece by Fysaraki *et al.*,[17] which reported that 53.0% were females and 49.0% were males within HD patients, whereas the current results disagreed with the study investigated in Palestine by AbuTaha *et al.*[18] found the incidence rate in HD patients in males was 64% and 36% in females and their median age was 58 years. The results in the existing study agreed with the study done in China by Xu *et al.*[19] presented that females were 43.6% and males were 56.4% in non-HD patients. The clinical interpretation of sex differences in the outcome of bacteremia in HD patients is still uncertain. Some studies paid attention to this issue and observed that there were strong differences in infection sites based on sex, different levels of hormones in the body such as estrogen have also been confirmed to have a direct protective effect on vascular endothelial cells and the effects on the inflammatory response[20] environmental influences, lifestyle between men and women such as hand-hygiene behavior, genetic, environmental influences, and physiological factors.[21] The

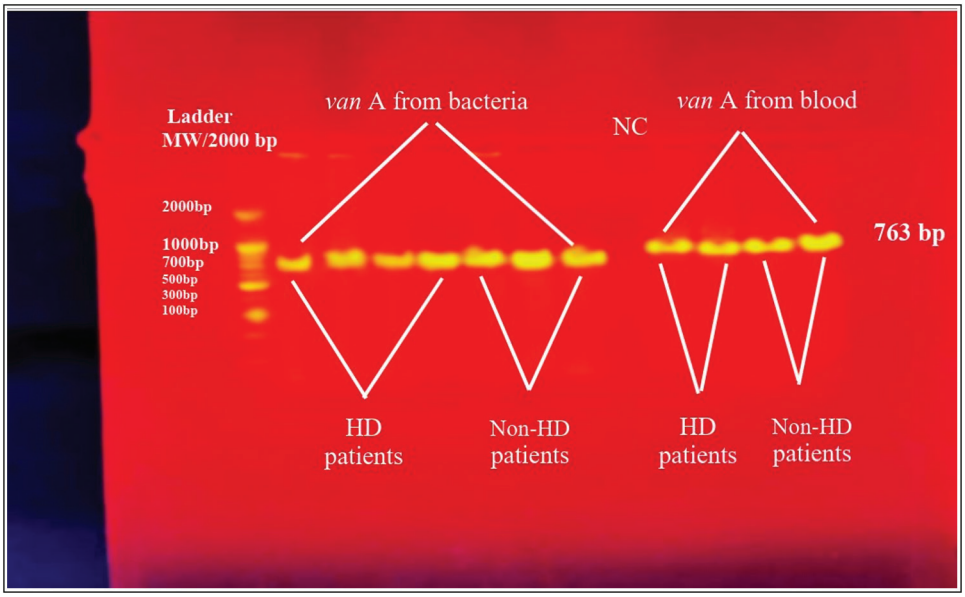

Figure 4: Gel electrophoresis of PCR products (763 bp) for *van A* resistance gene. Lane 1: 2000 bp ladder; Lane 8: negative control; Lanes 2–12: PCR products of *Staphylococcus* spp. isolates (1.5% agarose, 7 V/cm^2, 120 min)

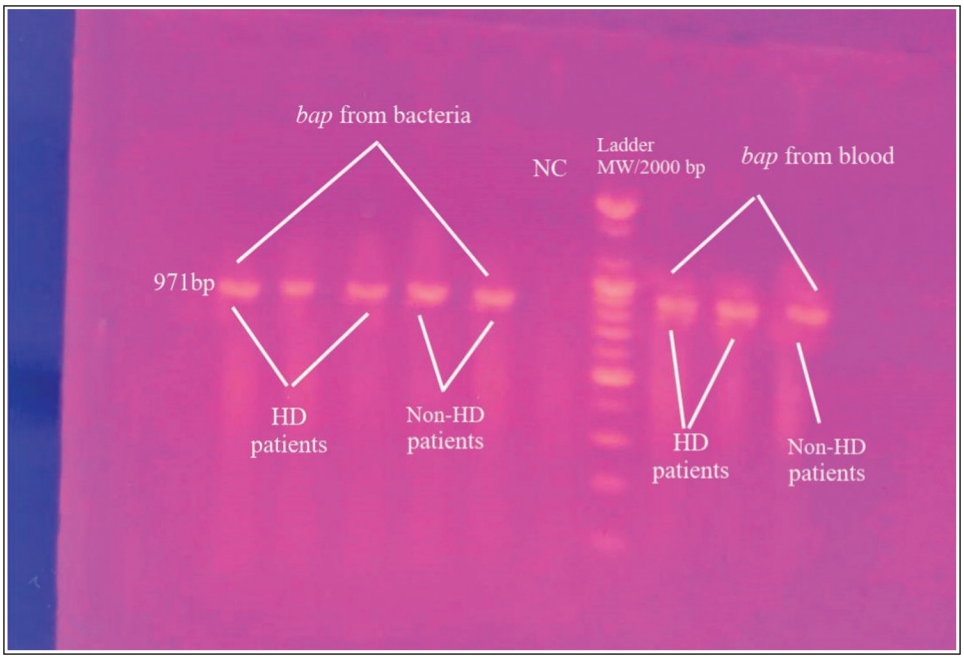

Figure 5: Gel electrophoresis of PCR products (971 bp) for *bap* virulence gene. Lane 4: 2000 bp ladder; Lane 5: negative control; Lanes 2–10: PCR products of *Staphylococcus* spp. isolates (1% agarose, 7 V/cm^2, 45 min)

Table 6: Association of *van A* gene from bacteria within groups

			van A from bacteria		Total
			Positive	Negative	
Group	HD	Count	31	3	34
		% within group	91.2%	8.8%	100.0%
		% within *van A* from bacteria	75.6%	60.0%	73.9%
	Non-HD	Count	10	2	12
		% within group	83.3%	16.7%	100.0%
		% within *van A* from bacteria	24.4%	40.0%	26.1%
Total		Count	41	5	46
		% within group	89.1%	10.9%	100.0%
		% within *van A* from bacteria	100.0%	100.0%	100.0%

Table 7: Association of *van A* gene from the blood within groups

			van A from blood		Total
			Positive	Negative	
Group	HD	Count	20	14	34
		% within group	58.8%	41.2%	100.0%
		% within *van A* from blood	71.4%	77.8%	73.9%
	Non-HD	Count	8	4	12
		% within group	66.7%	33.3%	100.0%
		% within *van A* from blood	28.6%	22.2%	26.1%
Total		Count	28	18	46
		% within group	60.9%	39.1%	100.0%
		% within *van A* from blood	100.0%	100.0%	100.0%

percentage of bacterial species in the present study, in the HD patients' group, was *S. aureus* 64.7%, *S. lentus* 11.8%, *S. hemolyticus*, *K. kristinae* 8.8%, and *S. hominis* 5.9% and in non-HD patients' group, *S. aureus* 50.0%, *S. lentus* 25.0%, and *K. kristinae* 25.0%. Similar findings in a study reported in Greece by Fysaraki *et al.*,[17] found that 55% of HD patients were *S. aureus* episodes in HD patients. A study published in Hungary by Kristóf *et al.*[22] showed

Table 8: Association of *bap* gene from bacteria within groups

			bap from bacteria		Total
			Positive	Negative	
Group	HD	Count	19	15	34
		% within group	55.9%	44.1%	100.0%
		% within *Bap* from bacteria	73.1%	75.0%	73.9%
	Non-HD	Count	7	5	12
		% within group	58.3%	41.7%	100.0%
		% within *Bap* from bacteria	26.9%	25.0%	26.1%
Total		Count	26	20	46
		% within group	56.5%	43.5%	100.0%
		% within *Bap* from bacteria	100.0%	100.0%	100.0%

Table 9: Association of *bap* gene from the blood within groups

			bap from blood		Total
			Positive	Negative	
Group	HD	Count	8	26	34
		% within group	23.5%	76.5%	100.0%
		% within *Bap* from blood	61.5%	78.8%	73.9%
	Non-HD	Count	5	7	12
		% within group	41.7%	58.3%	100.0%
		% within *Bap* from blood	38.5%	21.2%	26.1%
Total		Count	13	33	46
		% within group	28.3%	71.7%	100.0%
		% within *Bap* from blood	100.0%	100.0%	100.0%

Table 10: The biofilm mean in HD patients

		N	Mean	*P* value
Sex	Males	16	0.27	0.81
	Females	18	0.51	
Results of blood culture	Positive	34	0.40	
	Negative	0		
van A from bacteria	Positive	31	0.40	0.21
	Negative	3	0.37	
van A from blood	Positive	20	0.43	0.75
	Negative	14	0.34	
bap from bacteria	Positive	19	0.45	0.84
	Negative	15	0.33	
bap from blood	Positive	8	0.46	0.42
	Negative	26	0.38	

6% *S. haemolyticus* of HD patients which agreed with the current study. Other study was investigated in Germany by Scheuch *et al.*[23] found that 40% of HD patients were *S. aureus* carriers compared to 27% in non-HD patients. These discrepancies in the prevalence of *Staphylococcus* spp. in the current study and previous studies are due to the differences in sample size and geographical areas, diagnostic techniques, and infection control policies in the hospitals.[24] The results of this study showed that 100% of *Staphylococcus* spp. were resistant to vancomycin, cefoxitin screen, oxacillin, and benzylpenicillin in both HD and non-HD patients. These results agreed with a study investigated in Iraq by Rafiq *et al.*[25] which found that 78.94% of the isolates were resistant to vancomycin with similar findings to cefoxitin screen and benzylpenicillin, whereas this result disagreed with several studies that showed high sensitivity of Staphylococci to vancomycin such as a study published in Iran by Mamishi *et al.*,[26] in Kenya by Kyany'a *et al.*,[27] and in Barbados by Gittens *et al.*[28]The current study included a high rate of resistance in HD patients to teicoplanin (100%), erythromycin (97.06%), cefotaxime (88.24%), amoxicillin/clavulanic acid (85.29%), and ceftriaxone (79.41%). The study was published in Haripur, Pakistan, by Jiang *et al.*[29] that found the MRSA showed complete resistance to amoxicillin–clavulanic acid, cefotaxime, ceftriaxone, and erythromycin. Also in the present study, there was high resistance to tetracycline in both HD and non-HD patients, 88.24% and 83.33%, respectively. A similar finding in a study presented in Iran by Dormanesh *et al.*[30] reported the highest levels of resistance against tetracycline (98.64%) in the non-HD group. On the other hand, the resistance to rifampicin was 91.18% and levofloxacin was 23.53% in the current study that disagreed with results investigated in Italy by Santella *et al.*[31] that found fluctuated lightly resistance, ranging from 7% to 7.8% to rifampin, whereas there were similar finding to levofloxacin was 27.8%–33.3%. High resistance to Amikacin was 85.29% in a current study that disagrees with the study done in India by Vasudeva

Table 11: The biofilm means in non-HD patients

		N	Mean	*P* value
Sex	Males	9	0.26	0.97
	Females	3	0.47	
Results of blood culture	Positive	11	0.33	0.72
	Negative	1	0.10	
van A from bacteria	Positive	10	0.36	0.16
	Negative	2	0.08	
van A from blood	Positive	8	0.34	0.97
	Negative	4	0.26	
bap from bacteria	Positive	7	0.31	0.77
	Negative	5	0.32	
bap from blood	Positive	4	0.26	0.97
	Negative	8	0.43	

et al.[32] that found low resistance of *Staphylococcus* spp. to this antibiotic which was 33.33%. In the current study, the resistance to imipenem was 61.76% and meropenem was 52.94%, which disagreed with a study conducted in New Zealand by Hurst *et al.*[33] that reported highly susceptible to meropenem than to imipenem in nonpatients. All previous studies until now were in agreement with the current study about the resistance to linezolid antibiotic that was 5.88%; it is considered as highly susceptible to treatment the infections caused by *Staphylococcus* spp. The results of this study revealed that the HD patients and the other patients had different levels of microbial susceptibility to the tested antibiotics. A selective antibiotic pressure may result from prolonged hospitalization as well as repeated use of antibiotics to treat other infectious complications.[34] The high rates of resistance make these antibiotics a poor choice for the treatment of infections with *Staphylococcus* spp. Antibiotic resistance has increased in Iraq as a result of these antibiotics being taken without a prescription, resulting in the development of MDR and XDR strains. The current study revealed that the percentage of *van A* from bacteria within HD patients was 91.2%, whereas the percentage of the gene using DNA extracted from blood was 58.8%. In comparison with non-HD, the percentage of *van A* from bacteria by conventional PCR was 83.3%, whereas the percentage of the gene from blood was 66.7%. A study performed in the South of Iran by Saadat *et al.*[35] detected *van A* gene in all isolated strains which approximately agreed with the present study while completely disagreed with research conducted in India by Rengaraj *et al.*[36] that found none of the isolates were positive for *van A*. The percentage of *bap* gene in bacteria isolated from HD patients in the current study was 55.9%, whereas the percentage of the gene from blood was 23.5%. In comparison to the non-HD patient group, the percentage of *bap* gene from bacteria by conventional PCR was 58.3%, whereas the percentage of the gene from blood was 41.7%. In addition, those genes in isolates from growth culture were more frequent in biofilm formation than genes in isolates from the blood by using DNA extracts. Away from the current results, the study published in Morocco and Palestine by Serray *et al.*[37] and Azmi *et al.*[38], respectively, showed that none of the isolates possessed the *bap* gene, and the absence of *bap* gene indicates that another gene-dependent mechanism might be responsible for adhesion and biofilm production in strains. In addition to the results of studies published in Iran by Goudarzi *et al.*[39] that detected *bap* genes in 1.3% of isolates showed weak ability in biofilm production. Also, another study in Iran by Zamani *et al.*[40] found that *bap* gene was 4.2% of isolates with low ability to biofilm formation.

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Conflicts of interest

There are no conflicts of interest.

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