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Molecular Detection of Some Antibiotic Resistant Genes of Bacteria Isolated from Bloodstream Infections of Hemodialysis Patients

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Abstract

A cross-sectional hospital based study was done from November 2017 to February 2019 including 240 patients with chronic renal disease (168 males and 72 females) whose ages were between 15-75 years and they admitted to hemodialysis unit of Kirkuk general hospital. The study also included 50 individuals of blood donors as control group. Ten ml of blood were collected by vein puncture each patient and control enrolled in this study, blood were injected in blood culture bottle sterilely for isolation of bacteria causing bacteremia, with detection of CTX-M-G2 and Van A genes by real time PCR. The study showed that 32.25% of HD patients have positive blood culture while no one of blood donors have a positive blood culture. The highest rate of isolated bacteria from HD patients (51.28%) was G-ve bacteria comparing with G+ve bacteria, while 42.86% of isolated bacteria from patients with UTI was G+ve bacteria. although the result was non-significant relation. The highest rate of bacteria isolated from HD patients (25.65%) was S. aureus followed by E. faecalis (17.95%). The study showed that 80% of S. aureus isolates who were resistant to ceftriaxone were positive for CTX-M-G2 gene comparing with 20% haven't CTX-M-G2 A gene. The highest rate of ceftriaxone resistant S. faecalis (77.78%) were positive for CTX-M-G2 gene comparing with 22.22% haven't the gene, although the results was non-significant. The study showed that the highest rate of ceftriaxone resistant K. pneumoniae (63.64%) were positive for CTX-M-G2 gene comparing with 36.36% haven't the gene. The study showed that 77.78% of S. aureus were positive for Van A gene comparing with 22.22% haven't Van A gene. The highest rate of vancomycin resistant S. faecalis (69.23%) were positive for Van A gene comparing with 30.77% haven't the gene. The study showed that all vancomycin resistant K. pneumoniae were negative for Van A gene. It was concluded that Most S. aureus and E. faecalis isolates were positive for CTX-M-G2 and vanA gene and most Klebsiella spp. isolates were harbored CTX-M-G2 gene.

Keyword: *Bacteremia;; hemodialysis, Blood culture; CTX-M-G2; Van A*

Introduction

Chronic kidney disease is a worldwide public health problem with an increasing incidence and prevalence, poor outcomes, and high cost. Outcomes of chronic kidney disease include not only kidney failure but also complications of decreased kidney function and cardiovascular disease(1). Current evidence suggests that some of these adverse outcomes can be prevented or delayed by early detection and treatment(2). The major outcomes of chronic kidney disease, regardless of cause, include progression to kidney failure, complications of decreased kidney function, and cardiovascular disease(CVD) (3). Increasing antimicrobial resistance is a worldwide concern. The prevalence of resistance of blood borne isolates is increasing and it also varies in accordance with geographical and regional location(4). The infection caused by multidrug resistance (MDR) organisms is more likely to prolong the hospital stay, increase the risk of death, and require treatment with more expensive antibiotics(5). The widespread use of vancomycin has led to increased number of infections by vancomycin resistant enterococci, which could transfer via plasmids the above resistance to S. aureus(6,7). The aim of the study was to isolate bacteria casing bacteremia in hemodialysis patients and molecular detection of CTX-M-G2 and Van A genes by real time PCR

Material and Method

A cross-sectional hospital based study was done from November 2017 to February 2019 including 240 patients with chronic renal disease (168 males and 72 females) whose ages were between 15-75 years and they admitted to hemodialysis unit of Kirkuk general hospital. The study also included the control group who were matched to the patients, included 50 individuals (blood donors). Ten ml of blood were collected by vein puncture using vacutainer syringe from each patient and control enrolled in this study, 10 ml of blood were injected in two 5 ml blood culture bottle sterilely (5 ml for aerobic and the other was for anaerobic culture) for isolation of bacteria causing bacteremia, blood is sampled directly into two aerobic and one anaerobic blood culture bottles containing culture broth.

Bacterial susceptibility test to antimicrobial agents.

Disk diffusion test were employed in the present study and it was carried out according to the modified Kirby-Bauer method. Pure inoculum were obtained by selecting pure colony from an agar plate and suspended in broth to achieve a turbid suspension, and allowing the culture to achieve good active growth, as indicated by observable turbidity in the broth.

Extraction of bacterial DNA

In this study, to view and determine the gene expression of isolated bacteria from HD patients responsible for resistance to ceftriaxone and vancomycin. The isolated bacteria (S. aureus, E. faecalis and K. pneumonia) which represent examples to most resistant bacteria, these bacteria were cultured separately on the Muller-Hinton agar with and without ceftriaxone and vancomycin for quantitative and qaulitative detection of CTX-M-G2 and Van A genes. According to manufacture instructions of ZR Fungal/Bacterial DNA MiniPrep™ kit (Zymoresearch, California- USA). The DNA is isolated and purified and then detected by using real-time PCR (Sacace Biotechnology-Italy) through out detection of the following primers:

A. CTX-M-G2 gene:

CGTTAACGGCACGATGAC

CGATATCGTTGGTGGTRCCA

B. VAN A gene:

CTACTCCCGCCTTTTGGGTT

TTCACACCGAAGGATGAGCC

Statistical analysis

Computerized statistically analysis was performed using Mintab ver 18.0 statistic program for determination of the P. value (P<0.05: significant).

Findings

The study showed that 32.25% of HD patients have positive blood culture while no one of blood donors have a positive blood culture. The study was highly significant (P :0.00001), (Table 1)

Table 1: Results of blood culture among the study groups.

Results of blood culture	**HD patients**		**Blood donors**	
	No.	**%**	**No.**	**%**
Positive	78	32.5	0	0
Negative	162	67.5	50	100
Total	240	100	50	100

P<0.05

The study demonstrated that the highest rate of isolated bacteria from HD patients (51.28%) was G-ve bacteria comparing with G+ve bacteria (Table 2).

Table 2: Types of isolated bacteria among study groups.

Results of blood culture	HD patients	
	No.	%
Gram positive	38	48.72
Gram negative	40	51.28
Total	78	100

The study showed that the highest rate of bacteria isolated from HD patients (25.65%) was S. aureus followed by E. faecalis (17.95%) and the lowest rates was 2.56% for each of S. warneri, S. hominis and A. cloaca. (Table 3).

Table 3: Distribution of isolated bacteria among study groups.

Isolated bacteria from blood culture	HD patients	
	No.	%
Staphylococcus aureus	20	25.65
Enterococcus faecalis	14	17.95
Klebsiella spp.	13	16.67
Escherichia coli	10	12.82
Pseudomonas aeruginosa	6	7.69
Salmonella typhi	6	7.69
Proteus mirablis	3	3.85
Staphylococcus warneri	2	2.56
Staphylococcus hominis	2	2.56
Acintobacter cloaca	2	2.56
Total	78	100

The study showed that 80% (16 of 20) of S. aureus isolated from HD patients were positive for CTX-M-G2 gene (gene responsible for ceftriaxone resistance) comparing with 20% haven't CTX-M-G2 gene, the result was highly significant (p <0.1), the highest rate of S. faecalis (77.78%) were positive for CTX-M-G2 gene comparing with 22.22% haven't the gene, although the results was non-significant (P >0.05), the highest rate of K. pneumoniae (63.64%) were positive for CTX-M-G2 gene comparing with 36.36% haven't the gene, although the result was non-significant (Table 4).

Table 4: Molecular detection of CTX-M-G2 gene resistance to ceftriaxone.

Bacteria	Total No.	No. of resistant isolate	CTX-M-G2				P. value
			Present		Absent		
			No.	%	No.	%	
S. aureus	20	20	16	80	2	20	0.001
S. faecalis	14	9	7	77.78	6	22.22	0.08
K. pneumoniae	13	11	7	63.64	4	36.36	0.15

The study showed that 77.78% of S. aureus isolated from HD patients were positive for Van A gene (one of the genes responsible for vancomycin resistance) comparing with 22.22% haven't Van A gene, the result was significant (P<0.05), the highest rate S. faecalis (69.23%) were positive for Van A gene comparing with 30.77% haven't the gene although the result was non-significant (P >0.05), The study showed that all vancomycin resistant K. pneumoniae were negative for Van A gene, (Table 7).

Table 5: Molecular detection of Van A gene resistance to vancomycin.

Bacteria	**Total No.**	**No. of resistant isolates**	**Van A gene**				**P. value**
			Present		**Absent**		
			No.	**%**	**No.**	**%**	
S. aureus	20	18	14	77.78	4	22.22	0.018
E. faecalis	14	13	9	69.23	4	30.77	0.16
K. pneumoniae	13	6	0	0	6	100	0.014

Discussion

The study showed that 32.25% of HD patients have positive blood culture while no one of blood donors have a positive blood culture. The study was highly significant. In agreement with this result, Hassoon et al (1) demonstrated that 40% of the blood samples of patients under hemodialysis was positive for blood culture. In agreement with the current results, Villalon et al (2) demonstrated that non significant difference was occurred in positive blood cultures of HD patients regarding G+ve and G+ve isolated bacteria and the main microorganisms isolated in blood culture were Staphylococcus aureus and Pseudomonas aeruginosa. Additionally, Mohsin (3) found that Gram negative microorganisms accounted for 61.5% of the events and Gram positive microorganism accounted for 38.5% and Staphylococcus aureus was the most common microorganism. In the study of Parameswaran et al (4), 64% of the pathogens causing CRBSI were Gram-positive and 36% were Gram negative and the commonest pathogen causing bacteremia in HD patients was S. aureus 40%, Pseudomonas aeruginosa 16%, coagulase negative staphylococci 8%, E. coli 8%, Klebsiellapneumoniae 8%, and Acinetobacterbaumanii 4%. Dalgaard et al (5) identified that the most common causative microorganisms in hemodialysis patients, were Staphylococcus aureus (43.8%) and Escherichia coli (12.6%). The most common isolates reported in other studies vary from S. aureus, E.coli, Pseudomonas and Klebsiella and Salmonella and Enterobacter cloacae bacteremia and the source of this bacteria was contaminated saline (6,7). While, Gauna et al (8) found Gram-positive cocci formed 27.7% of blood culture positive of patients with ESRD, also revealed that Staphylococcus aureus accounted for most of the gram-positive infections. Other studies indicated that bacteremia due to hemodialysis caused mostly by Gram positive S. aureus and non-fermenting Gram-negative bacilli, such as Acinetobacter baumannii and Pseudomonas aeruginosa, which are difficult to treat because they are highly resistant, can cause outbreaks of nosocomial infection, and are often associated with high mortality rates (9,10). Hassan et al (11) indicated that most S. aureus isolates harbor CTX-M antibiotic resistance gene and PCR technique is a fast, practical and appropriate method for determining the presence of antibiotic-resistance genes. Al-Marzooq et al (12) reported that, CTX-M genes was detected in 91.3% Malaysian multidrug-resistant K. pneumoniae isolates. Our finding was in agreement with most recent studies in Asia and worldwide(13,14). Moreover, Kpoda et al (15) reported a dissemination of CTX-M genes in ESBL producing Enterobacteriaceae and 58% of Klebsiella had CTX-M- gene. Mathlouthi et al (16) reported the distribution of CTX-M gene was 51.7% in Klebsiella isolated from hospitals in Tunisia and Libya. In a similar study in Burkina Faso Ouedraogo et al (17) reported CTX-M-1 and 2 groups as dominant in hospitals acquired infection. This is in line with the reports of Ibrahim et al (18). In regard of vancomycin resistance, several studies reported that The most common isolated E. faecalis was with vanA genes (19). Most VRE outbreaks in human populations are attributed to the vanA and vanB gene clusters (20). Saadat et al (21) demonstrated that 82.53% of VRSA S. aureus isolates were detected with vanA gene. Banerjee et al (22) also found a high rate of vanA-VRSA strains S. aureus isolated from post operative pus sample. In June 2002, the World's first reported clinical infection due to S. aureus with high resistance to vancomycin (VRSA) (vancomycin MIC>128 µg /ml) was diagnosed in a patient in the USA, this isolate contain the vanA genes from enterococci and the methicillin-resistance gene mecA(23). Bamigboye et al (24) also demonstrated that, significant rate of S. aureus VRSA isolated from different clinical samples were contain the vanA genes. The responsible mechanism for vancomycin resistance in these strains has been found to be the acquisition of resistance plasmids carrying vanA or vanB operon. The similar VRSA isolate in Europe was reported from Portugal in 2013, and this isolate carried mecA and vanA genes probably acquired from VRE that coinfected the patient from whom the isolate was recovered(25). During the past decade VRSA did not spread rapidly and there were only a few reports of this superbug. Until the end of 2012, 33 cases of vanA-type VRSA have been reported worldwide: 13 from the United States, 16 from India, 3 from Iran (2 from Tehran, 1 from Mashhad) and 1 from Pakistan(26). Limited spread of VRSA is attributed to the highly-costly vanA operon for S. aureus, which can be acquired from enterococcal conjugation(27,28,29).

Conclusion: Most S. aureus and E. faecalis isolates were positive for CTX-M-G2 and vanA gene and most Klebsiella spp. isolates were harbored CTX-M-G2 gene.

Conflict of Interest: Non

Source of Findings: Self

Ethical Clearance: Non

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