Plant Archives Vol. 20 Supplement 1, 2020 pp. 1573-1576 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

MOLECULAR INVESTIGATION OF GRAM NEGATIVE BACTERIA EXTENDED SPECTRUM β-LACTAMASE IN HAEMODIALYSIS PATIENTS IN BASRAH PROVINCE, IRAQ

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Abstract

The right medication can decreases of fee that take a lot of money when the rapid screening techniques for detection of extended spectrum β-lactamase (ESBL) producing by bacteria. In the current study, we used the genotypic methods for detection the prevalence of four genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{OXA}) in Gram-negative bacteria in haemodialysis patients that may be causing increases spend to treat of infection by bacteria that can produce ESBLs enzymes that lead to in many cases patient dead. A total of 27 clinical isolates of Gram negative bacteria that collected from haemodialysis (blood samples, were screened for the prevalence of ESBL genes by used four specific primers to amplification bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{OXA} genes. Results showed that 24 (88.9%) out of 27 Gram negative bacteria were ESBL positive. The highest rate of bla_{OXA} gene was 17 (70.8%) while bla_{TEM}, bla_{SHV} were 13 (54.2%) and bla_{CTX-M} was the lowest (8.3%) out of 24 ESBL positive isolates.

Key words*:* ESBLs, bla_{TEM}, bla_{SHV}, $bla_{CTX-M,}$ bla_{OXA}, Haemodialysis.

Introduction

Extended-spectrum β-lactamases (ESBLs) are classically defined as β-lactamases enzymes with the ability to hydrolyze extended-spectrum cephalosporins (ESCs), such as ceftriaxone (CRO), ceftazidime (CAZ), cefotaxime (CTX) and the aztreonam (ATM) (Livermore and Brown, 2001, Rupp and Paul, 2003, Lal *et al*., 2007, Peirano and Pitout, 2010). ESBLs are found in the Gram-negative bacteria and are plasmid-mediated enzymes, in addition, they have been derived from mutations that occurred to the original β-lactamases (Pfaller and Segreti, 2006, Kiiru *et al*., 2012). ESBLs can be blocked *in vitro* by β-lactamase inhibitors such as clavulanic acid and usually retain sensitivity to the cephamycins (*i.e*., cefoxetan and cefoxitin) or carbapenems (*i.e*., erthopenem, meropenem and imipenem) (Nathisuwan *et al*., 2001, Tham, 2012), that produced by the Gram negative bacteria and it have been identified among members of the family Enterobacteriaceae and Pseudomonadaceae in different sites but more frequently in Escherichia coli and *Klebsiella pneumonia* (Rupp and Paul, 2003, Lal *et al*., 2007, Peirano and Pitout, 2010). Major source of morbidity and mortality among hemodialysis (HD) patients the Infectious complications of the vascular access area. Abundant reports implicate the vascular access in up to 48-73% of all HD bacteriemia patients (Nassar and Ayus, 2001, Allon, 2004). Controversy remains regarding the optimal treatment and few clinical reports comparing the treatment efficacy of antibiotic in HD patients (Pitout and Laupland, 2008). Because not found real studies in Iraq that tackle the causes that lead of increased of morbidity and mortality among hemodialysis (HD) patients in Basrah province hospitals, in south of Iraq this study suggestion the screening techniques for detection of four genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{OXA}) producing by Gram-negative bacteria in haemodialysis patients that may be causes increases spend to treated of infection by bacteria that can producing ESBLs enzymes that lead to in many cases patient dead.

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Materials and Methods

Sample collection

A total of 27 Gram-negative bacteria isolated from 147 blood samples from 147 hemodialysis patients in the industrialized kidney unit in the Basrah General Hospital and AL-Sader teaching Hospital in Basrah province of Iraq during the period of October 2017 to January 2018, the patients aged between (9 -78) years old. 1cc of blood was collected from laboratory and nursing staff and before the patient was given anticoagulation substance.

Microbiology

The collected blood was inoculated directly into 10 ml of the brain, heart infusion broth in sterile condition, after that, the blood was transferred to the bacteriological laboratory at the biology department of science college in Basrah university and incubation in 37°C for 7 days with making a subculture each 24 h., on the MacConky agar and subculture repeated for 7 days before discarding the sample (Cheesbrough, 2000). Identification of bacterial isolates was done by using a Vitek2 system test.

Molecular study

Plasmid DNA extraction: Plasmid DNA was extracted from (27) bacterial samples according to (Pure Yield™ Plasmid Miniprep System, Promega, USA).

Detection of ESBLs types by PCR

Four specific Oligonucleotide primers was used to detect the prevalence of four genes (bla_{TEM} forward: (5'-GTATCCGCTCATGAGACAATAACCCTG-3'), reverse:(5'CCAATGCTTAATCAGTGAGGCACC-3'), bla_{SHV} forward:(5'-GGTTATGCGTTATA TTCGCC-3), reverse:(5'-TTAGCGTTGCCAGTGCTC-3'), bla_{CTX-M} foreword:(5'-CGCTTTGCGATGTGCAG-3'), reverse: (5'-ACCGCGATATCGTTGGT-3') and bla_{OXA} forward: (5'-ACACAATACATATC AACTTCGC-3'), reverse: (5'-AGTGTGTTTAGAATGGTGATC-3') with yielded a 918-bp, 842-bp, 550-bp and 885-bp respectively (Rupp and Paul, 2003, Pfaller and Segreti, 2006, Kiiru *et al*., 2012). Five microliters of the template DNA were added to each PCR mixture. The PCR mixture consisted of 25 µl of a mixture of 12.5 microliters Promiga Mastermix, one microliter for each forward and reverse and a final volume completed free nuclease water. The program that used for bla_{TEM}, bla_{SHV} and bla_{CTX-M} PCR amplification consisted of an initial heating step at 95°C for 2 minutes, followed by 30 cycles of 45 s denaturation step at 95°C, a 45s annealing step at 62°C and a 1min. extension at 72°C and a final 5 min. extension step at 72°C. While, the program that used for bla_{OXA} PCR amplification consisted of an initial heating step at 96°C for 5 minutes, followed by 35 cycles of 1 min. denaturation step at 96°C, a 1min. annealing step at 60°C and a 2 min. extension at 72°C and a final 10 min. extension step at 72°C. PCR products were resolved by electrophoresis and visualized with ethidium bromide on 1.5% agarose gels.

Results

The results of Vitek2 test show that *Acinetobacter spp*. Recorded the highest rates that represented 8\27(29.6%). *Pseudomonas spp*. was the second most frequent bacterial isolates with 7\27(25.9%) isolates, Whereas *Klebsiella pneumoniae* and *Burkholderia cepacia group* represented 4(14.8%) for each other, while *Sphingomonas paucimobilis* and *Ralstonia mannitoilyfica* represented 3(11.1%) and 1(3.7%) respectively out of 27 bacterial isolates.

The amplification genes that responsible for production of ESBLs enzymes showed that represent (bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{OXA}) genes in 24(88.9%) out of (27) Gram-negative bacterial isolates, highest incidence in *Pseudomonas luteola* which have 3 genes (bla_{TEM}, bla_{SHV} and bla_{OXA}) and two isolates of *Klebsiella pneumoniae* (18a) and (18b) which have (bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{OXA}) and (bla_{TEM}, bla_{SHV}, and bla_{CTX-M}) genes respectively, followed by one isolate

Table 1: The percentage of ESBLs genes in the isolates under study.

Gram negative bacteria isolated	Total no. of isolated	% of bla_{TEM}	% of bla_{SHV}	% of bla_{OXA}	% of bla_{CTX-M}	Total
Burkholderia cepacia group	4	3 (75%)	1 (25%)	1 (25%)	0 %	3 (75%)
Ralstonia mannitoilyfica	1	1 (100%)	0 %	1 (100%)	0 %	2 (50%)
Pseudomonas luteola	2	2 (100%)	2 (100%)	2 (100%)	0 %	3 (75%)
Sphingomonas paucimobilis	3	1 (33.3%)	1 (33.3%)	3 (100%)	0 %	3 (75%)
Acinetobacter baumanii complex	3	2 (66.7%)	1 (33.3%)	3 (100%)	0 %	3 (75%)
Klebsiella pneumonia	4	2 (50%)	2 (50%)	1 (25%)	2 (50%)	4 (100%)
Pseudomonas aeruginosa	5	2 (40%)	3 (60%)	2 (40%)	0 %	3 (75%)
Acinetobacter haemlyticus	4	0 %	2 (50%)	3 (75%)	0 %	2 (50%)
Acinetobacter lowffii	1	0 %	1 (100%)	1 (100%)	0 %	2 (50%)
Total	27	13 (54.2%)	13 (54.2%)	17 (70.8%)	2 (8.3%)	24 (88.9%)

of *Pseudomonas aeruginosa* (Isolate No. 132) and *Sphingomonas paucimobilis* (isolate No. 76) that have (3) genes (bla_{TEM}, bla_{SHV} and bla_{OXA}) for each other, whereas other isolates variance to have 1 or 2 ESBLs genes (bla_{TEM}, bla_{SHV} and/or bla_{OXA}), as shown in (Table 1).

Whereas the percentage of enzyme-encoded genes in isolates, among the 4 genes, bla_{OXA} recorded the highest percentage in 17 (70.8%), while bla_{TEM} and bla_{SHV} were 13(54.2%), while bla_{CTX-M} recorded the lowest percentage in 2(8.3%) out of 24 isolates.

Discussion

Recent research showed that the emergence of bacteria has ability to produce ESBL enzymes is more common in patients with a past history of exposure to antibiotics, hospitalization, urogenital surgical procedure, and catheterization (Azap and Arslan, 2010).

The study of enzyme detection at the gene level is one of the few studies carried out of Iraq, especially in the cases of hospital-acquired infections and the detection of four genes belonging to the ESBL enzyme groups, bla_{TEM}, bla_{SHV} and bla_{CTX-M} which inhabited by clavulanic acid and bla_{OXA} which low or not inhabited by clavulanic acid.

Although the phenotypic method is useful and certified to detect the presence of the ESBL enzyme, it is not possible through which any of the genes that belong to the ESBL enzymes are already present.

This study was conducted to investigate the production of the ESBL enzyme by the genes and using PCR technique to detect the spread of these enzymes. Numerous research or reference laboratories use genotypic techniques amplification of ESBL gene by PCR for the identity of the particular gene responsible for the production of the ESBL enzymes, that have the extra capacity to detect low-level resistance (*i.e.*, May be neglected by using phenotypic methods).

Moreover, molecular assays additionally have the ability to be accomplished without delay on clinical samples without culturing the bacteria, with the next reduction of time which needed for detection. In addition to the fact that genetic method to detect the production of ESBL enzyme is more efficient, accurate and give results more reliable than the phenotypic methods and avoid the problems that may occur when use phenotype methods such as technical failure and problems of storage. In this study, all Gram-negative bacteria obtained from the blood culture were genetically tested after extraction of plasmid and investigation of ESBL genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{OXA}) presence in plasmid by using PCR technique and the percentage of ESBL was 88.9% (24 out of 27).

There are many studies around the world that included study the prevalence of ESBL enzymes, in a study in 2010 by Bali and Co-workers detected the ESBL in Gram-negative bacteria deals with different samples (Urine, Blood, Sputum, Wound, Catheter, CSF and Abscess) by using genotypic methods was 71.3%. Another study by Osman e*t al*., 2017 also in the same line with our result that recorded (73.9%) 28. In Iran, 2009, bla_{TEM}, bla_{SHV} and bla_{CTX-M} was, 18%, 7.5% and 24.5%, respectively (Nasehi *et al*., 2010). In India both bla_{TEM} and bla_{SHV} were, 67.3%, 20%, respectively (Lal *et al*., 2007). While this study shows the 54.2% for bla_{TEM}, bla_{SHV} equally and 8.3% for bla_{CTX-M}. A study by Al-Diywania\Iraq recorded the prevalence of bla_{SHV} and bla_{CTX-M} in *K. Pneumonia* in patients undergoing urinary infections was 92% (35\38), 78.9% (30\38) respectively (Saleh *et al*., 2018). The prevalence of ESBL-Producing bacteria suggest over use and irrational use of third generation cephalosporins and fluoroquinolones, provide the world brand new resistance bacteria which can produce many different antibiotic resistance enzymes (Soraas *et al*., 2013).

Conclusions

Using the genetic method and gene amplification can avoid the technical errors that the phenotype requires. It is also a reliable and accurate method for detecting the spread of the enzyme even if the ESBL activity is low.

Conclusion

High prevalence of bla_{OXA} followed bla_{TEM} and bla_{SHV} and low incident of bla_{CTX-M} which, revealed by molecular analysis, among ESBL-Producing Gram-negative bacteria isolate collected from the blood of haemodialysis patients.

Acknowledgment

The authors kindly thank the Research council of Basrah University of life science and the industrial kidney unit in the Basrah General Hospital and AL-Sader teaching Hospital in Basrah province of Iraq to support and overcome difficulties to achieve this study.

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