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RESEARCH ARTICLE

Identification of *bla$_{OXA-1}$* genes in Klebsiella isolated from urinary tract infections.

Zahid S. Aziz
Dept. of Biology, Faculty of Science , University of Misan, Misan / Iraq

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Corresponding Author

Zahid S. Aziz
E.mail:
za_h_id1974@yahoo.com

Abstract

The study aimed to evaluate the distribution of *bla$_{oxa-1}$* genes which coding for oxacillinsaes β- lactamases which hydrolyzed oxacillin and cloxacillin β- lactams .

All urine samples taken from patients complained from urinary tract infections which collected during year 2014 in Hospitals of Al-Najaf province were cultured on MacConkey agar were the results revealed that 250 of isolates were lactose fermentative versus to 50 of isolates were lactose non fermentative.

Conventional tests IMViC (Indol, Methyl red, Voges- Proskauer and Citrate utilization) were implemented for preliminary identification of isolates, further more motility test was used crucially. Lactose fermentative and non motile isolates were candidated to be long-established by Vitek 2 system. The results revealed that 53 of isolates identified as Klebsiella spp.

The results of molecular study showed the prevalence of *bla$_{oxa}$* genes in 38(71.7%) of isolates which been screened by Polymerase Chain Reaction .

Introduction

The OXA β-lactamases differ from the TEM and SHV enzymes and they are a member of class D (2d) according to Ambler classification (Ambler et al., 1991). The OXA group mainly occurs in Acinetobacter and Pseudomonas species. The OXA β- lactamases attack the oxyimino-cephalosporins and have a high hydrolytic activity against oxacillin, methicillin and cloxacillin more than benzylpenicillin, inhibited less efficiently by clavulanate and their activity is inhibited by NaCl (Walther-Rasmussen and Hoiby, 2006).

OXA enzymes prevalent among various Gram-negative bacteria. The dissemination of plasmids, transposons, and integrons among bacteria and species contribute to so-called gene epidemics. Integrons have a alarming capacity for the enrollment, spread, and expression of resistance genes, and surveys show that they are widespread among gram-negative bacteria (Walsh**,** 2010 and Chen et al., 2010).

Walther- Rasmussen and Hoiby (2006) mentioned that more than 180 different variants of OXA enzymes have been identified on the protein level, most of the genes encoding class D oxacillinases have frequently been found on plasmids incorporated as gene cassettes in integrons, several chromosomal encoded oxacillinases have been described .

Materials and Methods

Plasmid DNA Extraction

Plasmid DNA extraction was fulfilled by using High-Speed Plasmid Mini Kit according to the protocol of manufactured company (Geneaid, South Korea).

Polymerase Chain Reaction Protocol

The extracted plasmid DNA were subjected to *bla*$_{oxa\text{-}1}$ genes amplifications.The primers of (Bioneer , South Korea) were used for *bla*$_{oxa\text{-}1}$ amplification:

OXA-1 F ACA CAA TA CAT ATC AAC TT CGC and OXA-1 R AGT GTG TTT AGA ATG GTG AT where PCR conditions used as suggested by Lim et al.(2009) as following: a cycle of initial denaturation temperature was 96 C^{o} for 5 minutes followed by 35 cycles of 96C^{o} for 1minutes, annealing temperature was 60C^{o} for 1 minutes, elongation temperature was 72C^{o} for 2 minutes followed by cycle of final elongation temperature 72C^{o} for 10 minutes.

The premix tube (1 µl Taq DNApolymerase, dNTPs each 250 µM, Tris - Hcl (pH = 9.0) 10mM, KCL30Mm, Mgcl2 1.5 Mm and trace of stabilizer and tracking dye1) completed to 20 µl volume of reaction with recommended amount of DNA template 5 µl of 5-50 ng , 2.5 µl for each primer of 5-10 pmole and 5 µl of deionized distilled water. The Program was running by Sure cycler 8800 (Agilent, USA) .

Gel electrophoresis and documentation

The amplified PCR products were separated in 1% agarose gel after staining with ethidium bromide 5 µl of 0.5 µg / ml. The electric current was set on 75 volt for 2 hrs. and visualized with UV light using gel documentation system. The positive results were distinguished when the DNA band base pairs of sample was equal to the target product size compared with molecular DNA ladder(100 bp DNA ladder , Geneaid , South Korea . Finally the gel was photographed using Cleaver gel documentation system.

Results and discussion

Out of 53 identified Klebsiella spp. which had been screened for the prevalence of *bla*$_{oxa}$ genes, 38(71.7 %) were positive as shown in Figures (1, 2 and 3).

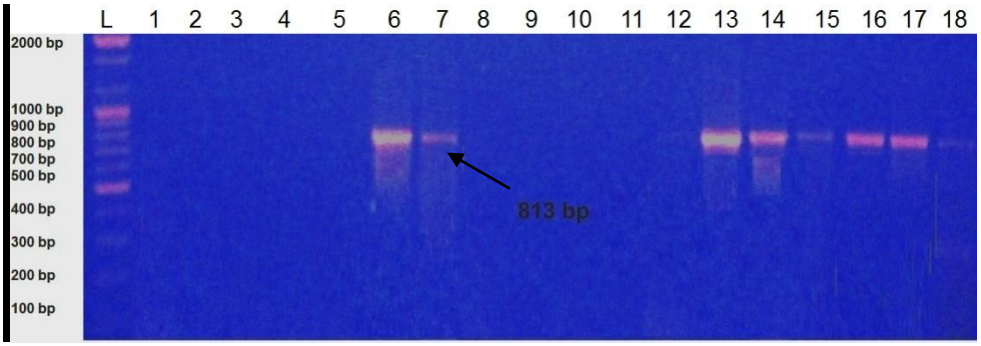

Figure (1) Electrophoresis diagram of bla $_{OXA\text{-}1}$ PCR amplicon (813bp) molecular size marker (100 bp). The electrophoresis was performed at 75 volt for 2hrs, agarose gel was stained with Ethidium bromide.

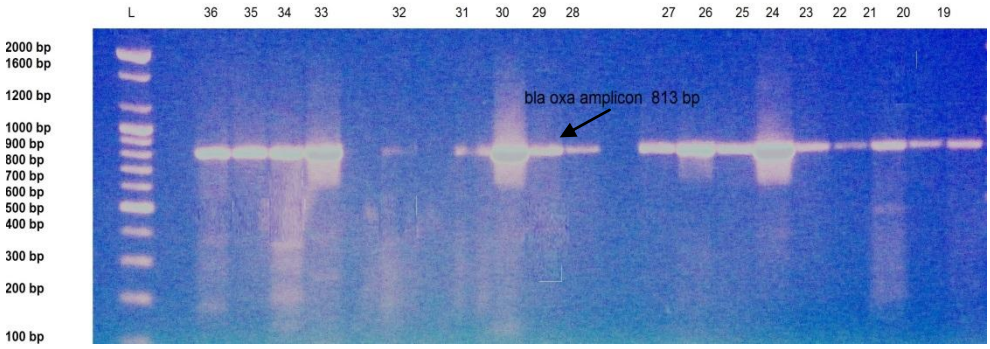

Figure (2) Electrophoresis diagram of bla $_{OXA\text{-}1}$ PCR amplicon (813bp) molecular size marker (100bp). The electrophoresis was performed at 75 volt for 2hrs, agarose gel was stained with Ethidium bromide.

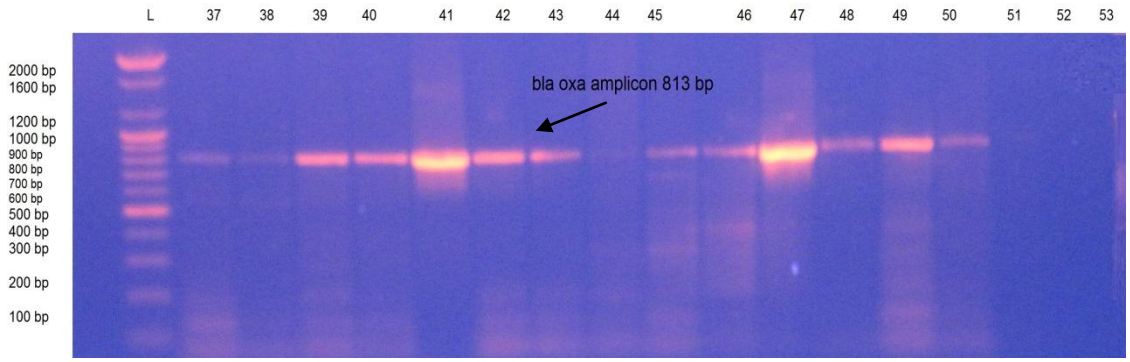

Figure (3) Electrophoresis diagram of *bla* $_{OXA\text{-}1}$ PCR amplicon (813bp), L molecular marker (100bp) The electrophoresis was performed at 75 volt for 2hrs, agarose gel was stained with Ethidium bromide.

Sana *et al.* (2011) stated in their study which conducted in North Lebanon that the results of screened samples by polymerase chain reaction showed high prevalence of $bla_{OXA\text{-}1}$ where 45.83% of samples were carried genes

Occurrence rate of bla_{OXA} genes in studied isolates varies widely. In Iran, Mostatabi et al. reported that 20.51% of ESBL-producing Serratia isolates carried bla_{OXA} gene (Mostatabi, 2013). In a study from Tunisia, Bourouis et al. (2013) showed presence of $bla_{OXA\text{-}1}$ genes among ESBL-producing *E. cloacae* .In Cameroon the results showed that $bla_{OXA\text{-}1}$ genes were present in all of isolates (Lonchel, 2012). In a study from Madagascar, Rakotonirina et al. (2013) reported that 14.28% of ESBL-producing isolates harbored $bla_{OXA\text{-}1}$ gene, which was lower than our findings. The antimicrobial resistance pattern among septicemia causing *K. pneumoniae* and the prevalence of inhibitor resistant OXA-1 β-lactamase genes among them. these isolates were further selected for $bla_{OXA\text{-}1}$ screening. Amplification of β-lactamases genes by conventional PCR showed the presence of $bla_{OXA\text{-}1}$ genes among 12 *K. pneumoniae* (20.3%) isolates (Sugumar et al., 2014). Ramazanzadeh (2010) indicated that genes encoding TEM, OXA-1 and OXA-2 were found in ESBL producing Klebsiella 14.85, 14.58 and 4.17%, respectively. Many researchers showed that these genes commonly present with the same genetic environment of other genes.The common combination of CTX-M-15, OXA-1, SHV-1 and TEM-1 β-lactamases and PMQR (plasmid mediated quinolones resistance) determinants aac(6')-Ib-cr and qnrS1 in a community *K. pneumoniae* strain (Abouddihaj et al., 2011). Isolates expressed CTXM- 15 and OXA-1 enzymes were multidrug-resistant (Woodford et al., 2007; Arpin et al., 2009). The combination of $bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$ and $bla_{TEM\text{-}1b}$ was reported in 30 strains from Portugal (Mendonc¸a et al., 2007), and an association between bla genes has been described in the Brazilian community (Minarini et al., 2007). The association of $bla_{CTX\text{-}M\text{-}15}$ and $bla_{OXA\text{-}1}$ in the same strain has also been described in Portugal (Mendonc¸a et al., 2007) and the USA (Hanson et al., 2008). Combined production of CTX-M and OXA enzymes by *E. coli* and *K. pneumoniae* also been reported (Livermore and Hawkey, 2005).
We go to concluding that there was high rate of occurrence of $bla_{oxa\text{-}1}$ genes among identified Klebsiella which might indicated the high level of pressure obtained from the use of related antibiotics.

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