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Molecular detection and genetic characterization of Resistance Genes in Shigella spp isolates Al-Diwaniyah city

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Abstract-- *Development of antibiotics resistance in bacteria, consider as a part of evolutionary process in them has now been declared as a matter of global crisis by the World Health Organization ,It has resulted in increased failure rates of treatment of infectious diseases caused by bacteria, as drugs to which they were previously susceptible no longer works. Bacteria can be intrinsically resistant owning to its resistant mechanisms.aim of current study to detection and genetics characterization of resistance genes in Shigella spp. Shigella isolates were investigated genotypically for harboring resistance genes including (bla $_{CTX-M-15}$, bla $_{OXA}$, ereA ,ereB) . The current study recorded the highest prevalence of bla $_{CTX-M-15}$ (60%) , bla $_{OXA}$ (70%) , ereA (45%) ,ereB (60%).*

Keywords: *Shigella spp, Antimicrobial Resistance, Resistance Genes, Shigella species*

I. Introduction

Shigella belongs to the phylum Proteobacteria , class Gammaproteobacteria, order Enterobacteriales, family Enterobacteriaceae. These are dividedinto multiple serotypes dependent on O-antigen and biochemical differences. Different species are linked to disease in varying geographical locations. *Shigella sonnei* has cells, which lead to severe inflammatory responses in become the most dominant serotype causing intestinal tissue , Intracellular *Shigella* movement is shigellosis in asian countries in recent years (Qu *et al* ., 2014) . facilitated by directing host cell actin polymerization *Shigella dysenteriae*, implicated in epidemics, leads to exclusively at one pole of the bacteria by a process death (Raja *et al* ., 2011). Environmental risk factors of shigellosis known as actin-based motility. The force generated by include water supply, sanitation, and household the polymerizing actin is sufficient to propel *Shigella* environment (Chompook , 2011).

Resistance is defined as bacteria that are not inhibited by usually achievable systemic concentration of an agent with normal dosage schedule and/ or fall in the minimum inhibitory concentration ranges. Likewise the multiple drug resistance is defined as the resistance to two or more drugs or drug classes (Roger *et al* ., 2003). Antibiotic resistance occurs when bacteria change in someway that reduces or eliminates the effectiveness of drugs,

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chemicals or other agents designed to cure or prevent the infection. Thus the bacteria survive and continue to multiply causing more harm. Widespread use of antibiotics promotes the spread of antibiotic resistance. Bacterial susceptibility to antibacterial agents is achieved by determining the minimum inhibitory concentration that inhibits the growth of bacteria (Blair *et al* ., 2018).

On the other hand, bacterial species seem to have evolved a preference for some mechanisms of resistance over others. For example, the predominant mechanism of resistance to β-lactams in gram-negative bacteria is the production of β-lactamases, whereas resistance to these compounds in gram-positive organisms is mostly achieved by modifications of their target site, the penicillin-binding proteins (PBPs) (Huang *et al* ., 2005). It has been argued that this phenomenon is likely due to major differences in the cell envelope between gram-negatives and gram-positives. In the former, the presence of an outer membrane permits to "control" the entry of molecules to the periplasmic space. Indeed, most β-lactams require specific porins to reach the PBPs, which are located in the inner membrane. Therefore, the bacterial cell controls the access of these molecules to the periplasmic space allowing the production of β-lactamases in sufficient concentrations to tip the kinetics in favor of the destruction of the antibiotic molecule. **)** and Methylation of the bacterial ribosome producing resistance to macrolides (Jose *et al* ., 2016),

II. Materials and methods

isolates of *Shigella* bacteria were diagnostic by different tests were twenty isolates , and they included biochemical tests,. The suspectaed coloniess identified by Api20 E systems to confirm the diagnosis of *Shigella* species isolates were differentiated molecularly to 20 *Shigella* isolates (12 *S. sonnei* and 8 *S. flexneri*) . Genomic DNA was extracted from obtained *Shigella* isolates according to manufacturer instructions of Genomic DNA purification kit (Geneaid, USA). The purity and concentration of DNA for each isolate were measured by Nonodrop instrument (THERMO, USA).

Table 1. Polymerase Chain Reaction master mix preparation.

PCR Master mix	Volume
DNA template	5µL
Forward primer (10pmol/µL)	1.5µL
Reveres primer (10pmol/µL)	1.5µL
Master mix	7.5 µL
PCR water	4.5µL
Total volume	20 µL

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PCR for detection of genes including (*bla* $_{CTX-M-15}$, *bla* $_{OXA}$, *ereA* ,*ereB*) genes was carried out using a Master Cycler gradient PCR machine (Eppendorf, Germany). Microbial DNA was extracted from the colonies grown overnight on xylose lysine deoxycholate (XLD) agar. All the *Shigella* isolates were investigated genotypically for harboring quinolones genes by PCR technique.

Table 2.Primers used in this study

gene	Sequence (5'-3')	Amplicon	Reference
bla-CTX-M-15	TAAAGCATTGGGCGACAG	200bp	(Sabra *et al* ., 2009)
	GGTGAAGTAAGTGACAATC		
bla-OXA	ACCAGATTCAACTTTCAA	598bp	Rahman, *et al* ., 2017
	TCTTGGCTTTTATGCTTG		
ere(A)	GCCGGTGCTCATGAACTTGAG	420bp	Rahman, *et al* ., 201
	CGACTCTATTCGATCAGAGGC		
ere(B)	TTGGAGATACCCAGATTGTAG	537bp	Rahman, *et al* ., 201
	GAGCCATAGCTTCAACGC		

III. Results and Discussion

The *CTX-M* group has become the most common type of ESBL in Latin America, but is also becoming more common in Europe and has been recently reported in the UK(Canton and Coque ,2006) Also, in Asia, previous reports have identified *bla*$_{Ctx-M}$ producing isolates in China, Korea, Japan, and India, Resistance to broad-spectrum β-lactams is becoming an ever-increasing problem in Iran(Behrooozi *et al* ., 2010).

Shigella species have been progressively acquiring resistance to several antimicrobial agents used for the treatment of infections with these bacteria (Kariuki *et al* ., 1996). Several reports have indicated an increase in cases of *Shigella* species resistant to beta-lactams, including third-generation cephalosporins (Levesque *et al* ., 1995).

Regarding to PCR results showed that *bla* $_{CTX-M-15}$ gene were present in investigated bacterial isolates in percentage of *bla* $_{CTX-M-15}$ (65%) present in each *S.sonnei* (8 isolates) and *S.flexneri* (5 isolates).

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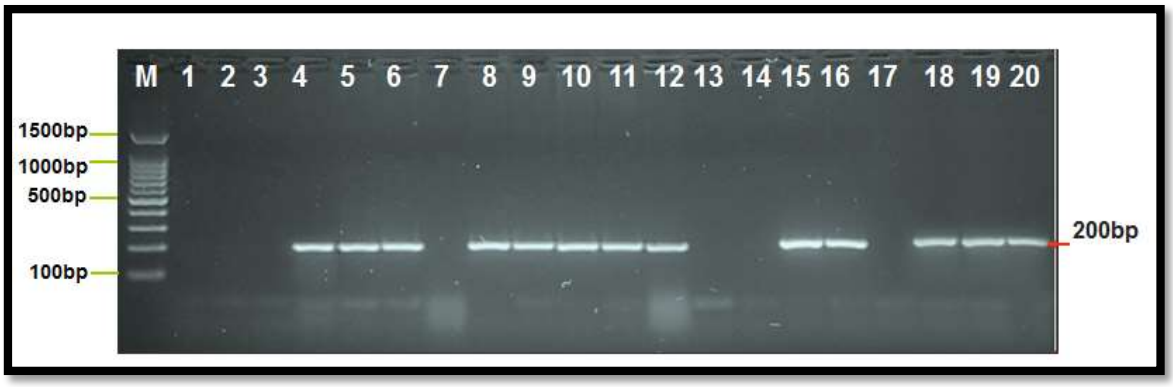

Figure (1): Agarose gel electrophoresis image that showed the PCR product analysis of *bla* $_{CTXM-15}$ gene in *Shigella spp*. isolates. Where Marker ladder (2000-100bp), Lane (4,5,6,8,9,10,11,12,15,16,18,19,20) only positive *bla* $_{CTXM-15}$ gene at 200bp PCR product size.

In a reported study in China by Qian *et al* (2018) the percentage of the presence of bla $_{CTX-M-15}$ is (10.7%). In irani studies by Ranjba *et al* (2013) and Tajbakhsh *et al* (2012) they detect the presence to bla $_{CTX-M-15}$ in *Shigella* species isolates. Molecular demonstration of resistant to third generation cephalosporin has revealed CTX-M type ESBLs, especially bla $_{CTX-M-15}$, as the most frequent ESBL determinants in different nations like India (Taneja *et al*., 2012) . The CTX-M types ESBLs are plasmid-mediated β-lactamases having higher hydrolytic action against cefotaxime. bla $_{CTX-M-15}$ has been observed as common genotype of ESBL among *Shigella* isolates (Parajuli *et al.*, 2017). Study by Zhang *et al* (2014) detection of bla $_{CTX-M-15}$ were (13.6%) and this contrast to present study. In china study by Wang *et al* (2019) bla $_{CTX-M-15}$ was (4.6%). In india study by Sethuve *et al* (2019) gen present in (13%) for both *S.sonnei* and *S.flexneri* .

bla $_{OXA}$ beta-lactamases were long recognized as a less common but also plasmid-mediated beta-lactamase variety where the percentage of bla_{OXA} (70%) Distributed between *S.sonnei* (8 isolates) and *S.flexneri* (6 isolates). .

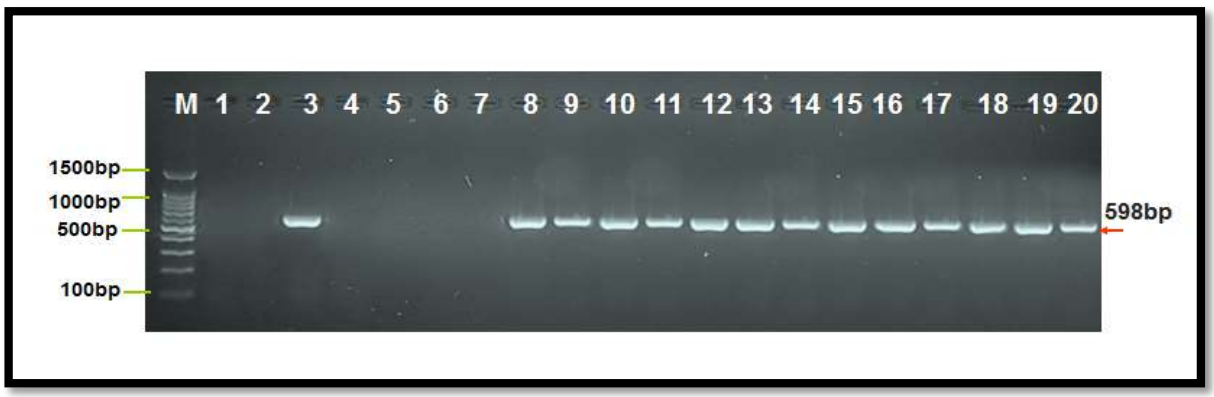

Figure (2): Agarose gel electrophoresis image that showed the PCR product analysis of *bla*-$_{OXA}$ gene in

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***Shigella spp*. isolates. Where Marker ladder (1500-100bp), Lane (3,8,9,10,11,12,13,14,15,16,17,18,19,20) only positive *bla-*$_{OXA}$ gene at 598bp PCR product size.**

The bla$_{OXA}$ gene is encoded for a class D β-lactamase, is also known as oxacillinase or OXA type β-lactamase (Poirel *et al* ., 2010). In Denmarki study by Peirano *et al* (2005) where the percentage of *bla* $_{OXA}$ gen in isolate was(69.3%) showed only in *Shigella flexneri* and not detecte *S.sonnei* in isolates This results are similar to the present study. other study in Japanese study by Ashraf *et al* (2006) no detection this gen between *Shigella* species and was (0.0%) and this contrast to the current study . in china study by Wang *et al* (2019) showed *bla* $_{OXA}$ in (39.8%).

Antimicrobial susceptibility of *Shigella* strains is related to general use of antimicrobials in population. There are many antibiotics not effective against shigellosis and it seems that this is the situation worldwide . in Northwest China study by Zhu *et al* (2017) were *bla* $_{OXA}$ (78.95%). in mexico study by Zaidi *et al* (2013) observed that *bla*$_{OXA}$ no detect in all isolates and this contrast to the present study . .

Erythromycin is an antibiotic used for the treatment of a number of bacterial infections. Erythromycin displays bacteriostatic activity or inhibits growth of bacteria, especially at higher concentrations, but the mechanism is not fully understood. By binding to the 50s subunit of the bacterial rRNA complex, protein synthesis and subsequent structure and function processes critical for life or replication are inhibited (Shafia *et al* ., 2016).

The current study showed present of *ereA* and *ereB* genes in isolates of *Shigella spp* in percentage (45%) and (60%) respectively. Where in France study by Bourtchai *et al* (2008) was negative for the *ereA* and *ereB* genes .

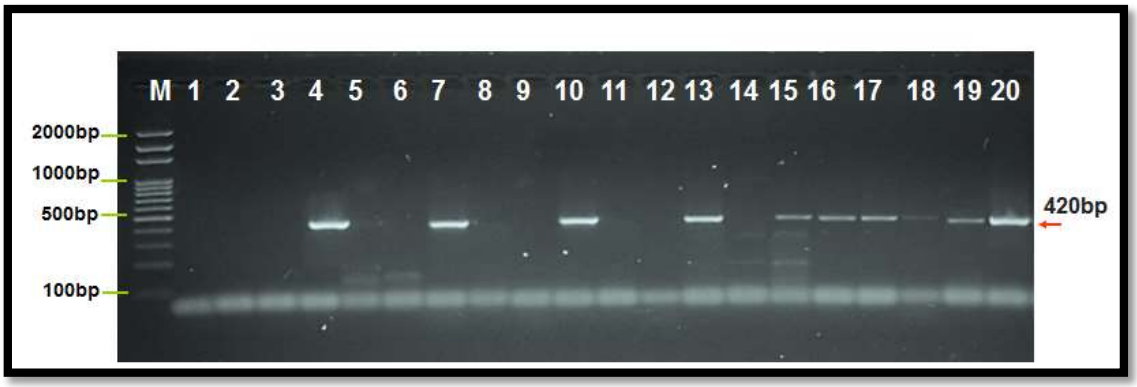

Figure (3): Agarose gel electrophoresis image that showed the PCR product analysis of *ere(A)* gene in *Shigella spp*. isolates. Where Marker ladder (2000-100bp), Lane (4,7,10,13,15,16,17,19,20) only positive *ere(A)* gene at 420bp PCR product size.

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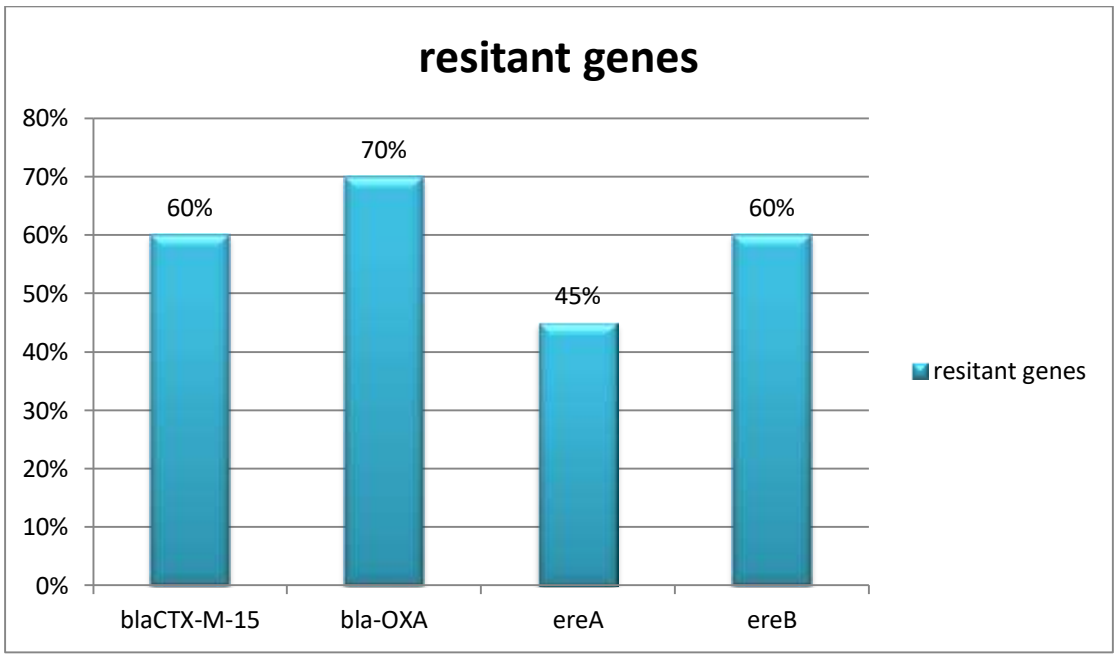
Figure (4): Agarose gel electrophoresis image that showed the PCR product analysis of *ere(B)* gene in *Shigella spp.* isolates. Where Marker ladder (2000-100bp), Lane (1,2,3,5,6,7,10,11,12,17,18,19) only positive *ere(B)* gene at 537bp PCR product size.

However, in the early 1960s, Erythromycin was as effective as chloramphenicol and tetracycline which were the first choice antibiotics for shigellosis at that time. Although *Shigella spp*. started to develop multiple drug resistance in the late 1960s due to massive use of antimicrobiotics in hospitals, Erythromycin was one of the few antimicrobiotics still as effective as previously (Honma *et al* ., 2000).



P value = 0.001 X2 = 20.02 (HS)

Figure (5) .The distribution of Antibiotic Resistance Genes

The minimum inhibitory concentration (MIC) of Erythromycin against *Shigella* is about 50 μg ml−1, which is likely to be achieved in the stool but not in the intestinal epithelial cells during the treatment of shigellosis (Higa *et al* ., 1995).

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IV. Conclusion

We conclude High occurrence rate of β-lactamases producing *Shigella* isolates was revealed, especially *bla* $_{CTX-M-15}$, *bla* $_{OXA}$ were the most common among investigated isolates , also resistance genes *ereA* and *ereB* were investigated. Molecular techniques is necessary for detection of pathogenic bacteria among clinical cases, There is a considerable genetic diversity among *Shigella* isolates in Al-Diwaniyah city.

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