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Detection of Genes Responsible for Resistance to Quinolones Antibiotic in Salmonella Enterica Using the Polymerase Chain Reaction (PCR)

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ABSTRACT

Standard microbiological procedures were used for the isolation and identification of the Salmonella enterica isolates. Salmonella strains resistant to quinolone antibiotics were tested and screened for according to CLSI standards for antibiotic susceptibility testing. employing qnrA, qnrB, and qnrs-specific primers in polymerase chain reaction (PCR) to identify qnr genes. The findings showed that 22 strains (59%) contained the qnr S gene, found that both the qnrA and qnrS genes were present in 7 strains (18%) of Salmonella. Two isolates (5%) had qnrB gene positive tests, while 11 strains did not have any stribany qnr genes at all. This approach allowed us to verify that the Quinolones genes are diluted throughout the examined salmonella strains.

Keywords- Quinolones antibiotic, Salmonella enterica, Polymerase chain reaction (PCR).

I. INTRODUCTION

SALMONELLA: Salmonella enteric a is one of the primary causes of human gastroenteritis globally, hence the growth of antimicrobial resistance among S. enteric in a number of countries over the last few years is cause for worry. Salmonella and other pathogens thrive in the environment, making it a major contributor to the contamination of food, poultry, and other products. In 2000 (1), Salmonella enterica serovar Typhi was responsible for 22 million cases of febrile illness and 217 thousand fatalities over the world. The sensitivity of the blood culture was assumed to be at 50% for the sake of this estimate. Antimicrobial pretreatment, blood volume collected, time and circumstances of sample transit, laboratory capabilities, and pathogen identification technique are all potential influences on the sensitivity of blood cultures for *S. Typhi.* There aren't many agnostic methods available for *S. Typhi* detection. Although the Widely test has a poor sensitivity of 39% (2,3), it has been one of the most frequently used diagnostics so far. antibiotics In both human and animal medicine, antimicrobials like quinolones and fluoroquinolones are often used. In 1962, nalidixicacid, the first quinolone, entered clinical use. By the mid- 1980s, ciprofloxacin, a fluoroquinolone with broad-spectrum in vitro antibacterial activity, had entered clinical use for the first time. The prevalence of enter bacterial resistance to quinolones is rapidly growing throughout the globe (22). Quinolones are widely utilized because of their broad range antimicrobial activity and their potent therapeutic impact on resistant bacteria. Fluor quinolones, which contain fluorine attached to the core ring structure and are often found at positions 6 or 7, make up the vast bulk of the quinolone family. The bacterial spectrum allows us to classify quinolones into four generations (4,5). Due of Salmonella's widespread prevalence, antimicrobials are used in animal husbandry for both therapeutic and growth-feed additive objectives. mutations in the DNA gyrase and DNA topoisomerase IV genes, the fluoroquinolones' target bacterial enzymes, provide protection against the inhibitory effect of quinolones and are the primary cause of quinolone resistance. However, the prevalence of this problem cannot be fully explained by the common view of quinolone resistance as a mutational phenomenon. Horizontally transferrable components may provide a better explanation for this phenomena. These provide a marginal amount of

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decreased quinolone. When quinolones are available, enough for bacteria to survive, susceptibility, and resistance mutations evolve sequentially rather than simultaneously. Since qnrA1's discovery, a number of plasmid-mediated quinolone resistance (PMQR) genes have been discovered.

Finding in 1998. According to recent studies, animals that dwell in or near water may have given origin to the qnr genes that are now in use. Extreme pressure from quinolones has resulted in the spread of such genes on mobile genetic elements. (6,7). PCR stands for polymerase chain reaction. Since the approach is highly sensitive, specific, and effective, it has significant promise as a diagnostic tool for the identification of antimicrobial genes and is straightforward to implement. The DNA copy number increases exponentially as the quantity of product doubles with each cycle of amplification. This implies that not only may a huge quantity of amplified DNA be generated in only a few hours, but that the reaction can be kicked off with as little as a few molecules of target DNA present in the sample. With primers of about 15 nucleotides and high annealing temperatures, the reaction is so precise that practically no "false priming" occurs, resulting in a very homogenous PCR product. Since the gene or genes of interest may be quickly amplified by PCR provided the surrounding sequences are known, acquiring DNA for gene cloning or sequencing purposes is facilitated by PCR. Gene amplification using PCR is standard practice in comparative and phylogenetic investigations (8,9). Recently, a new family of DNA components with the ability to resist Quinolones antibiotics through antimicrobial resistance genes has been discovered. The purpose of this research was to identify Salmonella enterica isolates from several locations in Mosul, Iraq, that have acquired resistance to the quinolone antibiotics qnrA, qnrB, and qnrS.

II. MATERIALS AND METHODS

Isolation and Identification of Bacterial:

The study included 93 *Salmonella enterica* isolates collected during November 2018 to January 2019 for several source (human, Poultry meat and enviromental samples). The isolates were obtained from clinical specimens that patients with suspected salmonella infections had provided. 30 examples, Poultry meat was 16 and environmental samples included sewage 27 and soil 20 samples. *Salmonella* was collected on selective media salmonella shigella agar. Standard microbiological and biochemical procedures, including gram staining and the basic Oxidase, Catalase, TSI, and IMViC tests, were used for bacterial isolation and identification. The O and H antigens were identified by serotyping after Salmonella was confirmed using biochemical tests(10,11).

Storage of salmonella isolates:

After confirmation of species, we cultured our Salmonella enterica isolates at 37°C for a full 24 hours in a nutrient broth inoculum of 5ml.Before undergoing PCR testing, the isolates were frozen in a solution of culture and glycerol (20% - 1 ml glycerol of 4 ml broth culture). The usual microbiological and biochemical procedures were used for isolation and identification. Following biochemical confirmation of the presence of Salmonella, serotyping was performed to identify the O and H antigens using specialist antiserum. (11,12).

Antimicrobial Suscptibility testing:

The susceptibility of bacteria to antibiotics was tested using a Kirby-bauer disc diffusion method with modifications according to NCCL2011 guidelines. Antibiotics g/disc Nalidixic acid NA 30 and ciprofloxacin CIP 10 obtained from the Bioanalyse firm in Turkey were used to cultivate the bacteria on Muller hinton agar(Oxid) (13,14).

III. MOLECULAR STUDY

DNA isolation and PCR:

The genomic DNA purification kit from Promega USA was used in the Wizard® DNA protocol's extraction step. dependent on the manufacture's instructions and the instrument's ability to detect the concentration (England's Nanodrop spectrometer).

PCR (Tabl 1 Primers used for PCR amplification of qnrA, qnrB and qnrS in Salmonella enterica) condition and Primers utilized for detection of qnrA, B and S genes in this investigation.

Origin	Expected size bp	Sequences 5→3	primers
Promega USA	516	F: 5′-ATT TCT CAC GCC AGG ATT TG-3′	qnrA
		R: 5′-GAT CGG CAA AGG TTA GGT CA-3′	
	526	F: 5′-GTT GGC GAA AAA ATT GAC AGA A-3′	qnrB
		R: 5′-ACT CCG AAT TGG TCA GAT CG-3′	
	417	F: 5′-ACG ACA TTC GTC AAC TGC AA-3′	qnrS
		R: 5′-TTA ATT GGC ACC CTG TAG GC-3′	

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PCR was conducted using premix (Promega USA) for the amplification of the qnrA and qnrB genes. a 25 l reaction mixture made of of:

1- 12.5 Go Taq G^2 Green master mix
2- 5 µl template DNA
3- 5 µl Upstream and Downstream primers
4- 2.5 Free nuclease water

IV. RESULTS AND DISCUSSION

Isolation and Identification of Bacterial

Isolating and identification, 93 isolates of *Salmonella enterica* different sources including human, meat chicken and environmental including sewage and soil , Microscopically , The single colony appeared black colour in Salmonella Shigella agar media when spreading in plate. Microscopic examination were gram negative, rod cell of bacteria Fig.1 The Biochemical tests were done to identify *Salmonella enterica* from different source shown in Table 2 (12,15).

Table 2: Biochemical tests

Sampling sources				
Test	Human	Meat chicken	Sewage	Soil
TSI	+	+	+	+
Indole	-	-	-	-
Methyl red	+	+	+	+
VP	-	-	-	-
Citrate	+ , -	+,-	+,-	+,-
Catalase	+	+	+	+
Oxidase	-	-	-	-

Positive = + , Negative = - , Variety = +,-

Antimicrobial Susceptibility testing:

From a total of 90 Salmonella enterica isolates, 42 (46% of the total) were resistant to Naldexic acid. whereas ciprofloxacin resistance was found in 13 (14%) of the isolates. Treatment for Salmonella using Ampicillin, Trimoxazole, and Chloramphenicol. However, in recent years, fluoroquinolones including naldixic acid, ciprofloxacin, and cephalosporin have been utilized to treat most instances of Salmonellosis infection. Multiple treatment failures with fluoroquinolone antibiotics in recent years indicate, antibiotic resistance is on the rise fluoroquinolones There is growing worry about the spread of antibiotic resistance in underdeveloped regions. Multiple processes contribute to antibiotic resistance, include chromosomal mutation, the spread of resistance among bacteria through transposable elements such plasmids and transposons, and a decrease in the cell wall's capacity to transmit antibiotics, and antibiotic enzymatic inactivation, With antibiotic removal mechanisms called efflux pumps, the Plasmid origin of this resistance increases the likelihood that quinolone-resistant Salmonella and other pathogens may spread across healthcare facilities and the general public (16,17,18).

Molecular study PCR:

In order to identify the qnr genes in 37 Salmonella isolates, polymerase chain reaction (PCR) amplification was used. The qnrS amplicon was 417 bp in length, the qnrA amplicon was 516 bp, and the qnrB amplicon was 526 bp in length, as predicted. Target genes from typical Salmonella isolates carrying a variety of qnr genes are shown to be amplified in Fig.1. Overall, qnrS genes were found in 22 of 40 strains (59%)., 7 (18%). Strains had both qnrA and qnr S gene together, Two (5%) isolates were positive for qnrB genes. None of the remaining isolates had qnr genes.The 68 salmonella isolates under study's gel electrophoresis findings revealed the molecular weight DNA bands in comparison to the 100bp ladder. The molecular weights of the DNA based on the distances made by these molecules in the gel, which are inversely proportional to their molecular weight DNA, are the volume indices. The DNA ladder 100bp was used, which gave macrophages eleven bands of known molecular weights in figure1. The 16SrRNA gene used in the current study used the Salmonella isolates if th volumes obtained were 317, 516 and 526, respectively in another study by (19,20) the genes were used to determine the genes. As result of the study, 16SrRNA preserved the genetic characteristics of microbial strains to the presence of certain sites within this gene sequences characterized by consistently nucleotides across the ages, which is the goal of genetic and species diagnosis utilizing molecular techniques like pcr. Antimicrobial gene identification by polymerase chain reaction.

(PCR) has recently showed significant promise as a diagnostic technique., because of the excellent levels of sensitivity and specificity [21] achieved by this technique. The sensitivity of Salmonella enterica detection has been worked on from several angles. Improved detection of DNA from this intracellular

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bacterium (22) is achieved using ox bile to lyse blood cells, then micrococcal nuclease to destroy human DNA

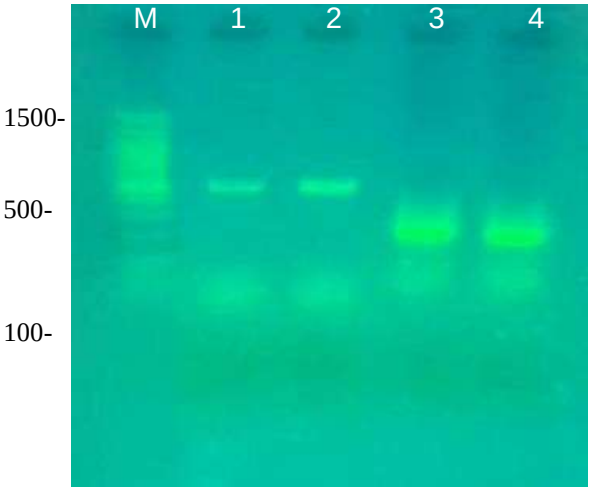
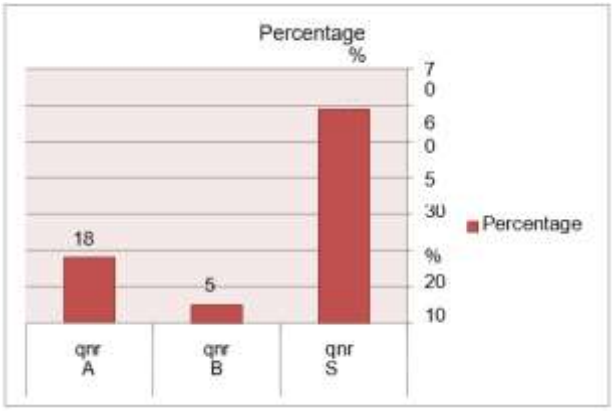


Figure (1) Based of PCR product of qnr S,A and B genes in *Salmonella enterica* isolated from different Source: M:DNA Ladder 100bp .



Percentage of genes responsible for resistance to Quinolones antibiotic in *Salmonella enterica*

V. DISCUSSION

About 90% of human gastroenteritis is brought on by the closely related serotypes *Salmonella typhi, S. typhimurium, S. enteritidis, and S. infantis*. [22]. In most cases, antibiotics are unnecessary for the treatment of non-typhoidal diseases. Patients with weakened immune systems, malnutrition, or cancer may benefit from antibiotics if they get enteric fever or septicemia. Treatment options for salmonellosis have included ampicillin, chloramphenicol, and co-trimoxazole; however, owing to rising resistance of these compounds, ciprofloxacin and cephalosporin use has grown in both adults and children [13].

Clinical salmonellosis infections are treated with fluoroquinolones, a class of broad- spectrum antimicrobials, by people of any age or immune system status who have enteric fever, invasive illness, or are chronic carriers of Salmonella. There is a critical need for rapid detection and molecular epidemiological research into bacterial resistance genes [23,24].

Since antimicrobials are used to either treat or prevent infections or to promote development (as feed additives), the prevalence of Salmonella has provided a major difficulty for animal husbandry. As a consequence, Salmonella species that are foodborne are inevitably going to develop antibiotic resistance (25). The advent of multidrug resistant (MDR) phenotypes has really had a big influence on the profession.

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