

**DETECTION OF SOME VIRULENCE, ANTIBACTERIAL RESISTANCE GENES FOR SALMONELLA ISOLATED FROM DOGS IN BAGHDAD CITY**Maher. S. Al – Rubaye  
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Email:mahernet32@gmail.com**ABSTRACT**

Two hundred and fifty (250) samples from both diarrheic and non-diarrheic dogs in both sex male and female multi breeds ( German shepherd and malinois ) in Academy for training police and military dogs for searching about drugs , explosives ,local dogs and pet dogs from different clinical cases in Baghdad city were tested. All samples are obtained in aseptically rectal swabs and transporting to the microbiology lab, for culturing and identification for salmonella species. The study gave 16 (6.4%) positive Salmonella. Virulent gene study gave us 16(6.4%) invA gene, invF 13(5.2%) and sit C 16 (6.4%).Antibiotic sensitivity test According to Bauer-Kirby method were done and gave 16 (6.4%) isolates resistance for ciprofloxacin which is the most clinical antibiotic drug of choice against Salmonella infection . Detection of gyrA gene resistance gene for ciprofloxacin using Polymerase chain reaction (PCR) gave 16 (6.4%) for all positive isolates.

**Keywords:** Salmonella, virulence genes, Antibacterial resistant genes, polymerase chain reaction

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الكشف عن بعض جينات الضراوة، المقاومة للمضادات الحياتية لبكتريا السالمونيلا المعزولة من الكلاب في مدينة بغداد

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باحث

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المستخلص

مئتان وخمسون عينة من كلاب غير مصابة و كلاب مصابة بالإسهال ومن كلا الجنسين ذكور واثان ومن مختلف السلالات (جيرمن شيفرد ومالينوس) في اكااديمية الشرطة لتدريب الكلاب البوليسية للبحث عن المخدرات والمتفجرات وكذلك كلاب محلية و كلاب زينة تعاني حالات مرضية مختلفة في مدينة بغداد تم فحصها في هذه الدراسة. اخذت جميع العينات بواسطة مسحات قطنية مخرجة في ظروف معقمة ونقلت الى مختبر الاحياء المجهرية لزراعة وتشخيص العينات والقيام بخطوات الفحص القياسية للبحث عن عينات السالمونيلا. لقد اثبتت الدراسة 16 (6.4%) عزلة موجبة للسالمونيلا , اما عن دراسة الضراوة فكانت النتائج 16 (6.4%) لجين الغزو invA وجين الغزو invF 13 (5.2%) اما فيما يخص جين اكتساب الحديد sitC فكانت 16 (6.4%) . تم اجراء فحص الحساسية للمضادات الحياتية طبقا لطريقة بيور وكيري واعطت نتائج إيجابية ل 16 (6.4%) عزلة مقاومة للمضاد الحياتي السايبروفلوكساسين والذي يمثل العلاج الأمثل والاكثر استعمالا لعلاج بكتريا السالمونيلا. وتم الكشف عن جين gyrA الخاص بالمقاومة للسايبروفلوكساسين بواسطة تفاعل سلسلة البلمرة واعطت النتائج 16 (6.4%) من ضمن العزلات الموجبة لبكتريا السالمونيلا.

كلمات مفتاحية: سالمونيلا، جينات ضراوة، جينات المقاومة للمضادات الحيوية، سلسلة تفاعل البلمرة

## INTRODUCTION

Salmonella SPP. were known as a causative agent for more than 100 years ago, the first description for these bacteria was in 1880 then it cultured in 1884 and named on the names of the pathologist Daniel E. Salmon who are the first scientist could isolate Salmonella from intestine of pigs, the bacteria is common in digestive tract for mammals, reptiles, birds and insects (28). Salmonella SPP. bacteria belongs to family of Enterobacteriaceae It is described to be as bacilli shape, gram negative and non-spore forming . This species known to cause disease for human and animals .This bacteria can withstands dehydration for obviously long period of time(8). The infection for animals having greater importance due to direct economic influence and this domestic animals constituting a vast greater reservoir for these bacteria causing human infection, also prepare food born zoonosis duo to contaminating animal products by this bacteria (3). The use of antibacterials in the treatment of diseases caused by bacterial strains has a significant impact on disease distribution and death rates, but incorrect or excessive use of antibiotics in treatment of diseases has resulted in an increase in antibiotic tolerance costly (17). Enterobacteriaceae antibiotic resistance is evolving globally, particularly in developing countries where antibacterial drugs are abused more frequently in humans and animals (25). The increasing of bacterial resistance is sometimes linked to higher pathogenicity (19). Several data points support the idea that highly pathogenic strains would require antibacterial treatment to reduce the severity of symptoms and would be subjected to antibacterial exposure's selective pressure. In a similar vein, the relationship between virulence and antibacterial resistance among the study isolates revealed that isolates resistant to three or more antibacterials had a higher number of virulence genes (14). The presence of pathogenicity island 1 (SPI-1) in all Salmonella serovars has been linked to pathogenicity of isolates invasion gene A (invA), which codes for type 3 secretion system protein, is found on plasmids in the SPI-1 (10). InvF and Salmonella iron transporter gene C are two further pathogenic genes, Salmonella serovars carry sitC, one of

the genes encoding for iron uptake (22). This study showed the presence of virulence genes in Salmonella that affected dogs and viewed the resistance genes of salmonella in Baghdad city for antibiotic ciprofloxacin the choice against Salmonellosis.

## MATERIALS AND METHODS:

**Sampling :** This study was conducted between October 2020 to June 2021. 250 of combination including household, training and police K9 units dogs diarrheic and non-diarrheic dogs multisex, different age and multibreed, these dogs are presents in Iraqi academy of police dog training and management. The other dogs are coming to veterinary Baghdad clinics for diagnosis and treatment for different illness , the sample were takes before any drug administration, rectal swabs were collected from these dogs by using sterile media swab sticks , the cotton swabs were transported aseptically condition in ice-packs to veterinary microbiology laboratory at department of laboratory researches of Iraqi ministry of agriculture.

**Isolation and identification of Salmonella isolates:** swabs were inoculated with 10 ml Selenite F. broth and incubated at 37 °c for 24 hours aerobically , then a loopful from the Selenite F. broth swab were subcultured on Mac Conkey agar (Oxoid , England ) , Brilliant green (Oxoid , England ) , xylose lysine deoxycholate agar (XLD) (Oxoid , England) and Salmonella shegella agar SS (Oxoid , England), all of these were incubated at 37°c for 24 hours aerobically . Then suspected colonies appeared on MacConkey pale (non-lactose fermenter), on Brilliant green alkaline reaction formed red colonies and on XLD agar were red colony/ black (H<sub>2</sub>S) center, on SS agar were pale with black center due to hydrogen sulfide production (18). For confirm diagnosis there were two ways , the first method by EPI 20 E test biochemical standard method from ( Biomerieux : France ) for diagnosis of isolated bacteria on base of Genus and Species consisting of a tape of dehydrated test substrates in individual micro tubes which reconstituted by adding suitable amount of bacterial syrup that's wanted to study , The second way by serological confirmation with polyvalent antisera this leading to definitive serotyping with specific (O) and (H) antisera

in central Iraqi laboratory public health following standard procedures .

### Detection virulence genes in Salmonella Spp.:

The DNA of Salmonella isolates were

extracted by using DNA extraction kit (Genaid, Taiwan) according to the manufactures instruction guide.

### Primers

**Table 1 Primer sequence for genes of the study**

primer	Sequence (5' → 3')	specifity	Product size (bp)	Refer ence
F	AAGGGATCCATGTCATTTTCTGAAAGCGAC	invF	918	(24)
R	AC			
F	GTTGTAGGGAAAGCTTCTCCAGTAATG	sitC	768	(23)
R	CAGTATATGCTCAACGCGATGTGGGTCTCC			
F	CGGGGCGAAAATAAAGGCTGTGATGAAC	inv A	284	(1)
R	GTGAAATTATCGCCACGTTCGGGCAA			
F	TCATCGCACCGTCAAAGGAACC	gyrA	313	(4)
R	TAC CGT CAT AGT TAT CCA CGA	for		
R	GTA CTT TAC GCC ATG AAC GT	ciprofloxacin		

### Reaction mixture

The reaction mixture for each gene done separately at total volume 25 µl for each gene as this rate volume (Go Tag R Green) Master Mix 12.5 µl, upstream primer 1 µl, downstream primer 1 µl, Nuclease – free water 2.5 µl, Q solution 3 µl, DNA template 5 µl

### Amplification condition

The amplification for inv A , inv F , sit C genes and for ciprofloxacin resistance genes is gyrA , the annealing temperature for invA gene is 60°C for 30 seconds with final extension 7 minutes , the annealing temperature for inv F gene is 55°C for 30 seconds with final extension time 10 minutes , the annealing temperature for sit C gene is 60°C for 30 seconds with final extension time 10 minutes , the annealing temperature for gyrA gene is 54°C for 1 minute with final extension time 7 minutes.

### RESULTS AND DISCUSSION

From 250 samples according to Iraqi central public health lab (CPHL) the results of biochemical test by using EPI 20 gave us 16 (6.4%) positive isolates, Three genus which are *Salmonella typhimurium* , *Salmonella ohio* and *Salmonella choleraesuis* from different healthy , non-healthy and multibreed dogs , all these 16 (6.4%) Salmonella isolates (100%) were positive for PCR of invA gene , while 13 (5.2%) were positive for PCR of invF gene , and 16 (6.4%) positive for the PCR of sitC gene, while the resistance gene gyrA for ciprofloxacin antibiotic was 16 (6.4 %) for all Salmonella isolates. This study is deal with the presence of virulent and antibacterial resistant

genes, which harbored by Salmonella Spp. isolates that were taken from healthy and diseased dogs (different causes brought to clinics including diarrhea) in Iraq, Baghdad city was investigated. Isolation was at rate 16 (6.4%) positive case from 250 multi sex , age and breed but the majority of them are police dogs German shepherd , Belgium ( Malinouse) ,It is possible that Salmonella Spp. were the primary etiological causes of diarrhea or septicemia following the ingestion of this bacteria in contaminating food or drinking water or environmental surrounding objects (11). However, it is known that dogs were shedding Salmonella without showing any clinical signs for Salmonellosis disease (27) . Also it is known that dogs are possible for shedding Salmonella with episodes of clinical diarrhea that can caused by abundance causes of clinical conditions like Canine distemper, Canine parvovirus, Canine corona virus infection , different infection with bacteriosis (such as colibacillosis, combilobacteriosis ), Catching parasite helminthes, several intoxications, Pancreatic disorder and other causes that are inducing canine diarrhea and its consequences, also there are some causes make dogs in health and diarrheic condition for shedding Salmonella which are include antimicrobial therapeutics and immunosuppressive dogs (24). The rate of isolation 6.4% in this study appear to be higher when it compared 4% isolation rate of Salmonella that reported by Ojo and Adetsoye in Nigeria (16) also it is prepared higher rate when it comparing with 3.6% and 0.09% of Salmonella isolation rate in Japan and Trinidad

respectively (7 , 21). The cause of higher isolation rate of Salmonella may be due to the positive samples dogs in this study have an immune suppression resulting to increased Salmonella shedding than the clearly healthy animals in previous lower rate studies, Also may be due to better environmental standard condition that lowering the levels of contamination for kennels, food and water sources for supplying animals (9). Also may be due to the multi sex , age and breeding of the dogs sampling in other various studies , Could also have influenced the rate of isolation , Since Salmonella shedding have been recorded likely more in pappies , young dogs and female animals which are weak immune response (21).The isolation rate of Salmonella 6.4% in our study are lower or little incidence than other studies 43.7% , 11% ,23.5% and 10.5% in Nigeria ,Turkey , Sudan , Iran respectively(9 , 12 , 21, 27). All above are apparently healthy dogs and this rate are due to the reasons that mentioned above, and the management state, also may be better in our area of study at Baghdad than those of higher isolation rate of Salmonella, this study, the existence of three virulence genes *invA*, *invF* and *sitC* were expressed by Salmonella bacteria isolates, so all these three virulent genes have previously been shown to be necessary required for full Salmonella virulence in an murine model (17). The *sitC* gene is very essential for acquisition of Iron (29). All of Salmonella isolates that tested have possessed *sitC* gene at rate 100% and this result are superposes identical with previous studies which are investigated the distribution for this essential gene since iron is a limiting nutrient for bacterial growth in Salmonella isolates from dogs that reported 85%\_ 100% *sitC* detection rate that results could be suggests that *sitC* gene locate on virulence plasmid that not always present and this plasmids of virulence serovars are specific for it and not all plasmids serovar bears containing these virulence plasmids (15). The detection of invasion A (*invA* ) gene which are all positive isolates 16 from 16 positive isolates that

contains *invA* 100% , and thus confidence that they were Salmonella spp. (14) . The unique sequences to genus Salmonella are present in *invA* gene; therefore, it considered the international standard for its identification (13). The rate of isolates for Salmonella 100% of *invA* gene for all 16 isolates against 13 (81.25%) from 16 isolates for *invF* gene that's suggest that *invA* is the predominate invasion gene that carried by Salmonella bacteria, Also this results is common as *invA* has reported to be contained in virulence plasmid carried by SPI-1 which occur in all Salmonella bacteria (24). Discovering the *invA* gene is represented the standard role for detection of invasion genes in these bacteria (7). There are a special relation between Salmonella *invA* gene with Salmonella *invF* gene that they are all of them encoding for a protein in type3 secretion system in the inner membranes which act as helping the organism to invade the host epithelial cells of intestine (10) . The rate 100% of *invA* gene detection in our study is similar to the reporting of many studies, that *invA* gene is 100% of Salmonella isolates from healthy dogs and other animals , So the other studies approach to same results for Salmonella isolates from another animals (12 , 15) .About the antibacterial resistance gene *gyrA* for ciprofloxacin , The causative agent that are giving this gene the resistance character belongs to there are single chromosomal mutation for *gyrA* gene encodes for changing , since this mutation happened in substitute of amino acid in (ser 83 phe ) (3) . Causing to decrease the response in a continuous manner for fluoroquinolones and that is leading to resistance character to appear in this gene (26). In vitro analysis of *hilA*, *hilC*, *hilP*, and *invF* suggests that these regulators work in a hierarchy to modify invasion gene expression, with *hilA* serving as the master regulator (20). Darwin and Miller (5) found that *invF* and its putative chaperone protein *sicA* are responsible for activating and regulating the expression of TTS-associated proteins expressed both inside and outside of Salmonella Pathogenicity Island.

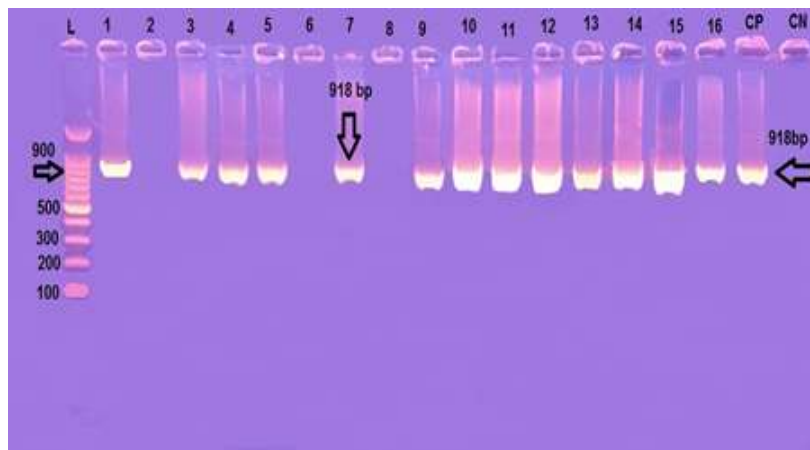


Figure 1. PCR amplification of 918 base pair fragments of invasion F (*invF*) gene , lanes (2,6,8) shows negative result, (1,3,4,5,7 up to 16) shows positive results (Salmonella species *invF*) , Lane L shows PCR markers ladder100bp, CP represents control positive, CN shows control negative.

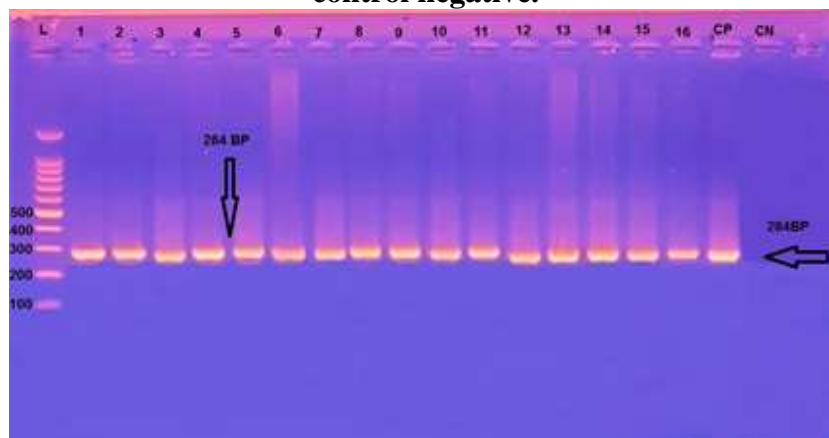


Figure 2. PCR amplification of 284 base pair fragments of invasion A (*invA*) gene , lanes (1-16) shows positive results (Salmonella species) , Lane L shows PCR markers ladder100bp , CP represents control positive, CN shows control negative.

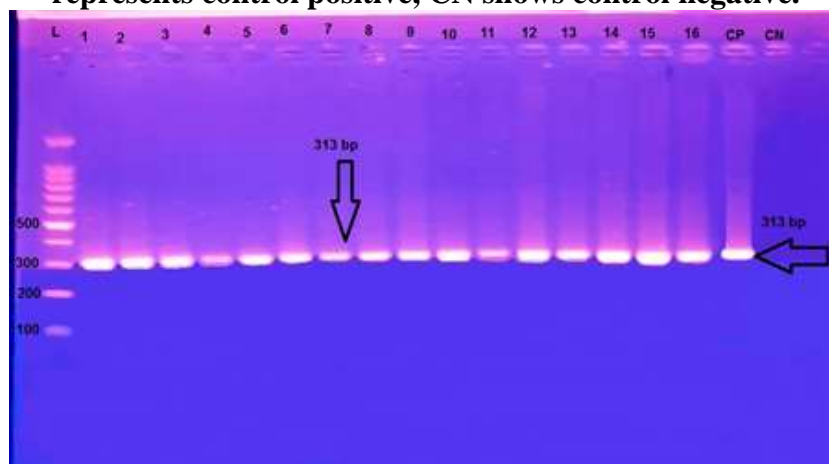
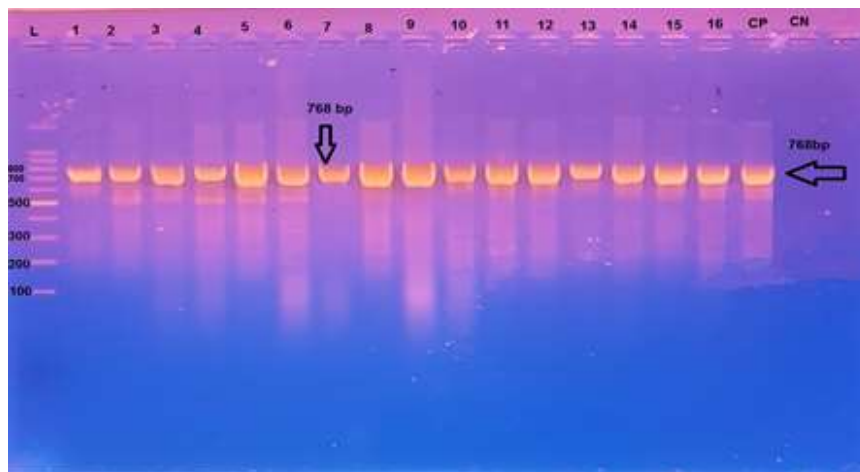


Figure 3. PCR amplification of 313 base pair fragments of ciprofloxacin resistance gene (*gyrA*) gene , lanes (1-16) shows positive results (Salmonella species *sitC* gene), Lane L shows PCR markers ladder100bp , CP represents control positive , CN shows control negative.



**Figure 4. PCR amplification of 768 base pair fragments of Iron transporter gene (sitC) gene, lanes (1-16) shows positive results (Salmonella species sitC gene), Lane L shows PCR markers ladder 100bp, CP represents control positive, CN shows control negative.**

### CONCLUSION

In this study, we concluded the prevalence of salmonella invasion gene A (invA) gene (invF) and (sitC) and detecting of drug resistance gene for ciprofloxacin gyrA gene in our isolates.

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