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DIAGNOSIS OF SALMONELLA FROM *MUSCA DOMESTICA* IN THI-QAR PROVINCE OF IRAQ

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ABSTRACT

The current study isolated and diagnosed pathogenic bacteria *Salmonella* from the domestic population of *Musca domestica* L collected from surrounding environments and overlapping with residential areas in Thi-Qar province. Thirty samples of *Salmonella* were isolated and diagnosed from 360 samples collected from markets, houses and hospitals of Thi-Qar provaince. The highest incidence of bacteria from external surface was during May and July (10.5%), while the lowest incidence was during January, February and November. This diagnosis was confirmed by a confirmatory test (API. 20) and the same diagnostic ratio given. All isolates were examined for their resistance to antibiotics which revealed that 46.6% of isolated *Salmonella* are resistant to nalidalic acid (86.7%), gentamicin (30%), amoxicillin (26.7%), norfloxacin and ampicillin (23.3 %). Diagnosed isolates were subjected to PCR, and this led to diagnosis of InvA gene in *Salmonella*.

Key words: *Salmonella* spp., ampicillin, norfloxacin, bacterial, *Musca domestica*, adults, PCR, InvA gene, Kirbery-Bauer, antibiotics, resistance

Musca domestica L accounts for almost 90% of the flies found in human and animal living places (Li et al., 2023; Al-Naeli, 2019). Adults are closely associated and feed specifically on the septic substrates, and as a result catch microbes, including pathogenic bacteria (Bertelloni et al., 2023). The hairy proboscis, feet with glandular hairs and pads excretes sticky material This characteristic together make flies catch the pathogen to her bodies, more than that during feeding process the regurgitation of vomits and deposit of faecal droplets gives the flies the ability to spread pathogens (Nayduch et al., 2023). The flies carry more than 100 pathogens which infect humans and animals, and there is a clear link between the number of human and animal disease cases including typhoid, cholera, bacillary dysentery, anthrax opthalmia and infantile diarrhea (Baker et al., 2018); and thus spreading diseases from animal and people (Förster et al., 2007). House flies have the ability to transport *Salmonella* (Baker et al., 2018). Infections by *Salmonella* can cause diarrhea, fever and abdominal cramps, and it is distinguished from *Shigella* that causes bloody diarrhea, while *Salmonella* does not (Hlashwayo et al., 2023). *Salmonella* is one of the most common diarrheal pathogens, Salmonellosis with symptoms such as high fever, toxicity, watery diarrhea or diarrhea with blood and mucus, abdominal cramps and tenesmus is quite common. It is estimated that 165 million people a year are infected, 99% of which are from developing countries (Hussain, 2018). This study is aimed to isolate and diagnose pathogenic bacteria from the domestic population of *M. domestica* collected from surrounding environments and overlapping with residential areas in the Thi-Qar province, and determine their antimicrobial susceptibility profiles.

MATERIALS AND METHODS

Three hundred and sixty house fly adults were collected from different locations (hospitals, homes and markets) of the Thi-Qar province from January to December 2019. The catch of flies was done using sterile nets during a specific time of the day (9:00 am to 1:0 pm) only when the flies are active. Sterile containers

(150 ml) were used to place the collected flies, and immediately transferred to bacteriological laboratory. Isolation and identification of *Salmonella* was done from these depending on cultural characteristics on differential and selective media. Biochemical identification using Api and molecular technique was followed. Isolate of *Salmonella* spp. from the outer surface of these was done while storing in sterile tube with (5 ml) normal saline and vortex for the release of bacterium attached to the body of the fly, especially the hairs covering the body. This extracted liquid was subjected to standard procedures for further tests Isolates of *Salmonella* spp. extracted and studied similarl from gut of house flies.

Antimicrobial susceptibility testing was done with all the isolated *Salmonella* focused on gentamicin (CN 30 µg), nalidixic acid (NA 30 µg), ampicillin (25) (AM 25 µg), cefixime (CFE 5 µg), norfloxacine (NOR 10 µg), amikacin (AK 10 µg) and amoxicillin AMC 30. These were determined using the Kirby-Bauer disc-diffusion method (CLSI, 2006). Isolated *Salmonella* were all screened for virulence genes (invA) by a simplex PCR (Hernández-Ledesma et al., 2023). From the isolates, overnight cultures total genomic DNA was extracted using the Presto Mini g DNA bacterial kit from (Geneaid,USA). The composition of the PCR mixture and 1 µl of template DNA1 µl PCR buffer, 200 mM of each dNTP, 0.25 mM of forward and reverse primers, 2.5 units of Taq DNA polymerase (Bioneer). The PCR cycling conditions were 5 min at 95°C; 30 cycles of 40 sec at : 94°C, 60 sec at 66.5°C, and 90 sec at 72°C, with an additional extension for 10 min at 72°C. PCR products were visualized by electrophoresis on 1.2% agarose gels in 1X TBE buffer at 50 V for 85 min. The data were analyzed according to ANOVA under complete randomized factorial design using X^2 value and LSD at $p \leq 0.05$

RESULTS AND DISCUSSION

The results showed that there were 30 samples positive for *Salmonella*, as seen in the Table 1; of these 47.4%, 18 *Salmonella* isolates from the external surface of 360 house flies, the highest rate of isolation was during May (10.5%) while the least was in January, February, November and December, where no isolation rate was recorded; there were no statistically significant differences. *Salmonella* was isolated from the outer surface and gut which showed that 30 (8.4%) samples were positive as observed by Förster et al. (2007); infection of flies could be external or internal (Sulaiman et al., 1998). *Salmonella* from gut of adults was to an extent of 31.6% maximum being during June and April with no statistically significant differences. De La Pa (1938) found that 42.86% of flies carried *Salmonella* internally. Sulaiman et al. (2000) isolated diversity of pathogenic organisms from the guts of several flies, including *M. domestica* observing *Salmonella arizonae* and *Shigella* spp. Ostrolenk and Welch (1942) observed that flies frequenting food contaminated with *Salmonella* were capable of infecting all surfaces, including food and water. The isolation frequency and microbial sensitivity profiles of *Salmonella* spp. Observed by Motta et al. (2023) revealed that isolation was seen from synanthropic fly on dairy farms located in Brazil Northern Paraná. Holt et al. (2007) showed that *Salmonella typhi* caused pollution, and is one of the most dangerous food-borne bacteria. Hassan (2009) isolated the bacteria *S. typhi* from the outer surface (25.2%). The difference in contamination and isolation of *Salmonella* was due to many reasons including the differences of sewage systems in houses, hospitals and market sites, in addition to the increase of waste in each of the above sites. However, it varied with months which was due to the difference in temperature and humidity.

Table 1. Isolates of *Salmonella* evaluated (2019)

Month	Salmonella	
	No.	%
Jan.	0	0
Feb.	0	0
Mar.	2	5.3
Apr.	1	2.6
May	4	10.5
Jun.	4	10.5
Jul.	2	5.3
Aug.	2	5.3
Sep.	1	2.6
Oct.	2	5.3
Nov.	0	0
Dec.	0	0
Total	18	47.4

X^2 value of external surface 30.82; X^2 value of ga Hut 3.24

A total of 30 bacterial isolates were identified by conventional biochemical test and API 20E- shape of the developing colonies for *Salmonella* on xylose lysine, desoxycholate agar (XLD) was small, smooth, rounded and red in colour with black center as shown

(Fig. 1); and in *Salmonella Shigella* Agar (SS) it was smooth, colourless. The β-lactam antibiotics basically work by inhibiting the cell wall synthesis, preventing terminal transpeptidation in the cell wall by binding and interfering with the structural cross linking of peptidoglycans, it weakens the cell wall of the bacterium and as results in cytolysis or death due to osmotic pressure (Zakir Hossain et al., 2023). This study observed that some isolates are strongly sensitive to gentamycin (CN), at (86.7%) and cefixim (CFM)- 70%. Debnath et al. (2018) observed that 100% isolates of *Salmonella* spp. were resistant to nalidixic acid and norfloxacin. Hussain (2018) showed that 40% isolates are sensitive to gentamicin; Gebreegziabher et al. (2018) showed that it was 84.2%. Yang et al. (2014) reported that only 34.5% were resistant to gentamicin. Jaran (2015) reported that all isolates were sensitive to amikacin, and only 4% resistant to gentamycin. Antibiotic susceptibility test by disc diffusion method as depicted in Table 2 show maximum *Salmonella* resistance to nalidixic acid (NA)- 46.6%, followed by cefixim and amikacin- 30%; while 86.7% are sensitive to gentamicin; and 26.7% are resistant to amoxicillin; 23.3% to ampicillin and norfloxacin. There are significant differences between resistant, sensitive and intermediate values.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

Table 2. Isolates identified as resistant, intermediate and sensitive to antibiotic

Antimicrobial agents	*Salmonella* isolates (n=30)					
	Resist		Inter		Sens	
	No.	%	No.	%	No.	%
GEN	4	13.3	0	0	26	86.7
NAL	14	46.6	2	6.7	14	46.6
AMP	7	23.3	4	13.3	19	63.4
CEF	9	30	0	0	21	70
NOR	7	23.3	5	16.7	18	60
AK	9	30	14	26.7	1	34.3
AMC	8	26.7	6	20	16	53.3
CalX² = 66.07			Df = 12			
TabX² = 21.03			P. value = (0.00)			

CONFLICT OF INTEREST

No conflict of interest.

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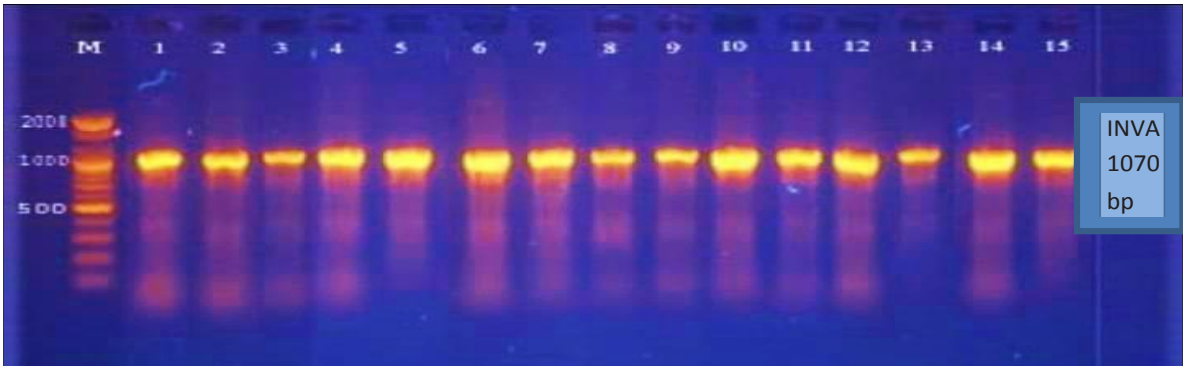

Fig. 1. The gel electrophoresis of invAgene. PCR products descant from *Salmonella*. M: marker (2 kb ladder); 1 to 15

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