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Molecular Identification of *Providencia* spp .in birds and their owners in Baghdad, Iraq

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Abstract : This study was conducted in different farms of birds to isolate *Providencia spp* from birds and their owners , A total of 100 samples were collected from different types of birds from cloacal swab of birds include pigeons (25), chickens (25) ,ducks(25), pet birds (15), parrots (10) collected from farms of bird and (50) stool samples from owners in different places in Baghdad city , Iraq. samples were inoculated in peptone water then cultured in Blood and MacConkeyagar. Identification of isolates was confirmed by conventional methods include (microscopic examination, cultural characteristic, biochemical tests), Vitek2, and 16S rRNA with sequencing. of 150 samples only five samples positive 5/150 (3.3%) *and P. vermicola* were the 2/5(0%), *P. stuartii* 3/5(60%) and number of isolate were in chickens 1/5(20%),in ducks 1/5(40%),in pet birds 1/5(20%) and in pigeons 1/5(20%) and in owners 1/5(20%).our results findings indicate that dropping of birds might be contaminated with *Providencia* spp.and this may leading to zoonosis transmission to all contact with birds causing infection like poisoning, diarrhea, urinary tract infections and other infections

Keywords: *Providencia,birds,* owner ,Vitek, 16S rRNA

INTRODUCTION

Bacteria are the most cause of septicaemia or bloodstream infection in humans and animals (Singh *et al*., 2023). *Providencia* is gram negative belong to enterobacteriaceae this species are opportunistic pathogens in immune compromised people which has been found in sputum ,urine,burns,wounds and stool specimens Additionally, it is found in fishes , beef meat and chicken meat as well as soil, water river, bird feces and waste water. resistance in *Providencia* is a public health. (Hammood N. W. and Ibrahim I. A. J. 2015; Foti , *et al*., 2017; Abd Al-Mayahi and Ali .2018). Providencia is pathogen causing urinary tract infections , in birds , egg shells , can be transmitted to humans,causing diarrhea. (Yuan *et al*., 2020; Wenxin Chen *et al*., 2022;Huang *et al*., 2022).Providencia has been explored sporadically for birds (McMullan &Donegan 2014; Al-Gburi, 2020). the high frequency of contact humans with birds and animals makes them more susceptible for infection (Salim, M. A. and Abed, S. A. 2017; Modupe, S.L.*et al*.,2021)

MATERIAL AND METHODS

Ethical approval

The animal care and use committee of the College of Veterinary Medicine, Baghdad University, approved the study protocols

I .Samples collection

Samples (150) collected from birds and owners included (100) cloacal swabs were collected from farms of birds include pigeons (25), chickens (25) ,ducks(25),pet birds(15), parrots (10) and (50) stool samples from owners were collected from farms using sterile materials and transmitted to the laboratory. (Al-Gburi, 2020):

II. Isolation and identification of Providencia spp

Inoculate sample in peptone water at 37 °c /24 hours, then cultured in MacConkey agar ,blood agar and colonies identifications by colonial morphology, staining. Cultivation on media , Triple sugar iron(TSI)urease,and phenylalanine. further identified using the VITEK®2 reactions formore differentiation according to(Quinn *et al*., 2002;Yi, *et al*., 2007 and Shukla and Mishra, 2015)

III. PCR

1 - DNA extraction: using Kit protocol(Promega, USA). Wizard Genomic DNA Purification PCR amplification(16S rRNA) with primer (5'-GGA TAA CC/TA TTG GAA ACG ATG-3') and R744 5'-CAT CTG AGT GTC AGT G/ATC TG- 3',

2 - Amplification: For conventional PCR.

3- PCR Products analysis: 1% agarose gel electrophoresis was applied after amplification. products separated by electrophoresis and analyzed data by computer software,.Table1

Table1 Primer

Primer Name	**Seq.**	**Annealing Temp. (°C**	**Product size (bp)**
27F	5`-AGAGTTTGATCCTGGCTCAG-3`	60	1500
1492R	5`-TACGGTTACCTTGTTACGACTT-3`	60	1500
F169	5`-GGATAACYATTGGAAACGATG-3`	53	617
R744	5`-CATCTGAGTGTCAGTRTCTG-3`	53	617

Table2PCR Program

Steps	°C	m: s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	53, 60	00:30	30
Extension	72	01:00	30
Final extension	72	07:00	30
Hold	10	10:00	1

RESULTS

From 150 samples collected five isolates positive identified *as Providencia*, in birds 4 isolates and one isolates from owners of birds , On MacConkey agar, non lactose fermenter, colonies (Figure 1).and circular, smooth, convex, non heamolysis on blood agar. it is motile, oxidase negative, positive results in catalase and tryptophan deaminase, TSI, (pink/yellow without gases ,H2S, Vitek 2, five samples (3.3%) *and P. vermicola* were the 2/5(40%), *P. stuartii* 3/5(60%) and one isolate *of P. vermicola* were in chickens 1/5(20%),in ducks one *isolate of P. vermicola* 1/5(20%),in pet birds *P. stuartii* 1/5(20%) , and in pigeons *P. stuartii* 1/5(20%) and *P. stuartii* in owners 1/5(20%), as in table (3) and a in PCR five positive results in sequence ID MN733989.1,MK641332.1,MH532483.1,MK641332.1,MK993518.1as in band of 617 bp (Figure 2).

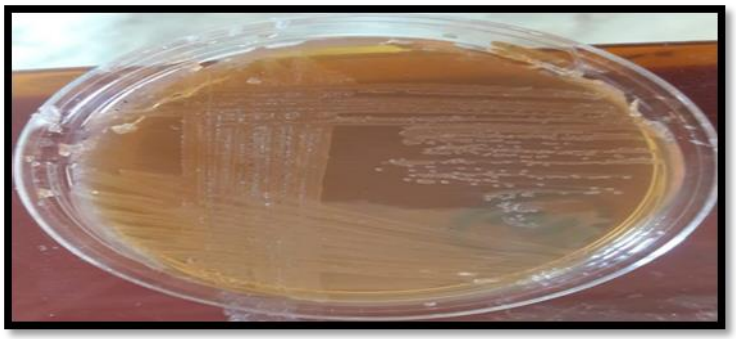

Figure 1 *Providencia* spp. colony on MacConkey agar

Table(3)percentage of *Providencia* spp isolation

Type of birds	Number Of samples	*P. stuartii*	*P. vermicola*
chickens	25	(0%)	1/5(20%)
ducks	25	(0%)	1/5(20%),
pigeons	25	1/5(20%)	(0%)
pet birds	15	1/5(20%)	(0%)
parrots	10	(0%)	(0%)
owners	50	1/5(20%)	(0%)

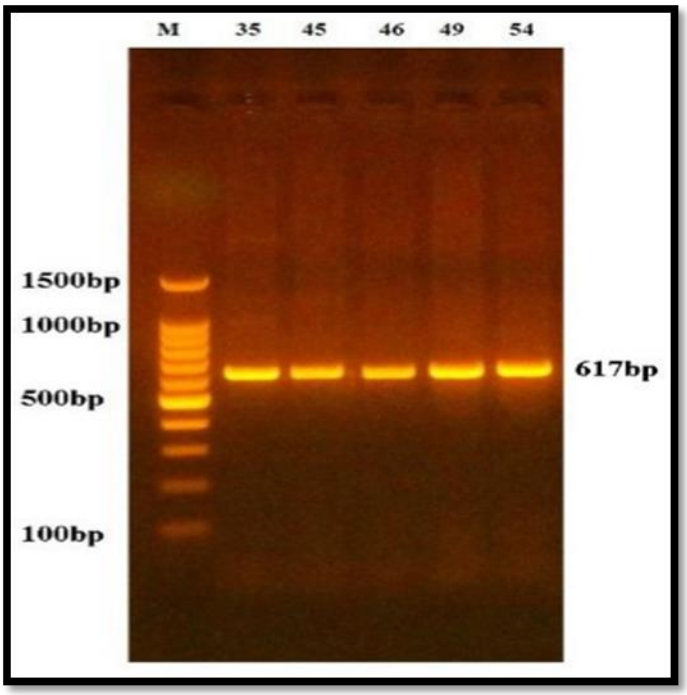

Figure (1) five positive results as in band of 617 bp

DISCUSSION

Providencia contribute in dissemination of zoonotic pathogens, spreading in working areas leading to public health hazards. (Cheng *et al.*,2013; Mellata *et al.*,2013). Our results was the first in Iraq indicate that birds and owners caries *Providencia*is ,In agreement with the present study, water polluted with faeces contains of pathogenic bacteria of animal and human origin, is transport to birds by food (Pinto *et al*, 2015).and *Providencia spp* from humans, chickens, soil, andwastewater(Abd Al-Mayahi and Ali 2018). The findings of other investigation show *Providencia spp* that the analyzed chicken samples (Shawish, R. R. ; Eltahan,A.; and Elbayoumi, Z. H. 2023). also *Providencia* was isolated from 21 strains in Wild birds (Foti, *et al.*, 2017).Over 55% of the *P. stuartii* isolates in study

of (Arifatun Nahar *et al.*,2016). *P. stuartii* isolate from droppings of chicken spreading by human to animal interface and from person-to-person transmission and impose cross-resistance to other pathogens (Chander *etal.*,2006).Ayaka Shima *etal.*,(2016) study the prevalence of Providencia spp. (17/26) of the chicken samples, Myonsun Yoh *etal.*, (2005) study *Providencia* importance as a cause diarrhoea five isolates of *Providencia stuartii* from 130 specimens, Providencia species, cause diarrhoea, and these species are important pathogens. Infections with Providencia which cause food poisoning, diarrhea, and UTI have been increased in the world, especially in developed countries.

CONCLUSION

Our study proved that *Providencia spp* isolated from birds and owners play a role in zoonosis diseases, biosafety in farms cleaning and disinfection will reduce *Providencia spp* spreading in the farms.

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Authors Contribution

The Author collect samples ,diagnosis of *Providencia spp* ,and written the article

Conflict of interest

The authors have declared no conflict of interest.

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