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Detection of *Salmonella* spp. in exotic pigeons of North Kerala and its antibiogram

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Abstract

Pigeon breeding has transformed from being a mere hobby to becoming established as an industry. The increased trade of pigeons inadvertently invites the risk of dissemination of infections including zoonoses like salmonellosis. Pigeons once infected remain carriers for life. This coupled with the ability of the organism to acquire antimicrobial resistance makes salmonellosis, particularly from pigeons an important, public health risk for pigeon handlers. Cloacal swabs from a total of 200 exotic pigeons belonging to 24 lofts from Northern districts of Kerala were collected and attempted to isolate Salmonella and understand its antimicrobial resistance profile. Five isolates of salmonella could be obtained from four of the lofts studied. A prevalence of 2.5 per cent was identified for salmonellosis with 16.67 per cent of the lofts affected. Antimicrobial sensitivity based on disk diffusion assay revealed that all the five isolates were sensitive to amoxicillin-clavulanate and all were resistant to tetracycline and streptomycin. Sixty per cent of the isolates were sensitive to co-trimoxazole, chloramphenicol, ampicillin, cefoperazone, amikacin and gentamicin.

Keywords: *Salmonellosis, exotic pigeons, antibiogram*

Pigeon breeding requires minimal investment for housing, feed cost and veterinary care and its reproduction management is comparatively easy. These factors along with high sales return makes pigeon breeding a lucrative business and a sustainable entrepreneurship. Increasing volume of pigeon trade has resulted in increased trading of birds across the countries, which inadvertently invites the risk of dissemination of infections including zoonoses. Salmonellosis is one such common disease that gets traded along with pigeons. Pigeons once infected could remain as lifelong carriers. People who handle the infected stock can easily acquire the infection from

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pigeons. Salmonellosis is a major threat from the public health perspective as the organism is notorious in acquiring antimicrobial resistance (Arcangioli *et al*., 1999). Antimicrobials are extensively used in pigeon breeding industry to improve the performance and health of the birds. Unscrupulous and unsupervised use of antimicrobials can induce a drug induced selection pressure that results in the evolution of multiple drug resistant bacteria. Studies are lacking regarding the prevalence of salmonella among fancy pigeons in India, particularly in Kerala. Lack of systematic studies adds to the knowledge gap that exists regarding the occurrence and prevalence of multiple drug resistant Salmonellae among domestic pigeons in the state.

Materials and methods

A total of 200 cloacal swabs of exotic pigeons were collected as per García *et al*. (2011) from 24 different pigeon lofts from the Northern Districts of Kerala namely, Kannur, Kozhikode, Wayanad and Malappuram. Loft owners were interviewed to understand the antimicrobials used in the respective lofts. Samples were collected aseptically in buffered peptone water and incubated at 37ºC for 24 hours followed by selective enrichment in Rappaport-Vassiliadis (RV) broth at 42ºC for 24 hours. Plating was done on Salmonella Shigella (SS) agar, Xylose Lysine Deoxycholate (XLD) agar, Brilliant Green Agar (BGA) plates at 37ºC. The resultant colonies were analysed after 24 to 48 h.

Biochemical tests of the isolated *Salmonella* spp. were done according to the standard protocols (Edward and Ewing, 1986). Salmonella was identified based on colony morphology, Gram staining characters and biochemical tests *viz.* catalase test, oxidase test, IMViC test, urease test and Triple Sugar Iron (TSI) agar test.

Antibiogram of the salmonella isolates were studied using disc diffusion (HiMedia) technique (Bauer *et al.,* 1966) using amikacin (30µg), amoxicillin-clavulanate (20/10µg), ampicillin (25µg), cefoperazone (75µg), chloramphenicol (30µg), trimethoprim-sulphamethoxazole (1.25/23.75µg), gentamicin (10µg), streptomycin (10µg) and tetracycline (30µg). The zone of inhibition of bacterial growth around each disc including the diameter of the disc was measured and interpreted as sensitive or resistant by comparing the ranges given by the manufacturer. Polymerase chain reaction was done targeting *aadA2*, bla_{CARB-2}, *cmlA*, *sull, tetA* and *tetR* which encodes resistance against streptomycin, ampicillin, chloramphenicol, sulfamethoxazole and tetracycline respectively (Table 1, Table 2).

Table 1: Primers used for the molecular characterization of the isolates

Sl. No	Gene	Primer sequence 5'-3'	Protocol	Product size (bp)	Reference
1	*aadA2*	F- GTACGGCTCCGCAGTGGA TGGCGG	1	522	Briggs and Fratamico (1999)
		R-GCCCAGTCGGCAGCGACA TCCTTC			
2	bla_{CARB-2}	F- CAATGGCAATCAGCGCTTCCCGTT	1	639	
		R- CGCTCTGCCATTGAAGCCTGTGTT			
3	*cmlA*	F- CGC CAC GGT GTT GTTGTT AT	2	394	Chen *et al*., 2003
		R- GCG ACC TGC GTA AAT GTC AC			
4	*sull*	F- TCA CCG AGG ACT CCT TCT TC	2	331	
		R- CAG TCC GCC TCA GCA ATA TC			
5	*tetA*	F- GCG CCT TTC CTT TGG GTT CT	2	831	
		R- CCA CCC GTT CCA CGT TGT TA			
6	*tetR*	F- CGCTCCTTCGATCCCGT	3	260	Yang *et al*.,2001
		R- GCTGCGTTCATCTACAACAGAT			

Results and discussion

Among the 200 samples tested, five isolates produced typical colonies with biochemical properties suggestive of *Salmonella* spp. The isolates formed colourless and transparent colonies with black center in SS agar (Fig. 1A), red colonies with black centre in XLD agar (Fig. 1B) and pink colonies with pink coloration surrounding the media in BGA (Fig. 1C). The findings are in accordance with Rahman *et al.* (2016) and El-Prince *et al*. (2019) who also reported similar colony characteristics exhibited by *Salmonella* spp. in their studies. The biochemical characters of the isolates obtained are in agreement with Rajagopal and Mini (2013) and Rahman *et al*. (2016) who reported that the isolates of salmonella were gram negative, small bacilli with specific IMViC reaction results (-+-+) as all were found to be indole negative, methyl red positive, voges-proskaur negative and utilized citrate (Fig. 2). The findings also agree with Sharma *et al*. (2019) and Ranjbar *et al*. (2020) who reported that salmonella isolates produced acid butt and alkaline slant in TSI agar slants (Fig. 2).

A prevalence of 2.5 per cent was identified for salmonellosis with 16.67 per cent of the lofts affected. Comparable prevalence was reported by Casanovas *et al*. (1995) and Perez-Sancho *et al*. (2020) who reported a prevalence of 1.5 per cent and 4.41 per cent. However, higher prevalence were reported by Hosain *et al*. (2012) who reported a prevalence of 35.71 per cent and Saifullah *et al*. (2016) who reported prevalence of 34 per cent. Santos *et al*. (2020) suggested that samples should be collected every five days to detect the presence of *Salmonella* spp. due to the intermittent shedding nature of the bacteria.

Table 2. PCR cycling conditions used for the study

Protocol No.	Initial denaturation	Denaturation	Annealing	Extension	No. of cycles	Final extension
1	95°C, 5 min	95°C, 1 min	60°C, 1 min	72°C, 1 min	30	72°C, 10 min
2	95°C, 10 min	95°C, 30 sec	55°C, 1 min	72°C, 1 min	30	72°C, 7 min
3	95°C, 5 min	95°C, 1 min	48°C, 30 sec	72°C, 30 sec	40	72°C, 3 min

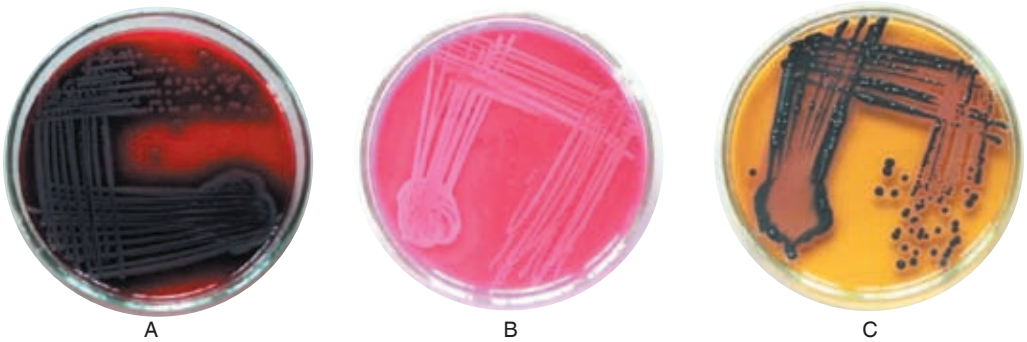
Table 3. Antimicrobial Sensitivity pattern of the isolates

Isolate	C	AMC	AMP	COT	CPZ	TE	S	AK	GEN
C1	R	S	R	R	R	R	R	R	R
C2	R	S	R	R	R	R	R	R	R
W1	S	S	S	S	S	R	R	S	S
W2	S	S	S	S	S	R	R	S	S
W3	S	S	S	S	S	R	R	S	S

C- Chloramphenicol; AMC- Amoxicillin-clavulanate; AMP- Ampicillin; COT- Trimethoprim-sulphamethoxazole; CPZ- Cefoperazone; TE- Tetracycline; S- Streptomycin; AK- Amikacin; GEN- Gentamicin; S - Sensitive; R - Resistant

Table 4. Antimicrobial resistance genes in the *Salmonella* isolates

Genes	ISOLATES				
	C1	C2	W1	W2	W3
aadA2	+	+	-	-	-
bla_{CARB-2}	-	-	-	-	-
cmlA	+	+	-	-	-
sulI	-	-	-	-	-
tetA	+	+	+	+	+
tetR	-	-	-	-	-



A B C

Fig. 1. *Salmonella* isolate on selective Media
A: SS Agar B: XLD Agar C: BGA

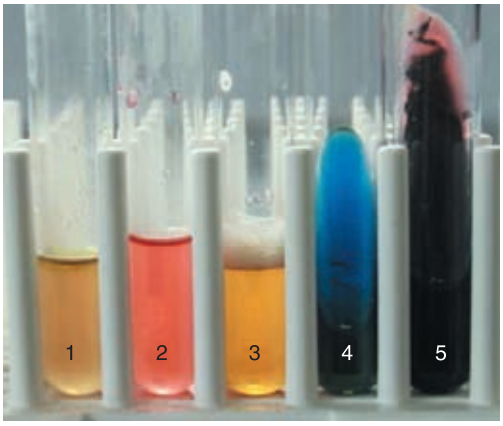


Fig. 2. IMViC test and TSI of Salmonella isolate
1. Indole: Negative 2. Methyl red: Positive
3. Voges- proskauer: Negative
4. Citrate utilisation: Positive
5. Triple Sugar iron with H2S production

Similar findings were also reported by Teske *et al*. (2013). Repeated sampling of all birds in the selected lofts during the present study might have improved the detection rate.

Among the five isolates tested for antimicrobial sensitivity, all (100 per cent) were sensitive to amoxicillin-clavulanate. Three isolates (60 per cent) each was sensitive to chloramphenicol, ampicillin, co-trimoxazole, cefaperazone, amikacin and gentamicin. All isolates were resistant to tetracycline and streptomycin. The details regarding the antimicrobial sensitivity pattern of the isolates are given in the Table 3.

High sensitivity towards amoxicillin-clavulanate was reported by Stenzel *et al*. (2014). Since none of the lofts surveyed for the present work used amoxicillin-clavulanate, absence of resistance to the drug could be explained to be due to poor selection pressure induced by the drug. The findings also agree with that of Jahantigh and Nili (2010) who reported high level of resistance towards tetracycline among salmonella in pigeons. The varying resistance to antimicrobials among different populations of fancy pigeons could be because of different preferences of antimicrobials being used in different localities (Kaczorek-Lukowska *et al*., 2020).

Among the six genes tested for detecting antimicrobial resistance, amplification was obtained in three. All of the isolates were positive for *tetA* gene and produced 831 bp sized amplicons (Fig. 3). The *aadA2* gene (522 bp amplicon) was detected in two isolates (40 per cent) (Fig. 4). The *clmA* gene (394 bp amplicon) was detected in two isolates (40 per cent) (Fig.5). No amplification was obtained for *sulI, tetR and* bla_{CARB-2}genes in any of the isolates (Table 4).

Kaczorek-Lukowska *et al*. (2020) reported that *tetA* gene was among the most common antibiotic resistance genes that were isolated from domestic pigeons. Extensive use of tetracycline could have contributed to the selection of isolates with *tetA* gene. Yousef and Mamdouh (2016) reported the presence of Class I integrons in *Salmonella* Enteritidis isolated from pigeons, which was associated with a variety of resistance genes including the *aadA*. Absence of *sul1* gene even in presence of isolates resistant to co-trimoxazole could be because of the involvement of other genes belonging to the *sul* family (Kozak *et al*., 2009; Xu *et al*., 2020).

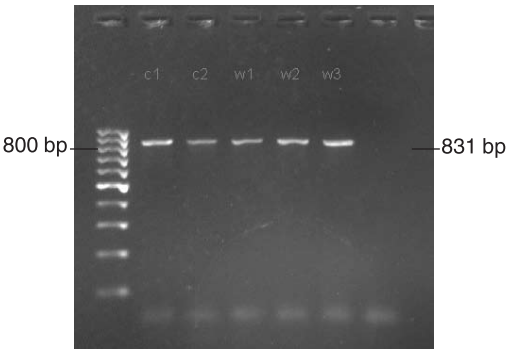


Fig. 3. Agarose gel electrophoresis of *tetA* specific PCR
Lane1: DNA marker 100bp
Lane 2-6: Positive samples (831 bp)

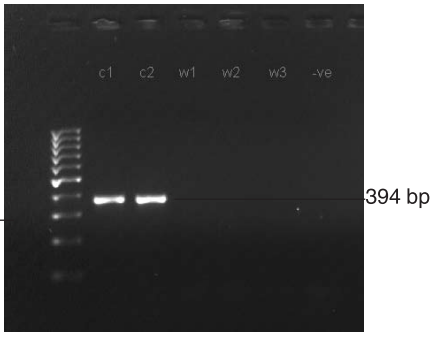


Fig. 5. Agarose gel electrophoresis of *clmA* specific PCR
Lane1: DNA marker 100bp
Lane 2.3: Positive samples (394 bp)
Lane 4-6: Negative samples

Conclusion

Among 200 cloacal samples of exotic pigeons tested for salmonellosis from 24 lofts, five positive isolates could be obtained. The study showed a prevalence of 2.5 per cent of salmonellosis among exotic pigeons with 16.67 per cent of the lofts affected. The antimicrobial sensitivity tests revealed that all the five isolates positive for salmonellosis were sensitive to amoxicillin-clavulanate and all were resistant to tetracycline and streptomycin. The same revealed that 60 per cent of the positive isolates were sensitive to co-trimoxazole, chloramphenicol, ampicillin, cefoperazone, amikacin and gentamicin.

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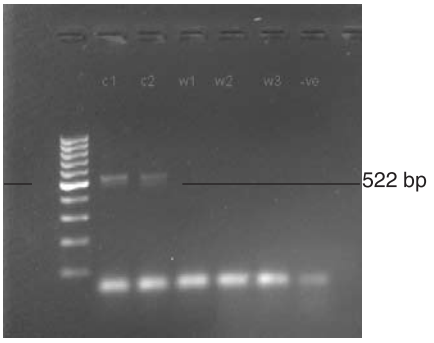

Fig. 4. Agarose gel electrophoresis of *aadA2* specific PCR
Lane1: DNA marker 100bp
Lane 2.3: Positive samples (522 bp)
Lane 4-6: Negative samples

Conflict of interest

The authors declare that they have no conflict of interest.

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