Med. Weter. 2019, 75 (12), 735-737 DOI: dx.doi.org/10.21521/mw.6280

Praca oryginalna *Original paper*

Occurrence and antimicrobial susceptibility of *Salmonella* spp. isolated from domestic pigeons *Columba livia* var. *domestica* in 2007-2017 in Poland

ALEKSANDRA LEDWOŃ, MAGDALENA RZEWUSKA*, MICHAŁ CZOPOWICZ**, MAGDALENA KIZERWETTER-ŚWIDA*, DOROTA CHROBAK-CHMIEL*, PIOTR SZELESZCZUK

Department of Pathology and Veterinary Diagnostics, Institute of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, 159c Nowoursynowska St. 02-776 Warsaw, Poland

*Department of Pre-Clinical Sciences, Institute of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Ciszewskiego St. 8, 02-787 Warsaw, Poland

**Laboratory of Veterinary Epidemiology and Economics, Institute of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, 159c Nowoursynowska St. 02-776 Warsaw, Poland

Received 11.02.2019 *Accepted 20.05.2019*

Ledwoń A., Rzewuska M., Czopowicz M., Kizerwetter-Świda M., Chrobak-Chmiel D., Szeleszczuk P.

Occurrence and antimicrobial susceptibility of *Salmonella* spp. isolated from domestic pigeons *Columba livia* var. *domestica* in 2007-2017 in Poland

Summary

Salmonellosis is one of the most important bacterial diseases in pigeons. The disease occurs in birds of different ages, and long-term *Salmonella* spp. carrier status is a major problem. The study included 499 samples from live pigeons and internal organs from 265 necropsied birds. The samples were cultured on a standard and selective media. Their antimicrobial susceptibility was carried out by the Kirby-Bauer diffusion method. Thirty one *Salmonella* spp. isolates were cultured. Most isolates were obtained from the internal organs of dead or euthanised pigeons, and only single isolates were obtained from droppings and cloacal swabs. A total of 4% of the samples tested were positive. The greatest percentage of isolates was susceptible to enrofloxacin (96.3%) and florfenicol (95.7%). Sulfamethoxazole and trimethoprim combination was effective against 73.9% of the isolates. Despite the fact of increasing antibiotic resistance of the investigated bacteria, it is comforting that enrofloxacin remains highly effective despite its widespread use in birds.

Keywords: *Salmonella* spp., pigeon, antimicrobial resistance, pigeon salmonellosis

Salmonella spp. is one of the most important bacterial pathogens of pigeons. The most common pigeon pathogen in Europe is *Salmonella typhimurium* var. Copenhagen, phage types PT2, PT99 and PT46, belonging to the serological group B (9, 14). Young pigeons are at the highest risk of infection, although deaths occur in birds of allages (5, 16). The main problem in combating salmonellosis is the long-term asymptomatic carrier status for this pathogen (5, 12). If *Salmonella* is isolated, treatment of all pigeons in the flock should be conducted, along with sanitisation/disinfection (5). The duration of the recommended antibiotic therapy is 14 to 21 days, and if a positive culture result is obtained again after 3 to 6 weeks, the therapy should be repeated (16). The antibiotic should be selected as indicated by antibiogram. In the studies of organs of clinically healthy wild pigeons conducted to date in Poland, *Salmonella* was isolated in approximately 11% of cases (11), while in the Czech Republic this bacterium was found in 33% of cases (7). In Poland, homing and ornamental pigeons are reared, and treatment of these birds is not subject to strict veterinary checks as in the case of pigeons reared for fattening. Therefore, the problem of excessive use of antibiotics by breeders exists.

The aim of this study was to assess the antimicrobial susceptibility of *Salmonella* spp. isolates obtained from domestic pigeons, and to analyse the dynamic changes in the profiles of resistance to the tested antibiotics throughout the ten-year duration of the study.

Material and methods

The tested material were 499 samples from live birds: 16 conjunctival swabs, 28 choaneswabs, 69 buccopharyngeal cavity swabs and 136 cloacal swabs, 250 droppings samples and 265 samples from necropsied pigeons: internal organs (mainly liver). The obtained material was cultured on microbiological media (Graso, Poland): Columbia Blood Agar, McConkey, SS (Salmonella Shigella Agar) and medium with sodium selenate (SF). The isolates were identified based on their growth characteristics and biochemical properties identified using the API 20E test (bioMerieux, France). Subsequently, serological assays were conducted using HM polyvalent serum and monovalent sera for group antigens: AO, BO, CO and DO (Biomed Lublin, Poland) to determine the serogroups.

Antimicrobial susceptibility testing was conducted by means of the agar disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2). The test was conducted for amoxicillin, amoxicillin with clavulanic acid, cefotaxime, colistin, streptomycin, gentamicin, neomycin tetracycline, florfenicol, enrofloxacin and sulfamethoxazole with trimethoprim (BD, USA and Graso, Poland).

The percentage of susceptible isolates was compared between two periods of study (2007-2012 and 2013-2017) using the Pearson's chi-square test, or Fisher's exact test if the expected count in cells of contingency table was below 5. Only those antibiotics for which at least 5 isolates were tested in each of the two time periods were included in the comparison. Significance level (α) was set at 0.05. The analysis was performedusing Statistica 12 (StatSoft Inc.).

Results and discussion

A total of 31 *Salmonella* isolates were obtained. No positive result was obtained for any of the tested samples of the conjunctival, choane and buccopharyngeal cavity swabs. In case of cloacal swabs *Salmonella* growth was obtained in only from one pigeon. Two samples of droppings were positive. The 28 isolates were obtained from internal organs collected during the necropsy of 265 pigeons. In one case, a positive liver culture was obtained from a clinically healthy pigeon euthanized due to a mechanical trauma, but in this case the isolated *Salmonella* strain was classified to the sero group D. Other isolates belonged to group B. The most isolates were obtained in the summer (38%), followed by spring (32%), autumn (26%) and winter (3%).

The greatest percentage of isolates was susceptible to enrofloxacin (96.3%) and florfenicol (95.8%) followed by sulfamethoxazole with trimethoprim (73.9%), gentamicin (54.8%), cefotaxime (52.4%), tetracycline (48.3), amoxicillin with clavulanic acid (43.3%), amoxicillin (41.4) and streptomycin (5.9%). Analysis of antimicrobial resistance of the isolates in two five-year periods (Fig. 1) revealed a significant increase in *Salmonella* spp. resistance to amoxicillin ($p = 0.005$), amoxicillin with clavulanic acid ($p = 0.003$), gentamicin ($p = 0.005$), tetracycline ($p = 0.039$) and neomycin ($p = 0.002$). For enrofloxacin and florfenicol p-values were 0.471 and 0.410, respectively.

The most available material for the study, usually obtained from healthy or ill pigeons, are droppings. In the case of droppings investigated in this study, only 0.8% of samples were positive for *Salmonella*. In turn, in a study conducted in Belgium by Pasmans et al. (10), samples taken from domestic pigeons yielded as many as 22.8% of positive results (10). From cloacal swabs, only one *Salmonella* isolate (0.73%) was obtained from a pigeon dead due to salmonellosis. In a study by

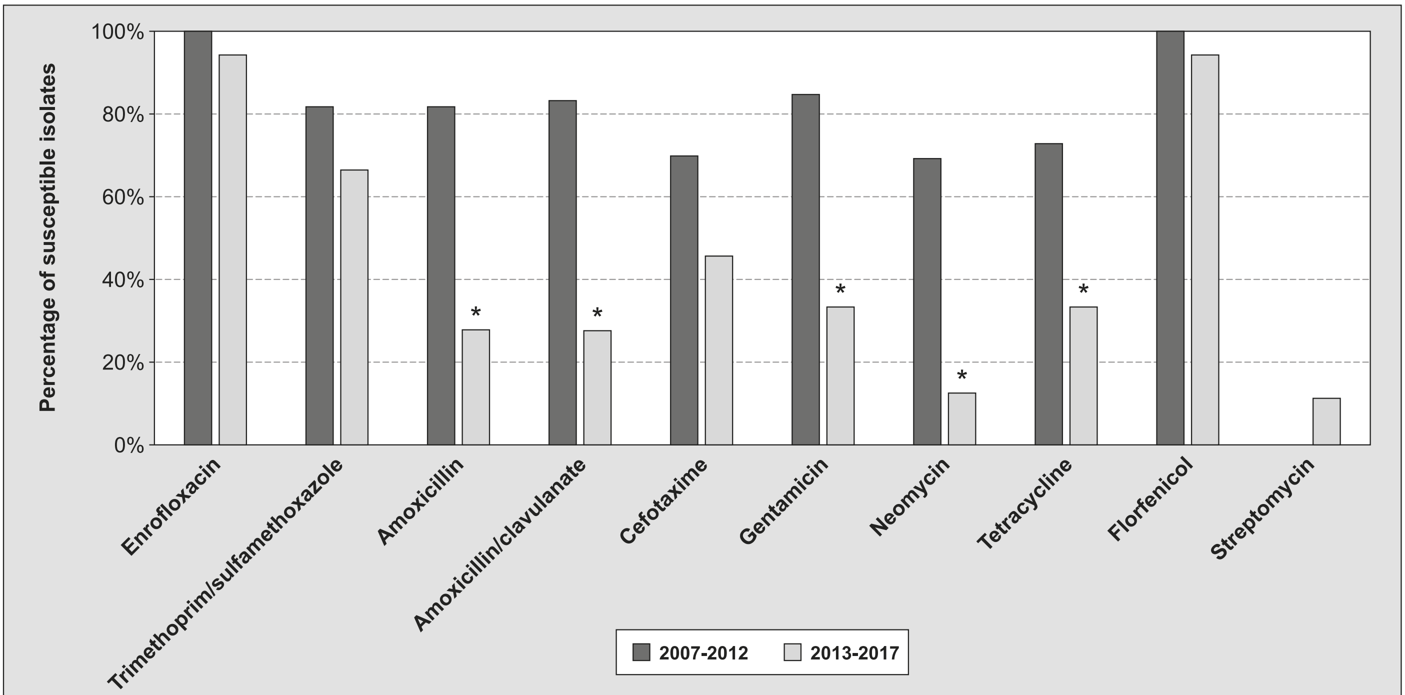

Fig. 1. Comparison of susceptibility of pigeon-derived *Salmonella* isolates to selected antibiotics between two five-year periods. Statistically significant differences are indicated by asterisks (*)

Gabriele-Rivet (4) conducted in Canada and a study by Lillehaug conducted in Norway (8), swabs taken from city pigeons yielded no *Salmonella* spp. isolates. In Germany, Teske et al. (14) obtained 0.9% to 3.7% of positive results from clinically healthy domestic pigeons. In Slovenia, Dovc et al. (3), analysing cloacal swabs from wild pigeons, demonstrated the presence of *Salmonella* in 5.4% of investigated birds. In our study, the most *Salmonella* isolates were cultured from the internal organs of 10.5% of necropsied pigeons. It should be noted that in most cases internal organs were collected from dead pigeons, therefore the chance of detecting infection was much greater in those cases. According to Pasmans et al. (10), pigeon-derived strains of *Salmonella* Typhimurium var. Copenhagen in murine tissues reach the highest density in the liver, followed by the spleen and the intestine. Conversely, the highest density of bacterial cells isolated from humans was observed in the spleen of infected mice. In the study by Ammar et al. (1), pigeon samples for *Salmonella* cultures were taken from the intestine, the liver and the caecal tonsils. The greatest number of positive results was obtained from the intestine, followed by the caecal tonsils and the liver. In our study mainly liver but no caecal tonsil samples were cultured.

Our results of the analysis of *Salmonella* antimicrobial susceptibility differ from those described in Poland by Stenzel et al. (13) who showed that 100% of isolates were susceptible to B-lactams, sulfamethoxazole with trimethoprim, flumequine, florfenicol and enrofloxacin. In turn, in a recent study conducted in Egypt by Yousef et al. (15), it was found that none of the *Salmonella* isolates from pigeons were susceptible to streptomycin, amoxicillin/clavulanic acid, amoxicillin, ampicillin and ceftazidime. In our study, susceptibility to amoxicillin with clavulanic acid in the 2007-2012 period was observed in 83% of isolates, but the percentage decreased more than 2-fold in the second decade of the 21st century. The greatest number of isolates were susceptible to enrofloxacin and florfenicol. In a study by Kimpe et al. (6) conducted in Belgium, all investigated pigeon-derived isolates of *Salmonella* Typhimurium var. Copenhagen were susceptible to ampicillin, trimethoprim and enrofloxacin. The most surprising is the fact that despite the very widespread use of enrofloxacin for treating birds, this antibiotic still demonstrates a high effectiveness against field isolates of *Salmonella*. Several preparations with enrofloxacin are approved for use in pigeons in Poland. Florfenicol is less available and more expensive, and not approved for use in pigeons in Poland, that can be the reason for its lower use and the still high susceptibility of *Salmonella* spp. to this antibiotic. According to the study by Pasmans et al. (9), oral application of florfenicol for the treatment of pigeon paratyphoid contributes to the development of carrier animals through sub-MIC concentrations in plasma that do not inhibit intracellular persistence. Therefore, the use of enrofloxacin remains the treatment of choice in pigeon salmonellosis.

Our study confirmed the problem of increasing resistance to antibiotics in pathogenic bacteria. This may often be due to starting treatment without bacteriological assessment, which causes the therapy to be too short in duration and contributes to the increasing resistance of *Salmonella* spp. strains. Another problem is detecting carrier status for these bacteria in live pigeons, and thereby the ease of transmitting the pathogens to other pigeons in the flock also by clinically healthy birds.

References

1. *Ammar A., Sultan H., El-Sayed I., Yousef S., Mamdouh R.*: Seroprevalence of Salmonellosis among Pigeon and its Surrounding Environment and Isolation of Salmonella Species. IJSR 2015, 9, 107-117.
2. CLSI. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. 4th. approved standard, CLSI document VET01-A4. Wayne, Pa, USA: Clinical and Laboratory Standards Institute 2013.
3. *Dovc A., Zorman-Rojs O., VerglesRataj A., Bole-Hribovsek V., Krapez U., Dobeic M.*: Health status of free-living pigeons (Columba livia domestica) in the city of Ljubljana. Acta Vet. Hung. 2004, 52, 219-226.
4. *Gabriele-Rivet V., Fairbrother J. H., Tremblay D., Harel J., Côté N., Arsenault J.*: Prevalence and risk factors for Campylobacter spp., Salmonella spp., Coxiella burnetii, and Newcastle disease virus in feral pigeons (Columba livia) in public areas of Montreal, Canada. Can. J. Vet. Res. 2016, 80, 81-85.
5. *Hooimeier J., Dorrestein M. G.*: Pigeons and Doves, [in:] Altman R. B., Clubb S. L., Dorrestein G. M., Quesenberry K. (eds): Avian Medicine and Surgery. W. B. Saunders Company, USA 1996, pp. 886-909.
6. *Kimpe A., Decostere A., Martel A., Haesebrouck F., Devriese L. A.*: Prevalence of antimicrobial resistance among pigeon isolates of Streptococcus gallolyticus, Escherichia coli and Salmonella enterica serotype Typhimurium. Avian Pathol. 2002, 31, 393-397.
7. *Kriz P., Sisak F., Slana I., Karpiskova R., Docekal J., Skoric M., Fictum P., Babak V., Pavlik I.*: Mycobacterium avium subsp. avium and Salmonella enterica serotype Typhimurium var. Copenhagen phage type DT2 in pigeons. Foodborne Pathog. Dis. 2011, 8, 1135-1137.
8. *Lillehaug A., Monceyron Jonassen C., Bergsjø B., Hofshagen M., Tharaldsen J., Nesse L. L., Handeland K.*: Screening of Feral Pigeon (Columba livia), Mallard (Anas platyrhynchos) and Graylag Goose (Anser anser) Populations for Campylobacter spp., Salmonella spp., Avian Influenza Virus and Avian Paramyxovirus. Acta Vet. Scand. 2005, 46, 193-202.
9. *Pasmans F., Baert K., Martel A., Bousquet-Melou A., Lanckriet R., De Boever S., Van Immerseel F., Eeckhaut V., de Backer P., Haesebrouck F.*: Induction of the Carrier State in Pigeons Infected with Salmonella enterica subspecies enteric serovar Typhimurium PT99 by Treatment with Florfenicol: a Matter of Pharmacokinetics. Antimicrob. Agents Chemother. 2008, 52, 954-961.
10. *Pasmans F., Van Immerseel F., Hermans K., Heyndrickx M., Collard J. M., Ducatelle R., Haesebrouck F.*: Assessment of virulence of pigeon isolates of Salmonella enterica subsp. enteric serovar Typhimurium variant Copenhagen for humans. J. Clin. Microbiol. 2004, 42, 2000-2002.
11. *Piasecki T.*: Evaluation of urban pigeon (Columba livia f. urbana) health status in relation to their threat to human’s health. Med. Weter. 2006, 62, 531-535.
12. *Rupiper D. J.*: Diseases that affect race performance of homing pigeons. Part II: bacterial, fungal, and parasitic diseases. J. Avian Med. Surg. 1998, 12, 138-148.
13. *Stenzel T., Bancerz-Kisiel A., Tykałowski B., Śmiałek M., Pestka D., Koncicki A.*: Antimicrobial resistance in bacteria isolated from pigeons in Poland. Pol. J. Vet. Sci. 2014, 17, 169-171.
14. *Teske L., Ryll M., Rubbenstroth D., Hänel I., Hartmann M., Kreienbrock L., Rautenschlein S.*: Epidemiological investigations on the possible risk of distribution of zoonotic bacteria through apparently healthy homing pigeons. Avian Pathol. 2013, 42, 397-407.
15. *Yousef S., Mamdouh R.*: Class I Integron and β-lactamase encoding genes of multidrug resistance Salmonella isolated from pigeons and their environments. Cell Mol. Biol. 2016, 62, 48-54.
16. *Zwart P.*: Bacterial Diseases, [in:] Samour J. (ed.): Avian Medicine. Elsevier, St Luis Missouri 2016, p. 456-457.

Corresponding author: Aleksandra Ledwoń, DVM, PhD, ul. Ciszewskiego 8/065, 02-787 Warszawa; e-mail: aledwonn@yahoo.pl