Original research

OCCURRENCE AND ANTIMICROBIAL RESISTANCE OF *SALMONELLA* AND *CAMPYLOBACTER* IN HUMANS AND BROILER CHICKEN IN UZBEKISTAN

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ABSTRACT

Background: Foodborne infections represent a significant public health burden. Moreover, antimicrobial resistance (AMR) in *Salmonella* and *Campylobacter* is a growing problem, which is linked to antimicrobial use in food animals. We aimed to get insight on the occurrence and AMR of *Salmonella* and *Campylobacter* isolated from humans with diarrhoea and broiler chicken in Uzbekistan, as such data can help inform national policy-making on food safety and AMR.

Methods: We conducted a survey during January–May 2015. We included a total of 84 intestinal samples from healthy broiler chicken and 81 samples from human patients with acute diarrhoea.

Results: *Salmonella* and *Campylobacter* were isolated from the diarrhoeal disease cases, and were the etiological agents in 27% and 9% of the cases, respectively. Of the broiler chicken samples, 30% were positive for *Salmonella* and 30% for *Campylobacter*. We observed a high level of multiresistance among the *Salmonella* isolates: 80% and 50% of isolates from broiler chicken and humans, respectively, were resistant to five or six antimicrobial classes. A large proportion of *Campylobacter* isolates from both humans and broiler chicken (71% and 92%, respectively) were resistant to fluoroquinolones. Antibiograms for the *Campylobacter* isolates from humans and broiler chicken showed comparable patterns.

Conclusion: Both *Salmonella* and *Campylobacter* seem to be important causes of diarrhoeal disease among humans in Uzbekistan, and broiler chicken seems to be a contributing source of infection. The level of AMR seems high among *Campylobacter* and *Salmonella* from both broiler chicken and humans, which may partly reflect the use of antimicrobial agents in the poultry industry in Uzbekistan.

Keywords: *SALMONELLA, CAMPYLOBACTER*, EPIDEMIOLOGY, UZBEKISTAN, ANTIMICROBIAL RESISTANCE

INTRODUCTION

Throughout the world, foodborne diseases, particularly zoonotic ones (i.e. diseases transmissible from animals to humans), represent a considerable public health burden and challenge. It is estimated that every year, more than 23 million people in the WHO European Region fall ill from eating contaminated food, resulting in about 5000 deaths per year *(1).* Diarrhoeal diseases account for the majority of these foodborne illnesses, the most common being norovirus infections, followed by campylobacteriosis, which causes close to 5 million cases annually. Non-typhoid salmonellosis causes the most deaths – almost 2000 annually. In 2014, in the European Union (EU)/ European Economic Area alone, there were 236 851 reported confirmed cases of campylobacteriosis and 88 715 reported confirmed cases of salmonellosis *(2).* The cost of campylobacteriosis to public health systems and to lost productivity in the EU is estimated by the European Food Safety Authority (EFSA) to be around €2.4 billion a year *(3).* EFSA has estimated that the overall economic annual burden of human salmonellosis could be as high as €3 billion *(4)*.

Antimicrobial resistance (AMR) is a considerable public health problem resulting from the use and misuse of antimicrobial agents. Use of any kind of antimicrobial in people, animals or plants can promote the selection and spread of AMR. In addition, AMR does not respect geographical nor biological borders. Thus, the use of antimicrobial agents in one sector, setting or country affects the spread of AMR in others. AMR is also a food safety issue, as antimicrobials are used in food animals for treatment, disease prevention or, in some places, even growth promotion, thus allowing resistant bacteria and resistance genes to pass through the food chain from food animals to humans *(5,6).* Resistance in the foodborne zoonotic bacteria *Salmonella* and *Campylobacter* is linked to antimicrobial use in food animals, and foodborne diseases caused by such resistant bacteria are well documented in humans. EU data show that resistance to widely used antimicrobials is commonly detected in *Salmonella* and *Campylobacter* from humans and poultry *(7).*

Salmonellosis is considered an important cause of diarrhoeal disease among humans in Uzbekistan (Gulnara Abdukhalilova, unpublished observations). However, limited information is available on the occurrence of *Salmonella* in the food chain in Uzbekistan. No information is available on the occurrence of *Campylobacter* infection in Uzbekistan, either from humans or from poultry. Generally, data are scarce regarding antimicrobial usage and AMR in Uzbekistan, both in food animals and in humans *(8,9).* Antimicrobials for both human and veterinary use are sold over the counter without prescription. Thus, there is reason to believe that there is misuse of antimicrobial agents in Uzbekistan, both in humans and in animals , as is the case in many other countries.

We conducted a survey to obtain a preliminary understanding of the occurrence and AMR of *Salmonella* and *Campylobacter* among human patients with diarrhoea and healthy broiler chicken in Uzbekistan, with the overall objective of informing policy-making on food safety and AMR in Uzbekistan.

MATERIALS AND METHODS

We conducted a survey on the occurrence and AMR of *Salmonella* and *Campylobacter* among humans and broiler chicken in Uzbekistan during January–May 2015 within the framework of the biennial collaborative agreement for 2014/15 between the Ministry of Health of Uzbekistan and the WHO Regional Office for Europe.

From 13 January to 27 April 2015, we collected faecal samples from 81 human patients with acute diarrhoea admitted to the clinic of the Research Institute of Epidemiology and Microbiology of Communicable Diseases (RIEMCD) in Tashkent, which is under the Ministry of Health, Uzbekistan. These samples were collected before any antimicrobial treatment was given. The patients were Uzbek citizens from the broader Tashkent area.

In collaboration with the Food and Agriculture Organization Country Office in Uzbekistan and the Ministry of Agriculture, we selected a large poultry farm in the Tashkent region of Uzbekistan for the collection of broiler chicken samples. This typical Uzbek broiler chicken-producing farm can be considered representative of broiler chicken production in Uzbekistan. Fresh broiler meat in the Uzbek market is of domestic origin. Linked to the farm is a slaughterhouse, which we visited four times during the study period to collect samples from four different broiler flocks at the point of slaughter. We randomly collected 84 broiler carcasses from healthy animals weighing 1000±100 g, and analysed intestinal (caecal) contents from each sampled carcass on the day of collection.

We analyzed the 81 faecal samples from human patients and 84 caecal samples from broiler chicken to isolate and identify *Salmonella* and *Campylobacter* according to ISO protocols *(10–15)* in the laboratory of RIEMCD in Tashkent.

For identification of *Salmonella* at the serogroup level, we used agglutination testing according to WHO protocols *(16)*. We agglutinated all isolates of *Salmonella* with polyvalent antiserum (including groups 2 (A), 4 (B), 7 (C_1), 8 (C_2), 9 (D) and 3, 10 (E)) and they all tested positive. We also agglutinated the isolates with monovalent antisera O1, O4, O5, O9, O12, Hi, H a, H d, H 1.2, with negative results. We observed that the isolates did not belong to groups A, B and D; thus, the isolates must belong to serogroup C or E. We did not conduct further identification due to unavailability of the specific monoagglutinating sera.

To identify *Campylobacter* spp., we tested suspected colonies with a combination of available laboratory tests: Gram-staining, testing for mobility using the "hanging drop" method, potassium hydroxide tests, cytochrome oxidase tests, catalase tests and aerotolerance tests. We could not identify the organisms at the species level due to a lack of necessary reagents.

We tested the antimicrobial susceptibility of *Salmonella* isolates according to the protocols of the Clinical and Laboratory Standards Institute by the disc-diffusion method *(17–19)* using commercial standard discs (HiMedia, Mumbai, India) with the following antimicrobial agents: ampicillin, cefotaxime, ciprofloxacin, gentamicin, doxycycline, chloramphenicol and trimethoprim/sulfamethoxazole. Gentamicin was included only for the isolates from broiler chicken. *Escherichia coli* 25922 was used for quality control.

We tested the antimicrobial susceptibility of *Campylobacter* isolates using the disc-diffusion method with commercial discs (HiMedia) containing erythromycin (15 µg disc load), tetracycline (30 µg disc load), and ciprofloxacin (5 µg disc load), as per the protocol of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) *(20)*. We used *Campylobacter jejuni* ATCC 33560 for quality control.

RESULTS

ISOLATION OF *SALMONELLA* AND *CAMPYLOBACTER* FROM HUMAN PATIENTS AND HEALTHY BROILER CHICKENS

Of the 81 human samples, 22 (27.2%) were positive for *Salmonella* spp. and seven (8.6%) for *Campylobacter* spp. One sample (1.2%) was positive for both *Salmonella* spp. and *Campylobacter* spp.

Of the 84 samples from poultry, 25 (29.8%) were positive for *Salmonella* spp. and 25 (29.8%) for *Campylobacter* spp. Seven samples (8.6%) were positive for both *Salmonella* spp. and *Campylobacter* spp. The results are presented in Table 1.

ANTIMICROBIAL SUSCEPTIBILITY TESTING OF *SALMONELLA* ISOLATES

Table 2 presents the summary results of antimicrobial susceptibility testing of the 25 *Salmonella* isolates from broiler chicken. In total, 88% of the isolates were resistant to doxycycline, 80% to ampicillin, 80% to ciprofloxacin, 80% to chloramphenicol and 80% to trimethoprim/sulfamethoxazole. With regard to cephalosporins, 8% were resistant to cefotaxime and produced extended-spectrum beta-lactamase enzymes (ESBLs) according to the double-disc synergy test. All isolates were susceptible to gentamicin.

TABLE 1. TYPE AND NUMBER OF SAMPLES AND THE FREQUENCY OF ISOLATION OF *SALMONELLA* AND *CAMPYLOBACTER*

Sample type	Samples tested	*Salmonella* positive		*Campylobacter* positive	
	n	n	%	n	%
Poultry, intestinal content (caecum)	84	25	29.8	25	29.8
Human, faeces from patients with diarrhoea	81	22	27.2	7	8.6

Table 2 also provides the summary results of antimicrobial susceptibility testing of the 22 *Salmonella* isolates from human patients. In total, 82% of isolates from humans were resistant to tetracyclines, 77% to chloramphenicol, 55% to ampicillin and 55% to trimethoprim/sulfamethoxazole. Of these isolates, 55% were susceptible to ciprofloxacin and none was resistant; however, 45% were intermediate susceptible. Half of the isolates were resistant to cefotaxime and produced ESBLs.

Table 3 provides an overview of the AMR profiles and the frequencies observed for the *Salmonella* isolates from broiler chicken and humans, respectively. Only 12% and 14% of the *Salmonella* isolates from broiler chicken and humans, respectively, were susceptible to all antimicrobials included in the susceptibility testing. More than half of the *Salmonella* isolates from both broiler chicken and humans were resistant to four or more antimicrobial classes concomitantly, and would thus be considered multiresistant. Of the *Salmonella* isolates from broiler chicken, 80% were resistant to five or six antimicrobial classes. Of the *Salmonella* isolates from humans, 50% were resistant to five antimicrobial classes. Overall, three and five AMR profiles were observed among the *Salmonella* isolates from broiler chicken and humans, respectively.

ANTIMICROBIAL SUSCEPTIBILITY TESTING OF *CAMPYLOBACTER* ISOLATES

Summary results of the antimicrobial susceptibility testing of the *Campylobacter* isolates are presented in Table 4. Of the 26 *Campylobacter* isolates from broiler chicken, 8% were resistant to erythromycin, 58% to tetracycline and 92% to ciprofloxacin. Of the seven *Campylobacter* isolates from human patients, 14% were resistant to erythromycin, 43% to tetracycline and 71% to ciprofloxacin.

Table 5 provides an overview of the AMR profiles and the frequencies observed for the *Campylobacter* isolates from broiler chicken and humans, respectively. All isolates from humans and 96% of isolates from broiler chicken were resistant to at least one antimicrobial class. In total, 4% of isolates from broiler chicken and none from humans were resistant to all three antimicrobial classes, i.e. multiresistant.

TABLE 2. ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA* SPP. ISOLATES FROM BROILER CHICKEN AND HUMANS

Antimicrobial	Isolates from broiler chicken (N=25)			Isolates from humans (N=22)		
	R	I	S	R	I	S
	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)
Beta-lactams: ampicillin	80 (20)	4 (1)	16 (4)	55 (12)	5 (1)	41 (9)
Cephalosporins: cefotaxime	8 (2)	8 (2)	84 (21)	50 (11)	0	50 (11)
Quinolones: ciprofloxacin	80 (20)	12 (3)	8 (2)	0	45 (10)	55 (12)
Aminoglycosides: gentamicin	0	0	100 (25)	–	–	–
Tetracyclines: doxycycline	88 (22)	0	12 (3)	82 (18)	9 (2)	9 (2)
Amphenicols: chloramphenicol	80 (20)	0	20 (5)	77 (17)	0	23 (5)
trimethoprim/ sulfamethoxazole	80 (20)	0	20 (5)	55 (12)	0	45 (10)

I: intermediate susceptible; R: resistant; S: susceptible

TABLE 3. RESISTANCE PROFILES OF *SALMONELLA* ISOLATES FROM BROILER CHICKEN AND HUMANS

AMR profile	*Salmonella* isolates from broiler chicken (N=25)		*Salmonella* isolates from humans (N=22)	
	n	%	n	%
Susceptible to all antimicrobial agents	3	12.0	3	13.6
Resistant to at least one antimicrobial agent	22	88.0	19	86.4
Resistant to one class of antimicrobial agents	2	8.0	3	13.6
DOX	*2*	*8.0*	*2*	*9.0*
C	*0*	*0*	1	*4.5*
Resistant to two classes of antimicrobial agents	0	0	4	18.3
DOX/C	*0*	*0*	*4*	*18.3*
Resistant to four classes of antimicrobial agents	0	0	1	4.5
DOX/C/AM/CO	*0*	*0*	*1*	*4.5*
Resistant to five classes of antimicrobial agents	18	72.0	11	50.0
DOX/C/AM/CO/ CIP	*18*	*72.0*	*0*	*0*
DOX/C/AM/CO/ CTX	*0*	*0*	*11*	*50.0*
Resistant to six classes of antimicrobial agents	2	8.0	0	0
DOX/C/AM/CO/ CIP/CTX	*2*	*8.0*	0	*0*

AM: ampicillin; AMR: antimicrobial resistance; C: chloramphenicol; CIP: ciprofloxacin; CO: trimethoprim/sulfamethoxazole; CTX: cefotaxime, DOX: doxycycline

TABLE 4. ANTIMICROBIAL SUSCEPTIBILITY OF *CAMPYLOBACTER* SPP. ISOLATES FROM BROILER CHICKEN AND HUMANS[a]

Antimicrobial agent	Isolates from broiler chicken (N=26)			Isolates from humans (N=7)		
	R	I	S	R	I	S
	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)
Macrolides: erythromycin	8 (2)	4 (1)	88 (23)	14 (1)	14 (1)	71 (5)
Tetracyclines: tetracycline	58 (15)	15 (4)	27 (7)	43 (3)	0	57 (4)
Quinolones: ciprofloxacin	92 (24)	4 (1)	4 (1)	71 (5)	14 (1)	14 (1)

I: intermediate susceptible; R: resistant; S: susceptible
[a] Disc-diffusion test method

TABLE 5. RESISTANCE PROFILES FOR *CAMPYLOBACTER* SPP. ISOLATES FROM BROILER CHICKEN AND HUMANS

AMR profile	Campylobacter isolates from broiler chicken (N=26)		Campylobacter isolates from humans (N=7)	
	n	%	n	%
Susceptible to all antimicrobial agents	1	3.9	0	0
Resistant to at least one antimicrobial agent	25	96.1	7	100
Resistant to one class of antimicrobial agents	10	38.5	5	71.4
CIP	9	34.6	4	57.1
TET	1	3.9	1	14.3
Resistant to two classes of antimicrobial agents	14	53.9	2	28.6
TET/CIP	13	50.0	1	14.3
ERY/CIP	1	3.9	0	0
ERY/TET	0	0	1	14.3
Resistant to three classes of antimicrobial agents	1	3.9	0	0
ERY/TET/CIP	1	3.9	0	0

AMR: antimicrobial resistance, CIP: ciprofloxacin, ERY: erythromycin, TET: tetracycline

DISCUSSION

Foodborne diseases caused by resistant bacteria are well documented in humans, and are linked to antimicrobial use in food animals. Tackling AMR requires a multifaceted holistic approach, which includes collaboration, cooperation and information-sharing between the public health and veterinary sectors. To combat AMR, we need to address the use of antimicrobial agents in food animals, and the occurrence and spread of AMR in the food chain. This is also emphasized in the global action plan on antimicrobial resistance *(21)*, which was endorsed by the World Health Assembly in May 2015. The World Health Assembly urged all Member States to develop and have in place, by 2017, national action plans on AMR that are aligned with the objectives of the global action plan. One of these objectives is "to strengthen knowledge through surveillance and research".

Despite the limited number of patients (i.e. only 81), the results of our survey confirm that *Salmonella* and *Campylobacter* are etiological agents of clinical diarrhoeal disease among humans in Tashkent, which is probably also the case in other parts of Uzbekistan. This is consistent with data from the EU and other countries *(1,22)*. Although the survey only included one large broiler chicken farm in the Tashkent region, the results also suggest that *Salmonella* and *Campylobacter* might be present in the broiler chicken population in other places in Uzbekistan because of the similarity in environmental conditions and production structures. The results indicate that broiler chicken may contribute to human infections with *Salmonella* and *Campylobacter* in the Tashkent region, which might also be the case in other parts of Uzbekistan, as in many other countries *(1,23)*.

Most *Salmonella* isolates from both humans and broiler chicken were multiresistant, with resistance to five or six antimicrobial classes. Such a high level of multiresistance can severely limit therapeutic options in invasive cases of salmonellosis. Resistance frequencies and AMR profiles for the *Salmonella* isolates from humans and broiler chicken varied, probably reflecting the complex epidemiology of *Salmonella* and patterns of antimicrobial use in both humans and poultry. It should also be noted that the serotypes of *Salmonella* spp. in the survey are missing and that a comparison should preferably be done according to serotype. Fluoroquinolone resistance was frequently observed in the *Salmonella* isolates from broiler chicken, whereas no such resistance was observed in isolates from humans. Notably, resistance to cephalosporins was observed in 50% of isolates from humans, but in only 8% of isolates from poultry.

The similarity in resistance frequencies and AMR profiles for *Campylobacter* from humans and broiler chicken suggests that broiler chicken may be a source of human infection with *Campylobacter*. However, the limited number of isolates from humans makes it difficult to draw conclusions from this comparison. Nevertheless, the high level of fluoroquinolone resistance and relatively low level of erythromycin resistance observed in both bacterial populations is consistent with data from the EU *(7)*.

The high level of fluoroquinolone resistance in *Campylobacter* isolates from both humans and *broiler* chicken and in *Salmonella* from broiler chicken is worrying, as fluoroquinolones are critically important antimicrobials in human medicine (24). The veterinary authorities in Uzbekistan confirmed that antimicrobials are routinely used in poultry production and are also available without a veterinary prescription. The antimicrobial classes most commonly used are tetracyclines, aminoglycosides (in particular, streptomycin), chloramphenicol, fluoroquinolones and penicillins. These classes are considered either critically important or highly important antimicrobials in human medicine *(24)*. The results obtained, combined with information on the use of antimicrobial agents in poultry production, suggest that AMR observed in *Campylobacter* and *Salmonella* from broiler chicken may reflect the use of antimicrobial agents in the poultry industry in Uzbekistan. Moreover, fluoroquinolone resistance in *Campylobacter* from humans in Uzbekistan may reflect the use of fluoroquinolones in the poultry industry.

LIMITATIONS

The survey included only patients who were admitted to a hospital clinic; however, many patients with salmonellosis and campylobacteriosis do not seek medical consultation and doctors often do not take samples. It should also be noted that the sampling period for the survey was January–April (which is not the main season of acute bacterial intestinal infections among humans in Uzbekistan), and not the summer when most cases of salmonellosis and campylobacteriosis are usually reported. It can thus be reasonably assumed that the real burden of salmonellosis and campylobacteriosis in the human population is higher.

Another limitation of the survey, which was a pilot study, is that it included only one broiler chicken farm in the Tashkent region, and four flocks. Therefore, the results of this demonstration project cannot be reliably extrapolated to all of Uzbekistan.

CONCLUSION

The survey sheds light on the epidemiology of *Salmonella* and *Campylobacter* and their AMR in the Tashkent area, which may be representative of Uzbekistan. The data advocate surveillance of salmonellosis and campylobacteriosis among humans and monitoring of *Salmonella* and *Campylobacter* in the food chain, including testing for antimicrobial susceptibility. The results highlight the importance of intersectoral collaboration, coordination and information-sharing, in particular between the health and the agriculture sectors, in the area of foodborne disease as well as AMR, to guide policy-making and risk management at the national level by adopting a “One health” approach *(25)*.

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