PS6.06 Antimicrobial Resistance among Campylobacter spp. Strains isolated from Portuguese Poultry at Slaughterhouse Level 428.00

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Abstract— The aim of work was to evaluate the antimicrobial susceptibility of Campylobacter spp. strains isolated from poultry samples at a slaughterhouse in 2008. Considering flocks' traceability, sampling was performed for caecum, carcass (neck skin) and breast meat at a poultry slaughterhouse in different days of work and a collection of 78 strains isolated and identified as C. coli and C. jejuni were tested for susceptibility to 11 antimicrobial agents (disk diffusion method). C. coli was more frequently isolated from caecum samples and C. jejuni from breast meat samples. An extremely high frequency of fluoroquinolone resistance was detected among Campylobacter strains, particularly for C. coli (100% resistant to norfloxacin, ofloxacin, nalidixic acid and ciprofloxacin). More than 60% of the isolated strains showed resistance to tetracycline and ampicilin. In this study, C. coli strains showed a higher level of resistance to erytromicin (46.9%) than C. jejuni strains (20.7%). Almost all isolated strains were sensitive to amoxicillin+clavulanic acid. Chloramphenicol and gentamicin were active against both C. coli and C. jejuni.

Index Terms— Antimicrobial resistance, Campylobacter coli, Campylobacter jejuni, Fluoroquinolones.

I. INTRODUCTION

Campylobacter species, namely Campylobacter jejuni and Campylobacter coli, can colonize the intestinal tract of most mammals and birds and are the most frequently isolated species in humans with gastro-enteritis. They are recognized as the most common cause of sporadic human bacterial foodborne illness, becoming an important public health problem in several countries.1,3,5,6,11,13 Despite of that, Campylobacter spp. does not generally trigger the same degree of concern as some other foodborne pathogens, since it rarely causes death and is not commonly associated with newsworthy outbreaks of food poisoning.1,3,11,14 Campylobacteriosis is frequently associated with the ingestion of improperly handled foods that are inadequately cooked and it has a high level of association with poultry products. This disease poses additional health risks besides acute gastro-enteritis, including chronic illness such as polyarthropathies or neuropathies, (Guillain-Barré syndrome and reactive arthritis) and in these complications antimicrobial treatment is often required.1,3,11,14 Recent studies indicated that antimicrobial resistance among Campylobacter spp. is growing, however few data are available from Portugal. Studies performed on 1992 and 2008 were published but more work has to be done to characterize the Portuguese Campylobacter strains and its level of antimicrobial resistance.4,8,9 In this context, this work was conducted to evaluate the antimicrobial resistance of Campylobacter strains isolated from poultry at a Portuguese slaughterhouse.

II. MATERIALS AND METHODS

A. Bacterial strains Seventy eight strains of Campylobacter jejuni and C. coli were isolated from poultry samples collected at a slaughterhouse between December 2007 and October 2008. Considering flocks' traceability sampling was performed for caecum, carcass (neck skin) and breast meat at a poultry slaughterhouse in different days of work. Detection and isolation of Campylobacter was performed according to EN/ISO 10272-1:2006 and strains were identified by multiplex PCR. 16

B. Antimicrobial susceptibility testing Antimicrobial susceptibility testing was carried out by using the agar disk diffusion method with Mueller-Hinton agar supplemented with 5% sheep blood. The plates were incubated at 42ºC for 48h in a microaerobic atmosphere. The antibiotics tested included ampicilin (10µg), tetracycline (30µg), chloramphenicol (30µg), erythromycin (15µg), gentamicin (120 µg,) ciprofloxacin (5µg), norfloxacin (5µg), nalidixic acid (10µg), ofloxacin (5µg), amoxycillin+clavulanic acid (20+10µg) and trimethoprim-sulfamethoxazole (25µg). Staphylococcus aureus 25923 ATCC and Enterococcus faecalis 29212 ATCC were used as quality control. Susceptibility categorization was carried out according

to National Committee of Clinical Laboratory Standards (2002) and Societé Française de Microbiologie (2008).

III. RESULTS AND DISCUSSION

Table 1 shows the resistances of seventy eight strains of Campylobacter jejuni and C. coli to the antimicrobial agents evaluated. Of the 49 C. coli isolates, the highest level of resistance was recorded to norfloxacin, ofloxacin, nalidixic acid and ciprofloxacin (100% of the isolates resistante).

Table 1 Number and frequency of *Campylobacter*-resistant strains isolated from poultry to different antimicrobial agents.

Antimicrobial agent	*C. coli*		*C. jejuni*		Total	
	(n= 49)	%	(n= 29)	%	(n= 78)	%
Ampicilin	41	83.67	18	62.07	59	75.64
Trimethoprim-sulfamethoxazole	29	59.18	9	31.03	38	48.72
Norfloxacin	49	100.0	26	89.66	75	96.15
Chloramphenicol	0	0.00	0	0.0 0	0	0.00
Ofloxacin	49	100.0	27	93.10	76	97.44
Erythromycin	23	46.94	6	20.69	29	37.18
Amoxycillin+clavulanic acid	3	6.12	0	0.0 0	3	3.85
Tetracycline	43	87.76	18	62.07	61	78.21
Ciprofloxacin	49	100.0	26	89.66	75	96.15
Nalidixic acid	49	100.0	27	93.10	76	97.44
Gentamicin	0	0.00	0	0.0 0	0	0.00

The frequency of resistance to the other antibiotics studied was variable, 87.76% to tetracycline, 83.67% to ampicilin, 59.18% to trimethoprim-sulfamethoxazole, 46.94% to erythromycin and 6.12% to amoxicillin+clavulanic acid. Regarding the C. jejuni isolates (n=29), the highest level of resistance was recorded to nalidixic acid and ofloxacin (93.1%) and to norfloxacin and ciprofloxacin with 89.66%. The number of strains with resistance to the other antibiotics studied was also variable, 62.07% to both ampicilin and tetracycline, 31.03% to trimethoprim-sulfamethoxazole and 20.69% to erythromycin. Amoxicillin+clavulanic acid was active against C. jejuni. Chloramphenicol and gentamicin were active against both C. coli and C. jejuni. The picture presented by the data obtain in this work seems to be similar or higher than that reported by EFSA (2009), were 94% of isolates from poultry origin were resistant to ciprofloxacin.6 This pose a particular risk to humans due to treatment failure, for these reasons the control of Campylobacter in the food chain must become a major target of all Portuguese intervenients (producers, distributers and agencie responsible for food safety). C. coli (more frequently isolated in the broilers caecum and carcass) and C. jejuni strains (Table 2) were resistant to fluoroquinolones group, independently of site of sample collection on slaughterhouse line (higher than 94%).

Table 2 Distribution (%) of *Campylobacter*-resistant strains according to poultry sample type (caecum, neck skin or breast).

Antimicrobial agent	Breast meat		Carcass (neck skin)		Caecum		Total	
	(n= 39)	%	(n= 21)	%	(n= 18)	%	(n= 78)	%
Ampicilin	29	74.36	16	76.19	14	77.78	59	75.64
Trimethoprim-sulfamethoxazole	15	38.46	9	42.86	14	77.78	42	53.85
Norfloxacin	39	100.00	20	95.24	18	100.00	77	98.72
Chloramphenicol	0	0.00	0	0.00	0	0.00	0	0.00
Ofloxacin	37	94.87	20	95.24	18	100.00	75	96.15
Erythromycin	13	33.33	6	28.57	10	55.56	29	37.18
Amoxycillin+clavulanic acid	1	2.56	1	4.76	1	5.56	3	3.85
Tetracycline	28	71.79	17	80.95	16	88.89	61	78.21
Ciprofloxacin	37	94.87	20	95.24	18	100.00	75	96.15
Nalidixic acid	38	97.44	21	100.00	18	100.00	77	98.72
Gentamicin	**0**	**0.00**	**0**	**0.00**	**0**	**0.00**	**0**	**0.00**

The level of strains resistant to ampicilin was very high, 74.64% of the Campylobacter strains isolated from breast were resistant to ampicilin. Tetracyclines have been named in the past as an alternative treatment for campylobacteriosis and are used widely as feed additives for livestock and poultry.1,3,13,14 In this study, resistance to tetracycline was high, 87.76% to C. coli and 62.07% to C. jejuni.

IV. CONCLUSION

An extremely high frequency of fluoroquinolone resistance was detected among Campylobacter strains, particularly for C. coli strains (100% resistant to norfloxacin, ofloxacin, nalidixic acid and ciprofloxacin). More than 60% of the isolated strains showed resistance to tetracycline and ampicilin. In this study, C. coli strains showed a higher level of resistance to erytromicin (46.9%) than C. jejuni strains (20.7%). Almost all isolated strains were sensitive to amoxicillin+clavulanic acid. Chloramphenicol and gentamicin were active against both C. coli and C. jejuni. The high rates of Campylobacter strains resistance to antimicrobials isolated from samples at slaughterhouse level make advisable a well stated policy for the use of antibiotics and the accomplishment of all preventive rules of good hygiene practices at farm level (producers) as well the good implementation of HACCP regarding the hazard Campylobacter at slaughter house level.

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