Prevalence and Antibiotic Susceptibility of Thermophilic *Campylobacter jejuni* from Commercial Broiler Farms in and Around Lahore

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ABSTRACT

Campylobacter jejuni is one of the most important poultry commensal causing food-borne zoonosis in human. The present study was conducted to determine the prevalence and the antibiotic resistance profile of multiple drugs resistant (MDR) strains of thermophilic *C. jejuni* from broiler chickens. A total of 375 cloacal swab samples were collected by systematic sampling method of commercial broiler farms in and around Lahore and examined for the presence of thermophilic *C. jejuni*. Their prevalence was found to be 53% (200/375). Confirmation was done by biochemical and molecular (Polymerase chain reaction) testing methods. Phylogenetic tree analysis was also performed for genetic identification of the species. *in vitro* antibiotic disc diffusion method of those selected isolates was performed against 20 most common human therapeutic antibiotics. The isolates were found to be highly resistant against most of the available antibiotics leaving behind very less choice of selection for treatment. The results are as follows: ciprofloxacin (91%), clindamycin (87%), moxifloxacin (84%), telithromycin (82%), erythromycin (82%), clarithromycin (76%), nalidixic acid (76%), azithromycin (69%), ampicillin (62%), co-amoxiclav (56%), chloramphenicol (51%), tetracycline (50%), tygecycline (45%), norfloxacin (27%), gentamicin (25%), levofloxacin (24%), ofloxacin (20%), imipenum (4%), meropenum (2%) and ceftazidime (1%). Carbapenums (imipenum and meropenum) and cephalosporin (ceftazidime) was found to be highly effective against these MDR thermophilic *C. jejuni* isolates. Its efficacy for the treatment of campylobacteriosis should be further evaluated in clinical trials.

INTRODUCTION

Campylobacter jejuni is a most important food-borne bacteria causing enteric disease in human. It is principally considered a pathogen with raw or under cooked chicken meat being a primary source of infection in humans (Gutiérrez-Martín *et al*., 2011). It causes a zoonotic illness with symptoms involving watery diarrhea, discontent, abdominal distress and fever (Cantero *et al*., 2018). Young adults are mostly affected in developed countries while the disease is predominantly existing in children of developing countries (Omara *et al*., 2015). Although most cases of campylobacteriosis are self-limiting, a small proportion needs medical involvement and antibiotics are suggested in severe and prolonged cases of enteritis, sepsis or other severe and persistent aggressive infections particularly in immuno-compromised patients as well as in infants and pregnant women (Weerasooriya *et al*., 2022).

During the last decade, concern about this food-borne pathogen has increased mostly because of consistent isolation of antimicrobial resistant strains of thermophilic *Campylobacter* spp. in human and animals worldwide (Elhadidy *et al*., 2018; Oh *et al*., 2015). For decades, macrolides had been the first while fluoroquinolones had been the second choice of replacement to the remedial therapeutics of *C. jejuni* infections (Lehtopolku *et al*., 2010; Kayman *et al*., 2019). Resistance of *C. jejuni* to both of these antibiotic classes is increasing in numerous countries (Dias *et al*., 2022). The rise in macrolide resistance is of grave concern as macrolide-resistant strains are usually resistant to fluoroquinolones and other antibiotic groups

(Sierra-Arguello *et al.*, 2016; Aksomaitiene *et al.*, 2019). Rapid emergence of multiple drug resistant (MDR) thermophilic *Campylobacter* strains is getting problematic because of the extremely limited range of treatment options in that situation (Venardou *et al.*, 2021). It has further complicated the cure of this disease (Melero *et al.*, 2012). It had been reported earlier that more than 94 percent of *Campylobacter* isolated from tourists had resistance to two or more antibiotics that are regarded as MDR (Abay *et al.*, 2014). MDR has become a global challenge and use of antibiotics has become a threat to human health. Even in the absence of antibiotic exposure, resistant strains from other farm animals and the farm environment may enter a contamination cycle infecting animal hosts (Maćkiw *et al.*, 2012).

The aim of the present study was to examine the prevalence of thermophilic *C. jejuni* and to identify antibiotics that could be useful against those strains.

MATERIALS AND METHODS

Collection of samples

A total of 375 samples were taken from 25 systematically selected commercial broiler farms in and around the Lahore area. For this purpose, cloacal samples of chicken (n= 15 per broiler farm) were obtained from every 10th farm selected from the list of broiler farms, in and around Lahore that were registered in Pakistan Poultry Association (PPA). The samples were collected from 3 to 3.5 weeks old chicken from different pens in a farm by using sterile cultural swab pre-moistened in sterile water. Each individual swab was kept in the pre-sterile and pre-labeled tube containing Carry-Blair transport medium, and transported on ice-packs, followed by refrigeration at appropriate temperature. Enrichment of each individual sample tube was done by placing it on vortex for 5 min then adding 1ml of that vortex sample into 9 ml of Bolton broth. Incubation was done at 42℃ in 2.5L anaerobic jar where Campy-Gen gas generating sachet (CampyGen, Oxoid) was introduced to provide microaerophillic conditions (5% O_2, 10% CO_2 and 85% N_2) for 24 h.

Isolation and identification of Campylobacter jejuni

After 24 h, enriched broth (100µl) was streaked onto separate, modified Charcoal-Cefoperazone-Deoxycholate-Agar (mCCDA) petri plates, which were incubated at 42˚C for 48 h under microaerophillic conditions. Microscopy of the suspected colonies were examined and thermophilic *C. jejuni* was identified by the biochemical tests of hippurate hydrolysis, indoxyl acetate hydrolysis, oxidase and catalase tests (Holt *et al.*, 2013). Confirmation of the positive samples was done by molecular tests.

Molecular confirmation of the isolates

DNA extraction was done by commercial nucleic acid extraction kit (Vivantis, USA) as per their instructions. The quantification of DNA (ng/µl) was done using Nanodrop 2000 (Thermofisher Scientific). The purity of the extracted DNA was checked at 260/280 ratio. Conventional polymerase chain reaction was done by using organism specific PCR kit (Genetic PCR solutions™ -CamJej epPCR Test - Code1117045) according to manufacturer's instructions. It was resolved on 1.2% TAE agarose gel to visualize the PCR amplified products. Band size of specie specific primer according to PCR kit was 242 bp. The positive samples were preserved at -70˚C in 80% nutrient broth with added 20% (v/v) glycerol for future research.

Phylogenetic studies

The PCR products were sent for sequencing to a commercial supplier (Advance Bioscience International, Pakistan). The reported sequences were downloaded from GenBank® and compared with the isolated sequence. The phylogenetic maximum likelihood tree for the *C. jejuni* genome was generated by using the Molecular Evolutionary Genetic Analysis (MEGA) software version 10 (X) software.

Antibiotic susceptibility testing

The *C. jejuni* isolates were tested by Kirby- Bauer disc diffusion method. Since, there are no antimicrobial resistance breakpoints specific of *Campylobacter* currently available (Lehtopolku *et al.*, 2010). The resistance breakpoints of enteric bacteria in the family non-Enterobacteriaceae were used to determine antimicrobial resistance of *Campylobacter* spp. according to the guidelines of Clinical Laboratory Standards Institute (Patel et al., 2017). *C. jejuni* was grown on nutrient agar and then in nutrient broth, under microaerophillic conditions at 42℃ for 24 h, to prepare 0.5 McFarland standard of cell density, which was obtained through spectrophotometer. 100 µl of that bacterial solution was swabbed onto the Mueller-Hinton agar plate. Known antibiotic discs were placed on that agar plate at defined distances and incubated overnight at 42˚C maintaining microaerophillic conditions. Results were interpreted by measuring the zones of inhibited growth for antibiotics corresponding to CLSI breakpoints criteria. All disc diffusion results were determined in triplicate using *C. jejuni* ATCC 33291 as control during this trial.

The results were interpreted as resistant or sensitive. The inhibition zone readings defined as intermediate were also classified as resistant. The strain that showed resistance to 3 or more antimicrobial agents was considered as multidrug resistant (MDR).

The concentrations of antimicrobial agents tested along with the CLSI zone diameter breakpoints interpretation used in this project are elaborated in Supplementary Table I.

RESULTS

Prevalence of C. jejuni

Out of 375 samples, 200 were found positive for thermophilic *C. jejuni*. A 242 bp amplicon was indicative for the presence of *C. jejuni* according to the instructions provided by organism specific PCR kit (Genetic PCR solutions™ - CamJej epPCR Test-Code 1117045). Additional genomic confirmation was done by sequencing the gene commercially (Advance Bioscience International, Pakistan). After sequencing of the gene, it was blasted on (BLAST) NCBI for confirmation that resulted as (*ompA*) gene, which produces the outer- membrane porin protein.

Phylogenetic analysis

The results of phylogenetic analysis showed that the local isolate of *C. jejuni* have more similarity with reported sequences of USA and UK (Fig. 1). Our isolated sequence has a little likelihood with three sequences (FDAARGOS422, NCTC13265 and FDAARGOS 263) but maintained its uniqueness at root 99.

Antibiotic resistance in C. jejuni

The isolates showed resistance against most of the antibiotics except few. Of all 200 *C. jejuni* isolates, 182 (90.90%) were highly resistant against ciprofloxacin, while 164 (81.81%) were resistant against erythromycin and also resistant against azithromycin 138 (69.09%), clarithromycin 153 (76.36%), telithromycin 165 (82.35%), clindamycin 174 (87.03%), nalidixic acid 153 (76.36%), moxifloxacin 167 (83.63%), ampicillin 124 (61.81%), co-amoxiclav 111 (55.55%), chloramphenicol 102 (50.90%), tetracycline 100 (50%) and tygecycline 91 (45.45%). Resistance against norfloxacin 55 (27.27%), ofloxacin 40 (20%), levofloxacin 47 (23.63%) and gentamicin 51 (25.45%) was less that 30%. Three antibiotics which are imipenum 7 (3.63%), meropenum 4 (1.81%) and ceftazidime 2 (1.06%) showed less than 5% resistance. The details of antibiotic resistance is shown (Fig. 2).

DISCUSSION

Food borne infections that are caused by *C. jejuni,* is one of the most frequently reported infections, that occurs all over the world (Kim *et al.*, 2018). The colonization of chicken gut is usually undetectable before the age of 7 days. It occurs between the second and fourth week of rearing.

It can reach the highest level at slaughter age (Rahimi *et al.*, 2013). The epidemiology of *Campylobacter* in broiler production is still unclear and the linear relationship occurs between the broiler flock prevalence and probability of human infection. Thus, reducing the *Campylobacter* at farm can contribute in reduction of human infection.
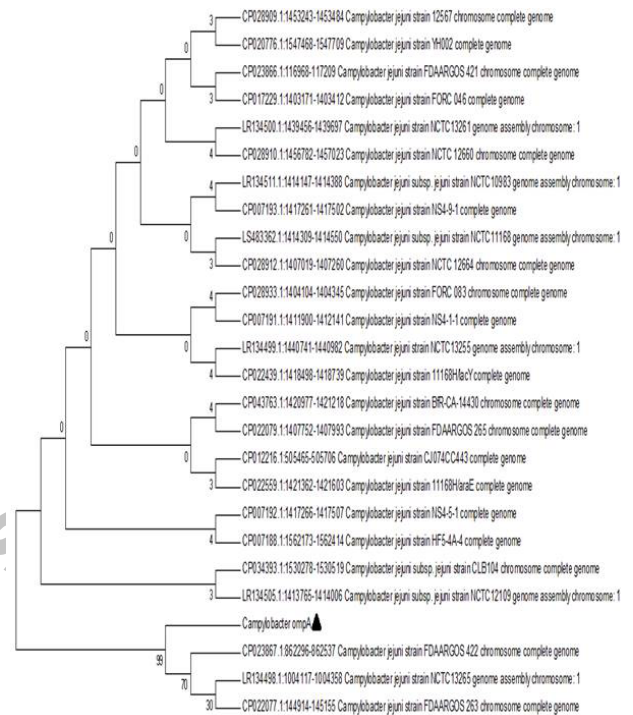

Fig. 1. *Compylobacter jejuni* phylogenetic analysis. The phylogenetic maximum likelihood tree for *Compylobacter jejuni* was generated by using the MEGA X software. Our isolated sequence showed with indication of triangle while other sequences were derived from GenBank®.
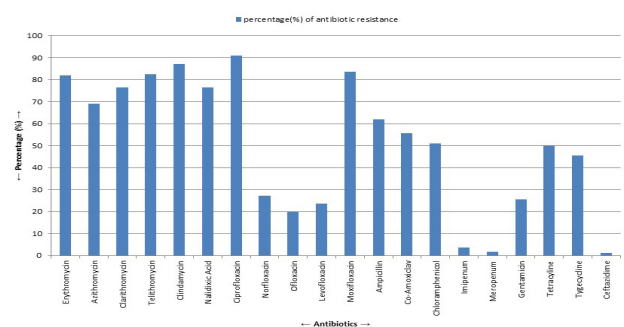

Fig. 2. Graphical presentation of antibiotic resistance (percentage) of *C. jejuni* against 20 antibiotics.

Multiple drug resistant strains of microbes are a major problem of the era (Elhadidy *et al.*, 2018) and in the

present study, the isolates of thermophilic *C. jejuni* were found to be uniformly resistant against multiple groups of antibiotics. There is an increase in the percentage of *Campylobacter* strains that are resistant to antibiotics and it is getting more difficult to control the management of infections caused by those antibiotic resistant strains in clinics (Maćkiw *et al*., 2012; Hameed *et al*., 2020). This study also showed antibiotic resistant *C. jejuni* are common among broilers that can easily find their way into our food chain. We have found MDR thermophilic *C. jejuni* to be highly prevalent during our study which is 53% (n=200) out of the total samples (375).

The percentage of thermophilic *C. jejuni* isolated in this study was found to be 53% (n=200) out of the total samples (375). Although the Minimum Inhibitory Concentration (MIC) method is preferred, World Health Organization (WHO) has suggested use of disc diffusion in limited resource conditions. Numerous authors had used disc diffusion method to study antibiotic resistance among *Campylobacter* isolated from poultry (Maćkiw *et al*., 2012; Ma *et al*., 2014; Nobile *et al*., 2013).

Both macrolides and floroquinones are drugs of choice for human in systemic infections or in severe and long lasting cases of enteritis (Rahimi *et al*., 2013). We found the resistance to macrolide to be highly frequent as 81.81 percent of 200 *C. jejuni* samples was resistant against erythromycin, also the resistance was found to be 69.09 percent against azithromycin, 76.36 percent against clarithromycin and 82.35 percent against telithromycin. This frequent resistance was also reported by (Pergola *et al*., 2017; Elhadidy *et al*., 2018). Among the fluororquinolone compounds, norfloxacin was 27.27% resistant; ofloxacin was 20% and levofloxacin was 23.63%. Ciprofloxacin was found to be 90.90% resistant, moxifloxacin was 83.63% and nalidixic acid was found to be 76.36% resistant. Frequent resistance to fluoroquinolones may be most likely due to its wide use in poultry industry particularly in feed and as biological fitter in chicken (Abay *et al*., 2014).

Moreover, the samples were also showing resistance against lincosamides (clindamycin) to be 87.03 percent. The level of resistance we found to penicillin (ampicillin 61.81%) and penicillin combination (co-amoxiclav/ augmentin 55.55%) for *C. jejuni* was more than 50 percent, while in chloramphenicol (50.90%), tetracycline (50%) and tygecycline (45.45%) were found to be around 50 percent. Mostly the strains were sensitive to carbapenems (imipenem 3.63% and meropenum 1.8%) and cephalosporin (cetazidime 1.06%). It may be because of their less use in the poultry industry. Successful control with carbapenems have been reported earlier (Kim *et al*., 2017; Geng *et al*., 2019; Roberts *et al*., 2020; Morita *et al*., 2022). It is of great note that cephalosporin (ceftazidime) was highly active against all isolates.

Among the older antibiotics, gentamicin (25.45%) was effective, but its usage is restricted in case of pregnancy. Still, gentamicin may be effective against septicemia and other general infections in combination with carbapenums or cephalosporin. The *in vitro* antibiotic resistance activity of tetracycline was found to be 50% while tigecycline resulted as 45.45%. Tetracycline can survive longer in an environment than do other antimicrobials consequently could cause bacterial resistant against it. Tigecycline is known for its best activity particularly for the treatment of problematic skin and soft tissue infections (Lawton *et al*., 2018), which is well known to be one of the manifestation of extra intestinal campylobacteriosis (Kim *et al*., 2017; Roberts *et al*., 2020). Tigecycline flows largely as unchanged drug and its major course of elimination is through the feces, likely via biliary defecation route (Lehtopolku *et al*., 2010; Quinteros *et al*., 2021). Based on this, it sounds sensible to assume that tigecycline might be effective even for patients with gastroenteritis if given in combination with carbapenums or cephalosporin.

With frequent occurrence of resistant *Campylobacter* against different antibiotics will ultimately lead to limited choice of drugs for treatment. Thus, antibiotic susceptibility test is required before any treatment that is to be initiated in *Campylobacter*-infected patients. The efficacy of carbapenums and ceftazidime for the treatment of human campylobacteriosis should also be evaluated in clinical trials.

CONCLUSION

In conclusion, we had found high contamination in commercial broiler farms by thermophilic *C. jejuni* and high resistance to antimicrobial groups. The study shows the need to establish an efficient system for cautious use of antibiotics in poultry production as resistance to most of the antibiotics is increasing. We have found the antibiotic disc diffusion method to be trustworthy and prompt, to check the resistance of any antibiotic that has been selected for the patient suffering from *Campylobacter* infection, before any treatment. The results also show the need to develop alternate antimicrobial methods to reduce the growth and spread of MDR thermophilic *C. jejuni* under careful guidance.

ACKNOWLEDGEMENT

IRB approval

The study was approved by Advanced Studies and Research Board in its meeting held on 30-09-2016 (Letter number DAS/9023).

Ethical statement

All the proceedures undertaken were in compliance with Institutional Guidelines for the Care and Use ofvEexperimental Animals of University of Veterinary and Animal Sciences, Lahore (DR/246, dated: 3-5-2016).

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20221017111032

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abay, S., Kayman, T., Otlu, B., Hizlisoy, H., Aydin, F., and Ertas, N., 2014. Genetic diversity and antibiotic resistance profiles of *Campylobacter jejuni* isolates from poultry and humans in Turkey. *Int. J. Fd. Microbiol*., **178**: 29-38. https://doi.org/10.1016/j.ijfoodmicro.2014.03.003

Aksomaitiene, J., Ramonaite, S., Tamuleviciene, E., Novoslavskij, A., Alter, T., and Malakauskas, M., 2019. Overlap of antibiotic resistant *Campylobacter jejuni* MLST genotypes isolated from humans, broiler products, dairy cattle and wild birds in Lithuania. *Front. Microbiol*., **10**: 1377. https://doi.org/10.3389/fmicb.2019.01377

Cantero, G., Correa-Fiz, F., Ronco, T., Strube, M., Cerdà-Cuéllar, M., and Pedersen, K., 2018. Characterization of *Campylobacter jejuni* and *Campylobacter coli* broiler isolates by whole-genome sequencing. *Foodborne Pathog. Dis*., **15**: 145-152. https://doi.org/10.1089/fpd.2017.2325

Dias, T.S., Costa, G.A., de Almeida, F.A., Dos Santos, M.L., da Cunha, N.C., do Nascimento, E.R., de Almeida P.V.L. and de Aquino, M.H.C., 2022. Molecular markers associated with antimicrobial resistance and genotypes of *Campylobacter jejuni*, and *Campylobacter coli* isolated from broiler and swine flocks in southeast Brazil. *Comp. Immunol. Microbiol. Infect. Dis*., **88**: 101866. https://doi.org/10.1016/j.cimid.2022.101866

Elhadidy, M., Miller, W.G., Arguello, H., Álvarez-Ordóñez, A., Duarte, A., Dierick, K., and Botteldoorn, N., 2018. Genetic basis and clonal population structure of antibiotic resistance in *Campylobacter jejuni* isolated from broiler carcasses in Belgium. *Front. Microbiol*., **9**: 1014. https://doi.org/10.3389/fmicb.2018.01014

Geng, Y., Liu, G., Liu, L., Deng, Q., Zhao, L., Xia-Sun, X., Wang, J., Zhao, B., and Wang, J., 2019. Real-time recombinase polymerase amplification assay for the rapid and sensitive detection of *Campylobacter jejuni* in food samples. *J. Microbiol. Methods*, **157**: 31-36. https://doi.org/10.1016/j.mimet.2018.12.017

Gutiérrez-Martín, C.B., Yubero, S., Martìnez, S., Frandoloso, R., and Rodrìguez-Ferri, E.F., 2011. Evaluation of efficacy of several disinfectants against *Campylobacter jejuni* strains by a suspension test. *Res. Vet. Sci*., **91**: e44-e47. https://doi.org/10.1016/j.rvsc.2011.01.020

Hameed, A., Woodacre, A., Machado, L.R., and Marsden, G.L., 2020. An updated classification system and review of the lipooligosaccharide biosynthesis gene locus in *Campylobacter jejuni*. *Front. Microbiol*., **11**: 677. https://doi.org/10.3389/fmicb.2020.00677

Kayman, T., Abay, S., Aydin, F., and Şahin, O., 2019. Antibiotic resistance of *Campylobacter jejuni* isolates recovered from humans with diarrhoea in Turkey. *J. med. Microbiol*., **68**: 136-142. https://doi.org/10.1099/jmm.0.000890

Kim, Y.J., Kim, H.S., Chon, J.W., Kim, D.H., Hyeon, J.Y., and Seo, K.H., 2018. New colorimetric aptasensor for rapid on-site detection of *Campylobacter jejuni* and *Campylobacter coli* in chicken carcass samples. *Anal. Chim. Acta*, **1029**: 78-85. https://doi.org/10.1016/j.aca.2018.04.059

Kim, Y., Shin, J.A., Han, S.B., Cho, B., Jeong, D.C., and Kang, J.H., 2017. Recurrent *Campylobacter jejuni* bacteremia in a patient with hypogammaglobulinemia. *Clin. Med. Case Rep*., **96**. https://doi.org/10.1097/MD.0000000000007238

Lawton, S.J., Weis, A.M., Byrne, B.A., Fritz, H., Taff, C.C., Townsend, A.K., Weimer, B.C., Mete, A., Wheeler, S., and Boyce, W.M., 2018. Comparative analysis of *Campylobacter* isolates from wild birds and chickens using MALDI-TOF

MS, biochemical testing, and DNA sequencing. *J. Vet. Diagn. Invest*., **30**: 354-361. https://doi.org/10.1177/1040638718762562

Lehtopolku, M., Nakari, U.M., Kotilainen, P., Huovinen, P., Siitonen, A., and Hakanen, A.J., 2010. Antimicrobial susceptibilities of multidrug-resistant *Campylobacter jejuni* and *C. coli* strains: *In vitro* activities of 20 antimicrobial agents. *Antimicrob. Agent. Chemother*., **54**: 1232-1236. https://doi.org/10.1128/AAC.00898-09

Ma, L., Wang, Y., Shen, J., Zhang, Q., and Wu, C., 2014. Tracking *Campylobacter* contamination along a broiler chicken production chain from the farm level to retail in China. *Int. J. Fd. Microbiol*., **181**: 77-84. https://doi.org/10.1016/j.ijfoodmicro.2014.04.023

Maćkiw, E., Korsak, D., Rzewuska, K., Tomczuk, K., and Rożynek, E., 2012. Antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli* isolated from food in Poland. *Fd. Contr*., **23**: 297-301. https://doi.org/10.1016/j.foodcont.2011.08.022

Melero, B., Juntunen, P., Hänninen, M.L., Jaime, I. and Rovira, J., 2012. Tracing *Campylobacter jejuni* strains along the poultry meat production chain from farm to retail by pulsed-field gel electrophoresis, and the antimicrobial resistance of isolates. *Fd. Microbiol*., **32**: 124-128. https://doi.org/10.1016/j.fm.2012.04.020

Morita, D., Arai, H., Isobe, J., Maenishi, E., Kumagai, T., Maruyama, F., and Kuroda, T., 2022. Diversity and characteristics of pTet family plasmids revealed by genomic epidemiology of *Campylobacter jejuni* from human patients in Toyama, Japan from 2015 to 2019. *bioRxiv*. https://doi.org/10.1101/2022.06.28.498051

Nobile, C.G., Costantino, R., Bianco, A., Pileggi, C., and Pavia, M., 2013. Prevalence and pattern of antibiotic resistance of *Campylobacter* spp. in poultry meat in Southern Italy. *Fd. Contr*., **32**: 715-718. https://doi.org/10.1016/j.foodcont.2013.02.011

Oh, E., McMullen, L., and Jeon, B., 2015. High prevalence of hyper-aerotolerant *Campylobacter jejuni* in retail poultry with potential implication in human infection. *Front. Microbiol*., **6**: 1263. https://doi.org/10.3389/fmicb.2015.01263

Omara, S.T., El-Fadaly, H.A. and Barakat, A.M.A., 2015. Public health hazard of zoonotic *Campylobacter jejuni* reference to Egyptian regional and seasonal variations. *Res. J. Microbiol*., **10**: 343. https://doi.org/10.3923/jm.2015.343.354

Patel, J.B., Cockerill, F.R., Bradford, P., Eliopoulos, G.M., Hindler, J.A., Jenkins, S.G., Lewis, J.S.,

Miller, L.A., Nikolau, D.P., Powel,l M. and Swenson, J.M., 2017. *Performance standards for antimicrobial susceptibility testing.* Clinical And Laboratory Standards Institute. pp. 42-45.

Pergola, S., Franciosini, M.P., Comitini, F., Ciani, M., De Luca, S., Bellucci, S., Menchetti, L., and Proietti, P.C., 2017. Genetic diversity and antimicrobial resistance profiles of *Campylobacter coli* and *Campylobacter jejuni* isolated from broiler chicken in farms and at time of slaughter in central Italy. *J. appl. Microbiol*., **122**: 1348-1356. https://doi.org/10.1111/jam.13419

Quinteros, J.A., Scott, P.C., Wilson, T.B., Anwar, A.M., Scott, T., Muralidharan, C., Van, T.T.H., and Moore, R.J., 2021. Isoquinoline alkaloids induce partial protection of laying hens from the impact of *Campylobacter hepaticus* (spotty liver disease) challenge. *Poult. Sci*., **100**: 101423. https://doi.org/10.1016/j.psj.2021.101423

Rahimi, E., Ameri, M., Alimoradi, M., Chakeri, A., and Bahrami, A.R., 2013. Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from raw camel, beef, and water buffalo meat in Iran. *Comp. clin. Pathol*., **22**: 467-473. https://doi.org/10.1007/s00580-012-1434-5

Roberts, S.C., Stone, S.M., Sutton, S.H., and Flaherty, J.P., 2020. Cutaneous manifestations of *Campylobacter jejuni* infection: A case report and review of the literature. *Infect. Dis. Clin. Pract. (Baltim. Md.)*. **28**: 61. https://doi.org/10.1097/IPC.0000000000000827

Sierra-Arguello, Y.M., Perdoncini, G., Morgan, R.B., Salle, C.T.P., Moraes, H.L.S., Gomes, M.J.P. and do Nascimento, V.P., 2016. Fluoroquinolone and macrolide resistance in *Campylobacter jejuni* isolated from broiler slaughterhouses in southern Brazil. *Avian Pathol*., **45**: 66-72. https://doi.org/10.1080/03079457.2015.1120272

Venardou, B., O'Doherty, J.V., Vigors, S., O'Shea, C.J., Burton, E.J., Ryan, M.T., and Sweeney, T., 2021. Effects of dietary supplementation with a laminarin-rich extract on the growth performance and gastrointestinal health in broilers. *Poult. Sci*., **100**: 101179. https://doi.org/10.1016/j.psj.2021.101179

Weerasooriya, G., Khan, S., Chousalkar, K.K., and McWhorter, A.R., 2022. Invasive potential of sub-lethally injured *Campylobacter jejuni* and *Salmonella typhimurium* during storage in chicken meat juice. *Fd. Contr*., **135**: 108823. https://doi.org/10.1016/j.foodcont.2022.108823

Supplementary Material

Prevalence and Antibiotic Susceptibility of Thermophilic *Campylobacter jejuni* from Commercial Broiler Farms in and Around Lahore

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Supplementary Table I. Standards for zone diameter breakpoints (nearest whole mm) of selected anitimicrobials according to resistance breakpoints of enteric bacteria in the family *non-enterobacteriaceae* of clinical laboratory standards institute (CLSI, 2017).

S.	Antimicrobial agent	Code	Company name	Disc content	Sensitive (S)	Intermediate (I)	Resistant (R)
1	Erythromycin	E	Liofilchem s.r.l Roseto (T.E) Italy	15mcg	> 23	14 – 22	< 13
2	Azithromycin	AZM	Bioanalyse	15mcg	> 13	12	< 11
3	Clarithromycin	CLR	Bioanalyse	15mcg	> 18	14 - 17	< 13
4	Telithromycin	TEL	Oxoid	15mcg	> 22	17 - 21	< 18
5	Clindamycin	DA	Bioanalyse	10mcg	> 21	13 - 20	< 14
6	Nalidixic Acid	NA	Bioanalyse	30mcg	> 19	14 - 18	< 13
7	Ciprofloxacin	CIP	Bioanalyse	5mcg	> 21	18 - 20	< 17
8	Norfloxacin	NOR	Bioanalyse	10mcg	> 17	13 - 16	< 12
9	Ofloxacin	OFX	Liofilchem s.r.l Roseto (T.E) Italy	5mcg	> 16	13 - 15	< 12
10	Levofloxacin	LEV	Bioanalyse	5mcg	> 17	14 - 16	< 13
11	Moxifloxacin	MXF	Bioanalyse	5mcg	> 24	21 - 23	< 20
12	Ampicillin	AMP	Liofilchem s.r.l Roseto (T.E) Italy	10mcg	> 17	14 - 16	< 13
13	Co-Amoxiclav	AMC	Bioanalyse	30mcg	> 18	14 – 17	< 13
14	Chloramphenicol	C	Bioanalyse	30mcg	> 18	13 – 17	< 12
15	Imipenum	IPM	Bioanalyse	10mcg	> 22	19 – 21	< 18
16	Meropenum	MEM	Bioanalyse	10mcg	> 18	15 – 17	< 14
17	Gentamicin	CN	Bioanalyse	10mcg	> 15	13 – 14	< 12
18	Tetracyline	TE	Bioanalyse®ASD	30mcg	> 15	12 – 14	< 11
19	Tygecycline	TGC	Oxoid	15mcg	> 18	16 – 17	< 15
20	Ceftazidime	CAZ	Oxoid	30mcg	> 21	18 – 20	< 17