AUTHOR: MARGARET ORDONEZ SMITH PhD

TITLE: *CAMPYLOBACTER*

TAXONOMY: *Campylobacter* is in the family of *Campylobacteraceae.* In 1963 E. J M. Sebald and M. Veron discovered it and was classified as a *Vibrio.* There are 24 species that affect humans and animals (73,75,103,117)

MORPHOLOGY: It is a bacteria Gram negative, curved, S shaped or spiral that can measure 0.2 to 0.9 *u*m wide and 0.5 to 5 *u*m long. It is a non-spore bacterium and in old cultures can have a coccus morphology. Found all over the world. (16, 67,70).

IDENTIFICATION: It can be a catalase positive, oxidase positive, motile, sensitive to nalidixic acid, usually they grow in microaerophilic atmosphere, but it can also grow on aerobic or anaerobic (122). A Gram stain can show in 100X the morphology of a *Campylobacter* bacteria (direct examination) (51). Figure No. 1.

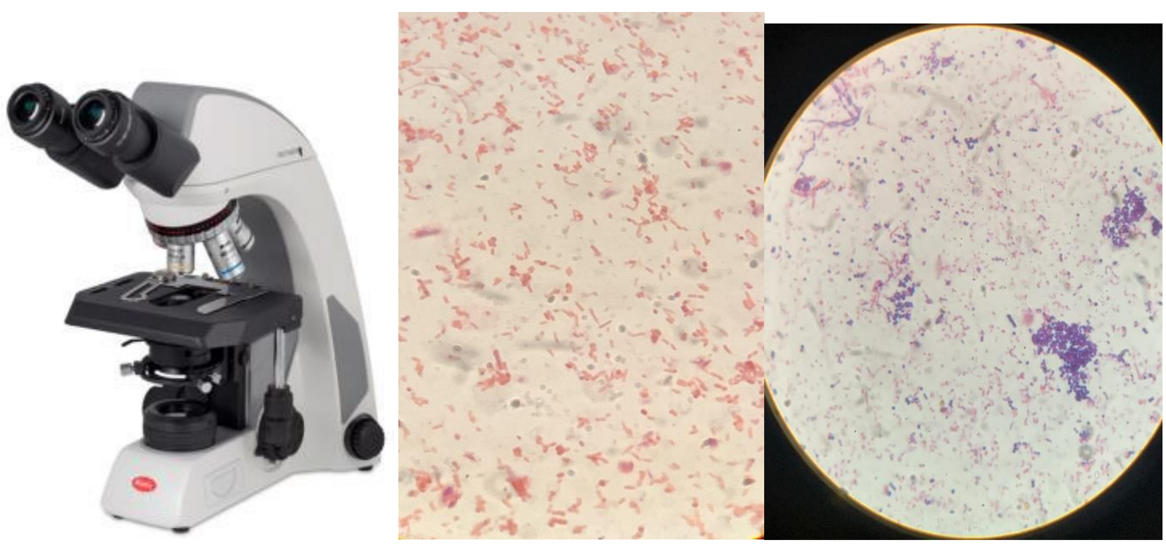

Figure No. 1 Microscope to see a Gram stain of feces.

CULTURES: Samples should be smeared onto the media before 1 or the most 2 hours after collection of the stool for a *Campylobacter's* isolation (6,93,94). Emulsifying feces in sterile saline solution has been recommended. It is necessary to use chocolate agar, an enrichment broth, or a *Campylobacter* selective media and must be incubated in a jar with microaerophilic (5% oxygen, 10% CO_2, and 85% nitrogen) atmosphere or gas generating kit for 24 to 48 hours at a temperature of 37°C. In case there is none of these items, the Petri dishes can be put in with a lit candle inside a box, this, may be enough to reduce the oxygen. Feces or the samples used to isolate the bacterium must be inoculated directly on to the selective media so that single isolated colonies are formed (48). A Gram stain of these colonies should be made in order, to confirmed the *Campylobacter´s* morphology. (Figure No. 2). There are many selective media: Skirrow (124), Butzler (17), Butzler and Dekeyser (19) Blaser-Wang (14), Karmali or Brillance Campy count (55) and the chromoagar (27,67).

Figure No. 2. A jar with a gas generating kit for microaerophilic atmosphere and the cultures with *Campylobacter* colonies in selective media chromoagar and Karmali (27,55,67)

OTHER METHODS: There are some expensive technics to identify *Campylobacter* but not all the laboratories can afford to do them: PCR (Polymerase Chain Reaction) Cohen 2023 (29), Heydari 2024 (50) Schiaffino 2024 (116) with Novel rpsK/rpsD primer-probe assay; it was found a 58.7%, Maldi Tof (Matrix-Assisted Laser Desorption/Ionization) and stool antigen tests: ProSpecT, Premier Campy, Xpect Campy, and Immuno Card Start! Campy (31,53,67). In 2023 Rousou et al described the Seeplex/Allplex, Amplidiag/Novodiag, BDMax and multiplex nucleic acid tests showed excellent accuracy to diagnose a *Campylobacter* infection.

SAMPLES: Fecal or feces samples or rectal swabs are acceptable for its isolation, skin (85), oral (99,126), saliva (4,72) periodontal (83,99,101), subgingival (64,107), synovial liquid (123) and blood samples can be tested (15, 54). A blood test is rarely done to diagnose *Campylobacter* diarrheal illness (129). In Germany Dammermann, studied in blood samples with an ELISA test. Since, this bacterium has been in foodborne outbreaks, it can be isolated from: water (3,35,80,100,131) chicken (30,63,34,68,79,106), milk (1,45,80,96,111), meat (5) vegetables (100), animal farms (130,132) and pets (81).

The preanalytical phase is very important in any procedure, in order, to have an accurate result. Every patient should have instructions how to take the sample and how to transport it to the laboratory. Time and temperature are very important: the ideal is to take it to the laboratory maximum after an hour of recollection. The sample must be kept at corporal temperature in a “kangaroo” style, never put it in the freeze (93,94).

CLINICAL SIGNIFICANCE: Since 1970 *Campylobacter jejuni* has been the most common causative agents of bacterial gastroenteritis in the world and an emerging zoonotic disease (9,34,40,41,43,131). Other symptoms are: fever, abdominal cramping, diarrhea (with or without blood and leucocytes) that lasts for several days to more than a week (2). Also, it can mimic acute appendicitis and extraintestinal do occur, such as bacteremia (95,129), reactive arthritis (ReA) (33, 46, 123), bursitis, urinary tract infection (39), meningitis, endocarditis, peritonitis, erythema nodosum, pancreatitis, abortion, neonatal sepsis (17), psoriasis and psoriatic arthritis (85). Deaths can occur rarely. Guillen-Barré syndrome (GBS) is one of the sequelae of infection, affects the peripheral nervous system, causing muscle weakness, temporary paralysis, and in severe cases, respiratory and cardiovascular effects (128). It can produce the Miller-Fisher syndrome: A variant of Guillain-Barré syndrome, characterized by the classic triad of symptoms: ataxia, ophthalmoplegia and areflexia (69,103,134). Also, it has been associated with cross-reactivity with autoimmune diseases: It has been observed that *Campylobacter jejuni* infections can trigger an autoimmune response in which the immune system attacks the body’s cells by mistake, which can lead to various autoimmune diseases, such as acute polyradiculoneuritis (82,76,127). In Sweden 2020 Melenotte (78) published an associated with non-Hodgkin's lymphoma (NHL), the most common haematologic malignance, in Japan with moyamoya disease (126). In Italy Pietropaoli (99) found an association between systemic exposure to periodontal microbiota and high blood pressure. In Canada patients untreated, periodontitis leads to destruction of the teeth-supporting tissues, bone resorption and ultimately loss of teeth (64).

TRANSMISSION: Campylobacteriosis is a zoonotic disease usually presents an acute gastroenteritis disease (43,49). The majority is infected by contaminated water (100) or food (12,47) as milk, chicken (68,70,79,106,122,131), meat (11), vegetables (75,100); less frequently with an infected animal or person (23,24). In United States waterborne and foodborne illnesses are caused by *Campylobacter* in 9% (60, 80). White (132) showed that people working with animal exposure a 13% had infections of *Campylobacter, Cryptosporidium*, Shiga toxin-producing *E coli*, and non-typhoidal *Salmonella*; and 14% in an industry with regular animal exposure. In United States Smith suspects that wild birds are sources for human infection, there is a prevalence of 27% with *Campylobacter spp.* The European Food Safety Authority (EFSA) estimates that birds as reservoirs of *Campylobacter* are responsible for 50 to 80% of cases, while handling and preparation may be responsible for 20 to 30% of cases (125). In United States MacCarthy (77) and in Denmark Kuhn 2021 (61) showed enteric infections in men who have sex with men had transmission of *Campylobacter, Giardia lamblia, Shigella.* In Japan Kumagai (63) with chicken products 80.3%. In South Africa Chibwe 2023 (25) studied the Bloukrans and Swartkops rivers and found *Campylobacer*. In many communities in the world, rivers play an important role in the social and cultural lives of people, and so it is important to monitor the quality of river water. Grace (45) and Ordonez Smith (87,88,89,91,92) had an incidence of 4%; Khan (55) and Abd El Ghany (1) had a 13%; and Fonseca (38) 63.8% in milk. Figure 3 and 4. In Colombia with fresh chickens in the Pontificia Universidad Javeriana of Bogotá had 54.8% (42 of 91 samples). The samples were detected by PCR and cultures (29,48).

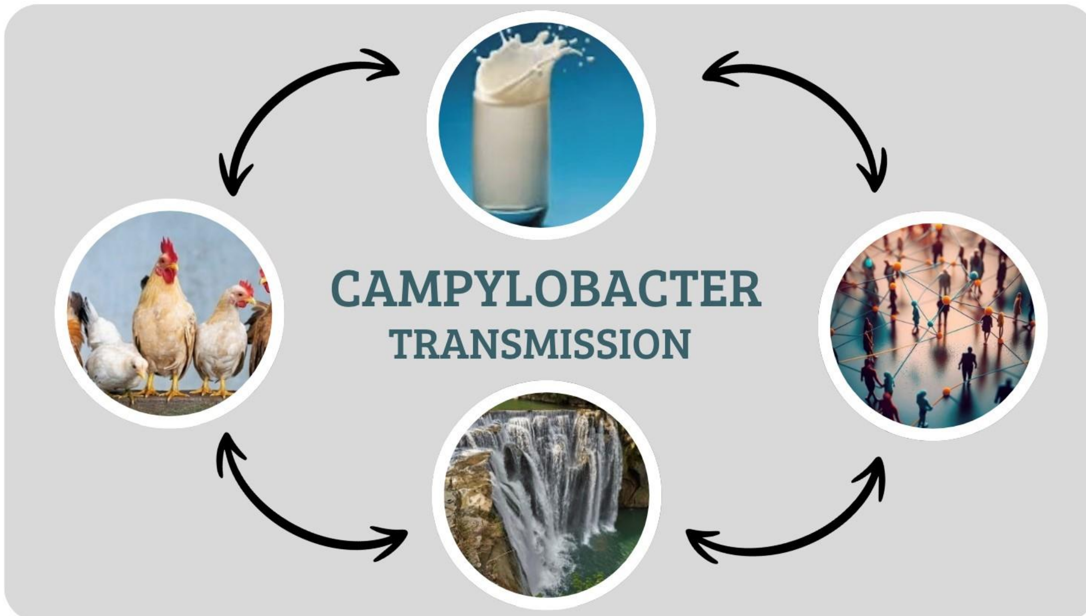

Figure No. 3 *Campylobacter*: Transmission with chicken, water, persons or milk.

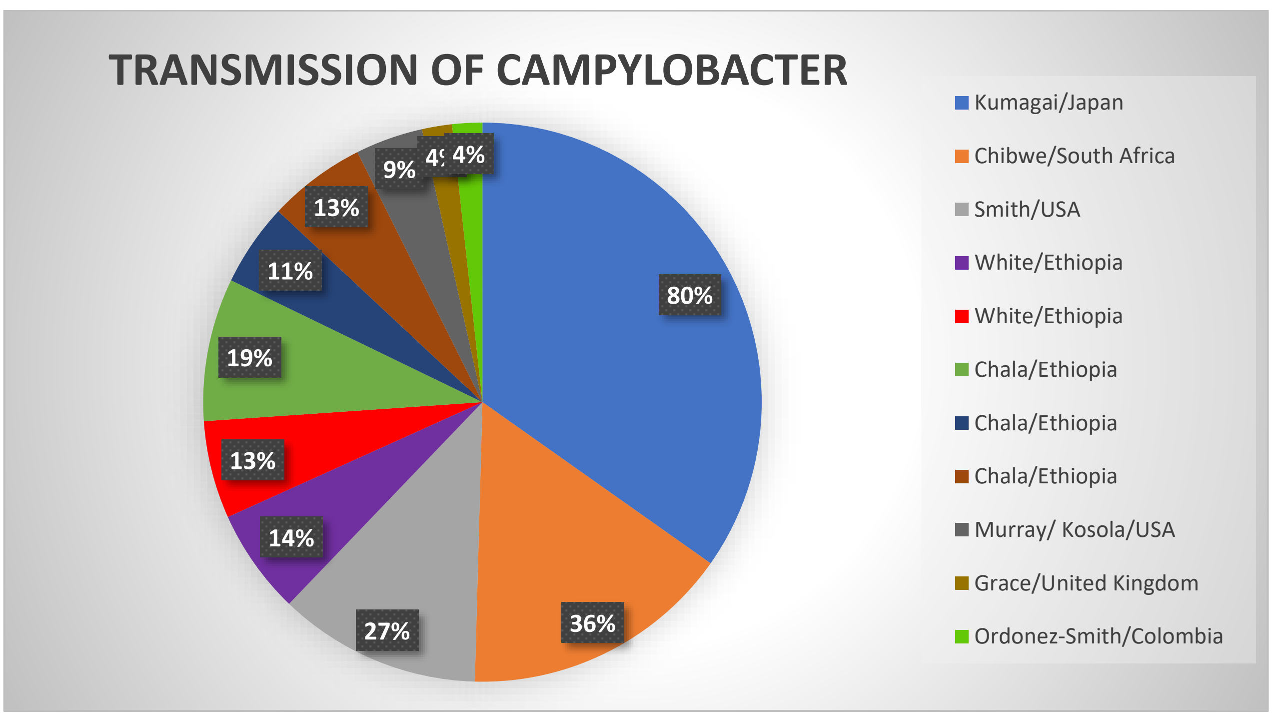

Figure No. 4 Authors and countries/percentages of the transmission with Campylobacteriosis in humans

***CAMPYLOBACTER* INCIDENCE**: In England and Wales between 1990 and 1999 was examined the *Campylobacter* incidence in conjunction with weather conditions. Over the 10-year interval, the average annual rate was determined to be 78.4 ± 15.0 cases per 100.000, ranging from 29.633 to 52.840 reported cases per year with an average of 40.127 cases per year, with an upward trend (66,69,70). Multiple regression analysis showed that daily average temperature, precipitation, and sunshine were significantly associated with *Campylobacter* rates. In the United States, England and Wales (1999) an estimated of 2.1-2.4 million cases of human Campylobacteriosis cases were reported, according to the National Infectious Disease Registry (National Public Health Institute) and a reduction after COVID-19 pandemic from April 19-33% to1-7% in August (86). In 2013 *Campylobacter* has been reported in Europe with 214.779 positive cases, that means 64.8 for every 100.000 persons and in Denmark Shioda (121) the costs per year 124 million of euros. The most common origin of contamination is with chicken or undercooked poultry, untreated water, animal contact, environmental factors, and contaminated milk. (31,53). Pires (100) in Denmark showed a 60% of gastrointestinal infections. Mozambique 52.2% (121), 14% in Colombia, gastroenteritis cases in 1984 (87,88,89,90,91,92). Chala (24) in Ethiopia reported of 519 samples a total of prevalence in *Campylobacter*: 42.4%, humans (10.1%), cattle 18.5% (77) and in Saudi Arabia a 3% (6), Australia 10% (26). In India Ghosh (42) had an incidence of 8.4% and Khan (56) a 13%. Results showed that *Campylobacter* leads the ranking of foodborne pathogens in almost all indicators explored. In 2019, *Campylobacter* caused nearly 59,000 cases, 41 premature deaths, 60% gastrointestinal infections (120).

In Colombia *Campylobacter* in children under 5 years of age, represents one of the most important etiologic agents of acute diarrheal, ranking the first place in morbidity and mortality. In Tunja a 2.3% (73), and the National Institute of Health between 2007 and 2011 there was an incidence of 3.3% and 6.8% (21). Postinfectious irritable bowel syndrome (PI-IBS) is an important sequela of *Campylobacter* infection (114); in Colombia, last year Ordonez-Smith (94) had an incidence of 64.4% (136 of 211 cases of irritable bowel syndrome). Parfenov (97) in Russia colitis.

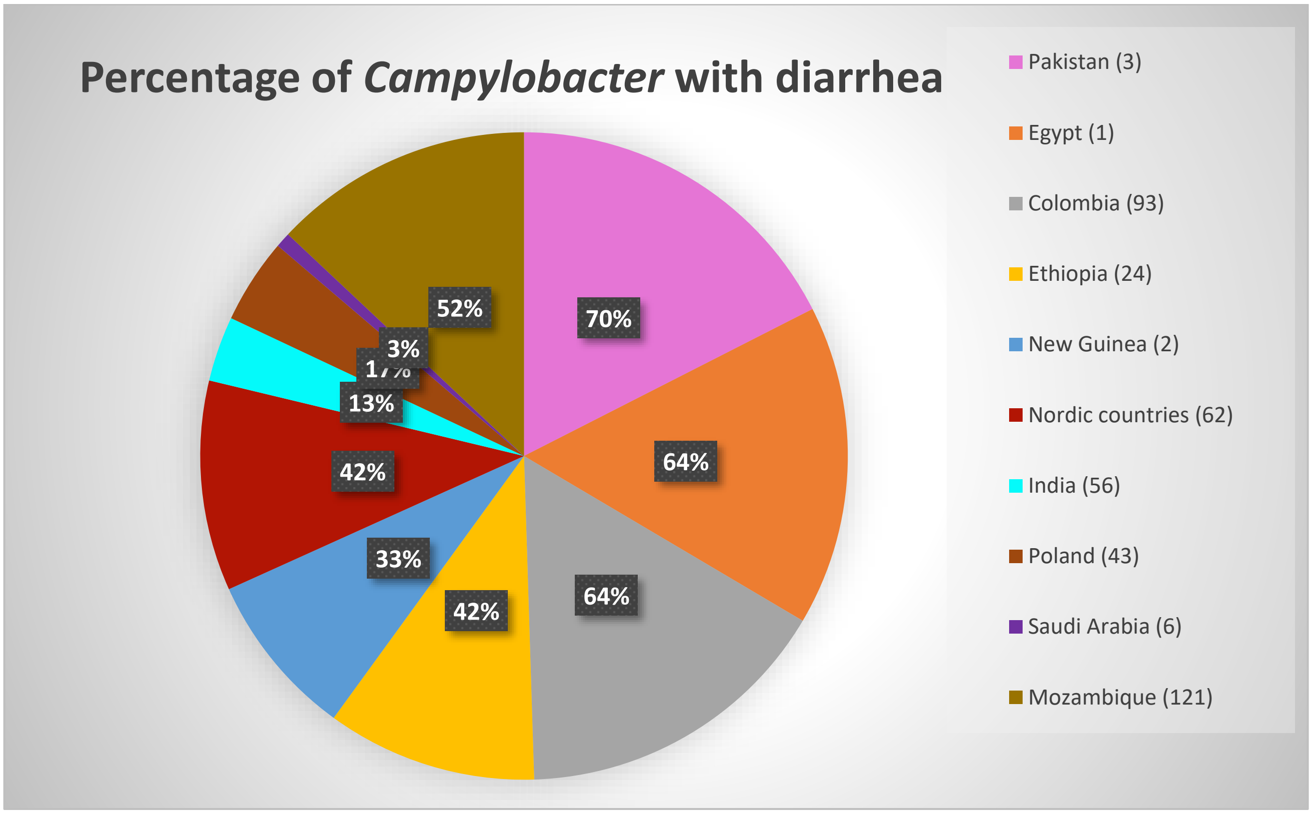

Figure 5. Percentages all over the world with Campylobacteriosis.

DIARRHEA INCIDENCE WITH *CAMPYLOBACTER*

COUNTRY	PERCENTAGES
Pakistan (3)	70%
Egypt (1)	64%
Colombia (93)	64%
Ethiopia (24)	42%
New Guinea (2)	33%
Nordic countries (62)	42%
India (56)	13%
Poland (43)	17%
Saudi Arabia (6)	3%
Mozambique (121)	52%

Guillain-Barré syndrome (GBS) is the most common cause of acute flaccid paralysis in polio-free regions, with incidence estimated at between 0.4 and 4 per 100,000 in different settings. The disease has an autoimmune pathology; following infection, antibodies produced against pathogen surface structures cross-react with nerve ending antigens, leading to neurologic damage (69,76,127) and in United States of America (USA) Scallan (113) found a 41% of *Campylobacter*-associated cases (82). Yamana (134) in Japan Guillain-Barré, in Sweden MacCartthy (77) one of every four cases had GBS. Pua Torrejón (103) in Spain published a case of Miller-Fisher Syndrome associated with *Campylobacter.* Pogreba-Brown (102) in USA and Germany Sharafutdinov (119) describes the incidence in sequelae in IBS (irritable bowel syndrome), IBD (inflammatory bowel disease), constipation, chronic diarrhea, Crohn´s disease, gastroesophageal reflux disease, ulcerative colitis, dyspepsia, reactive arthritis, Reiter's syndrome, joint pain (28). Norstrom (84) in Sweden found an incidence of 16% with joint symptoms following a *Campylobacter* infection, Reactive arthritis (ReA) has an annual incidence of 4.3 per 100.000, it is a non-purulent joint inflammation which can be triggered by infections in the gut or in the urogenital tract. Kirishima (57) et al in Japan 2022 described that *Campylobacter*, *Citrobacter* and *Leptotrichia* in the cholangiocarcinoma has an abundance increase between clinical stage with and without lymph node metastasis. Takayanagi (126) describes in Japan a higher incidence in the oral microbiome with moyamoya disease.

PUBLIC HEALTH IMPLICATIONS. Public health interventions aimed at controlling *Campylobacter* incidence have focused primarily on reducing food-borne infections, in particular those caused by contaminated poultry, cattle, birds (36). *Campylobacter* seasonality is clearly marked in chicken, as studies have shown in USA, Scandinavia, Netherlands and Wales (25,122). It is difficult to establish a direct correlation between human and chicken *Campylobacter* isolates. White (132) in USA published that pathogens with most foodborne illnesses 9% was of *Campylobacter* and Scallan (112) in USA with children a 63% of diarrhea cases. In Rwanda, Africa (111) a 41% of diarrhea with contaminated milk and other dairy products and in USA Palomares also in milk farms (96).

Peh (98) et al 2023 are studying an administering bacteriophages (phages), in order to, evaluate as a possible intervention strategy in primary poultry production to reduce the public health risk of human infection. Also, Kocot et al. (59) have used bacteriophages, which are ubiquitous bacterial viruses, and bacteriophage endolysins to eliminate Gram-negative pathogens, Kittler (58) in Germany they are working to use phages in chicken's intestine, and Chowdhury (26), has worked with bacteriophages, nanoparticles, probiotics in the poultry processing industry to prevent foodborne pathogens from reaching the consumer.

SUSCEPTIBIITY OF ANTIBIOTICS: Schiaffino in Peru (115) in 2019 published 77.4% resistance to ciprofloxacin, 33.1% to azithromycin, susceptibility to amoxicillin and clavulanic acid (AMC) was 94.%. It can be used erythromycin (E), tetracycline (TE), gentamicin, ampicillin (PN), ceftriaxone (CRO), trimethoprim-sulfamethoxazole (SXT), nalidixic acid (NA), and chloramphenicol (C), (21,131), with azithromycin (AZM) (29,32). The isolated *Campylobacter* species were found to be resistant to cephalothin (CEF) (100%), ampicillin (60.5%), cefotaxime (CTX) (60.5%), chloramphenicol (47.4%) and tetracycline (42.1%). On the other hand, the isolates were susceptible to nalidixic acid (86.8%), ciprofloxacin (CIP) (86.8%), sulphamethazole (84.2%), ceftriaxone (78.9%), clindamycin (68.4%) and cefixime (FEP) (65.8%), 84.2% of the isolates showed multi-drug resistance for three-to-six drug classes. Fonseca (38) in Canada from dairy herds: resistance to tetracycline was observed in 49.7% of the *Campylobacter* spp. isolates, followed by ciprofloxacin (19.9%) and nalidixic acid (19.3%). The proportion of multi-drug resistant (≥3 antimicrobial classes) *Campylobacter spp.* isolates was low (0.3%); however, 15.6% were resistant to two different classes of antimicrobials. In Kenia in diarrhoeic children Zachariah (136) *Campylobacter's* resistance to erythromycin (87.5%), doxycycline (DOX) (75.0%), ampicillin (73.7%), cotrimoxazole (73.3%) and minocycline (68.8%). In Costa Rica Lazo-Láscarez (68) chicken isolated isolates showed resistance to nalidixic acid (91.2%), ciprofloxacin (85.8%), enrofloxacin (85.8%), doxycline (25.0%), chloramphenicol (5.4%) and erythromycin (2.7%). In Greece 2024 Maraki 77.3% (74) was resistance to ciprofloxacin. Wieczorek (131) in Poland resistant to ciprofloxacin 49% and 89%, between 1994-1996 and 2005-2008, respectively. Musick (81) resistance to ciprofloxacin, azithromycin, carbapenem, tetracycline and sensible imipenem/cilastatin and oral Fosfomycin. In Colombia (94) had a sensitivity to AMC 63%, fosfomicina (FOS) 60%, cefuroxime (CXM) 50%, levofloxacina (LEV) 43% and ZM 20%. Figures 6,7.

Ribeiro (105) in Brazil published a screening of 28 vegetable oils with anti-Campylobacter activity using olibanum, salvia and candeia essential oils. Its treatment should be done with samples cultures, in order to do the sensitivity and it must be guided by a physician (37,94).

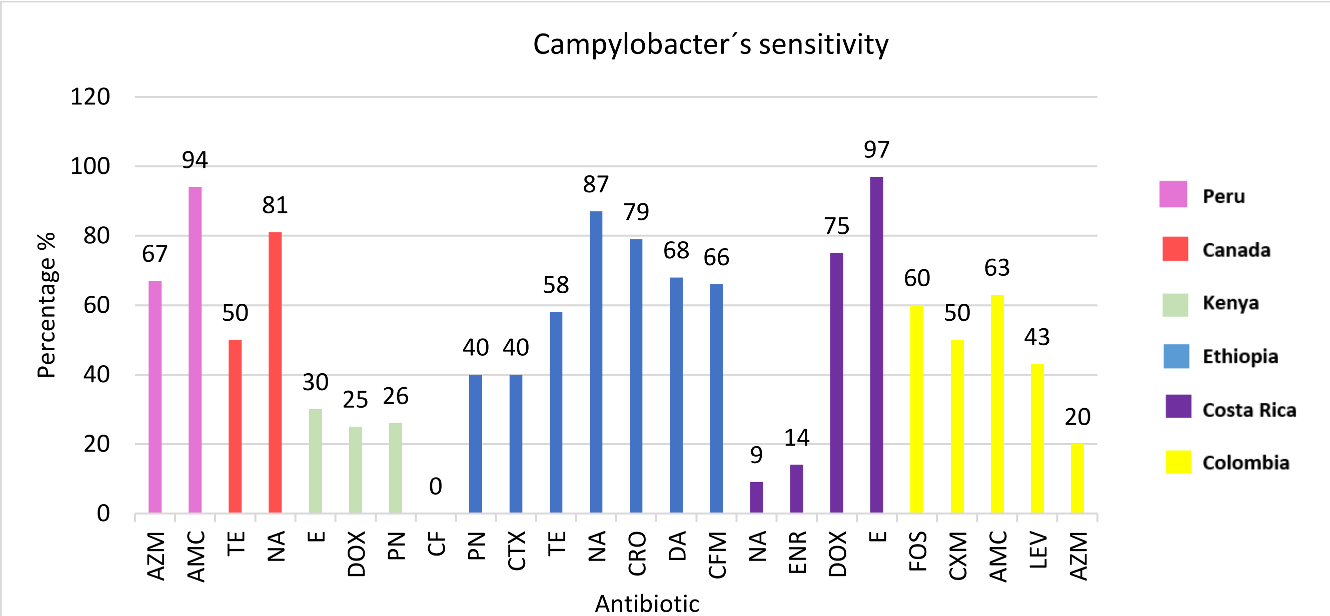

Figure 6. Susceptibility antibiotics in Campylobacteriosis in different countries.

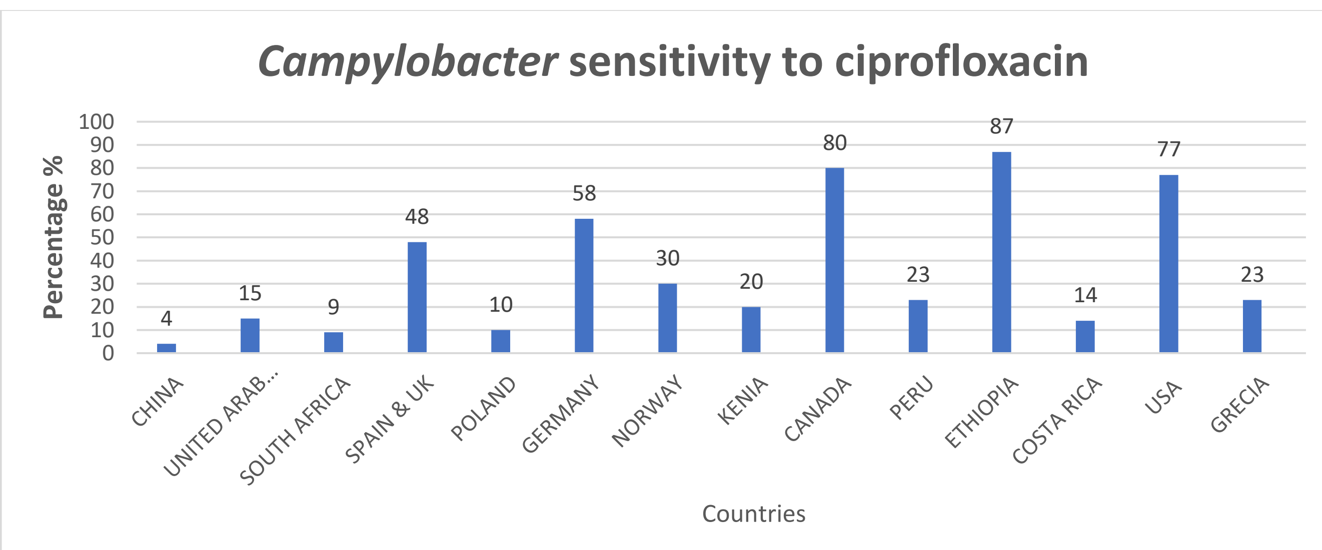

Figure 7. *Campylobacter* sensitivity to ciprofloxacin in different countries

VACCINES: Since viral (rotavirus, human calicivirus) and bacterial infections (*Campylobacter, Salmonella, Shigella, Vibrio cholera, Escherichia coli* (ETEC)) continue to be a leading cause of mortality and morbidity in young children in low income and middle income countries, in the elderly and immunocompromised individuals (8, 9). Grabowski (44), Islam (52), Kocot (59) used phage system in poultry. Seo (118) et al 2020 introduced a novel epitope- and structure-based vaccinology platform known as MEFA (multiepitope fusion antigen) and the application of MEFA for developing broadly protective multivalent vaccines against heterogenous pathogens.

CONCLUSION: *Campylobacter* is a bacterium that has to be monitored because it causes many pathologies and it is an etiology that involves many expenses in public health (10,30,70,120,128). It is also involved in carcinogenesis (135), Guillan-Barré (76,82,127,128,131,134), Re reactive arthritis (69,75,131), people working with animal exposure or industry (132), bacteremia (129). Campylobacteriosis represent the most common risk factor for developing IBD (irritable bowel disease) (119). Kuhn (62) et al 2020 showed in the Nordic countries that the incidences of *Campylobacter* are linked to increase in temperature and especially precipitation in the week before illness, suggesting a non-food transmission route (70).

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