

Antibiotic susceptibility profile of *Campylobacter spp* from poultry meat, Khartoum State, Sudan

Khalda. A. Khalifa¹, Elbrissi Atif², Igbal, S¹, Adel Rahim, Amgad, M. Abdel Rahman¹, Salwa, A. Ahmed¹, and Igbal, H. Munir¹

¹Central Veterinary Research Laboratory, Khartoum, Sudan

²Central Laboratory, Ministry of higher Education, Khartoum

Department of Avian Diseases and Diagnosis

ABSTRACT:

Campylobacteriosis is found among the four important worldwide food borne pathogens. In intensive poultry rearing systems in Sudan the use of oral antibiotics is essential to preserve health. The aim of this study was to determine the antimicrobial susceptibility profile of thermophilic *Campylobacter spp.* isolated from broiler in Khartoum.

Sensitivity of fourteen isolates against seven antibiotics namely Neomycin, Nitrofurantoin, Nalidixic acid, Gentamycin, Streptomycin, Tetracycline and Erythromycin were studied by disk diffusion. The result showed that Nitrofurantoin was found as highly sensitive (64.3%). It was also observed that Erythromycin, Gentamycin, Streptomycin and Neomycin verified the following resistant against isolates recovered 92.9%, 71.4%, 71.4%, 64.3% respectively. It was concluded that poultry meat in Khartoum could complicate the antimicrobial therapy in human as multidrug resistant of *campylobacter* was detected.

Key words: *Campylobacter*, Antibiotic Resistant, Disc Method, Broilers, Khartoum State

Introduction :

Poultry industry in Sudan witnessed considerable development only in the last 10 years, with production increasing from 5 million broilers in 2006 to close to 90 million in 2017. While several factors contributed to this increase, the two most important were the government decision to stop imports of frozen poultry in 2006 and the increase in red meat prices. Other factors that contribute to increase of poultry meat consumption are, urbanization, change in food habits, rising income and population growth (Nabil, 2017). Increasing meat consumption worldwide raises a lot of concerns and challenges to meat hygiene and safety (Sofos and Geornaras, 2010). One of the major concerns is contamination of meat by products by *campylobacter* particularly poultry meat. *Campylobacter spp* reside in the gut of domesticated animals and birds as part of the intestinal microflora (Salih et al, 2009).

The Liver and heart of chickens' carcass (known as giblets) are separated from the slaughtered bird during processing and often packaged with them. Giblets may also be purchased separately as livers, hearts, or a combination.

Campylobacter spp. is Gram negative, microaerophilic, curved or spiral rods in the family Campylobacteriaceae (OIE, 2004).

The organism is considered as one of the most common pathogen-related causes in diarrheal illness globally and has been recognized as a significant factor of human diseases over three decades (Bolton, 2005). Accordingly, there is a high threat for the thermophilic *Campylobacter spp* inhabitant in the intestinal tract of food animals to develop resistance to commonly used antibiotics. Contamination of broiler meat with pathogenic strains of resistant *Campylobacter* could, therefore, result in a form of campylobacteriosis in humans that is difficult to treat.

Most farmers in Sudan chose antibiotics depending on availability and cost and/or what the local sales person recommended rather than consulting a veterinarian. Knowledge of resistance to antibiotics was generally low. This study was conducted to find out the antibiotic susceptibility profile and resistant as no data was present.

Materials and Methods:

Source of samples:

Samples (livers, heart and intestines) of slaughtered broiler chickens were collected from fourteen slaughterhouses located in 5 different localities in Khartoum State namely (Gebel awlia1, bahri Alkadroo, Gebel awlia2, bahri Alsagai and Sharq alneel) (Table 1), during the year 2020 . Samples of livers, hearts and intestines organs were collected

during slaughtering, pooled, packed in sterile bags and then transferred on ice bags to the Central Veterinary Research Laboratories, Avian diseases and diagnosis, Soba-Khartoum.

Bacteriological examination:

Collected pooled samples were incubated in Cary Blair medium overnight at 42 °C, then streaked onto Skirrow's agar plates and charcoal-based media (CampyGen, Oxoid, CN35A) supplemented with antibiotic supplement mixture (vancomycin, polymyxin B and trimethoprim) were added to the media for primary isolation and incubated at 42°C and 37°C for 48- 72 h under micraerophilic conditions in an anaerobic jar (CampyGen, CN0225, Oxoid Ltd). Representatives of typical and well-isolated colonies were sub-cultured onto nutrient agar plates for purification which was checked by Gram staining. Heavy suspension of 24-h growth for each pure isolate was prepared in 10 ml of 20% glycerol-peptone medium, mixed well and suspended in 2.5 ml volume into four vials. All suspended colonies were identified by microscopic examination of morphology and motility, oxidase test, catalase test and standard biochemical methods as described previously (Atabay and Corry, 1997).

Antibiotic Susceptibility Test:

Resistance of *Campylobacter* isolates to certain antibiotics was determined by Kirby-Bauer disk diffusion method (OIE, 2004) on Mueller-Hinton agar (Liofilchem, Italy) supplemented with 5% sheep blood following CLSI 2016 guidelines. Seven antibiotics tested and their corresponding concentrations were Neomycin (30 mg), Nitrofurantoin (300 mg), Nalidixic acid (30 mg), Gentamycin (10 mg), Streptomycin (10 mg), Tetracycline (30 mg), and Erythromycin (25 mg). For the disk diffusion method, sterile cotton-tipped swabs were used to transfer the inoculum onto Mueller-Hinton plates to produce a confluent lawn of bacterial growth. After the inoculum on the plates was dried, the above mentioned antibiotic disks were distributed over the inoculated plates. The inhibition zones were recorded and interpreted following CLSI breakpoints.

Results:

Identification of isolated bacteria:

All collected samples (livers, hearts, and intestines) revealed *Campylobacter spp* by cultural methods. Colonies of *Campylobacter spp* on Skirrow selective medium appeared as pink while light grey colonies on charcoal-based medium. Microscopic examinations revealed Gram-negative small spiral curved bacilli with comma-shaped (S) or gull wing-shaped cells that showed high motility and cork-screw like, all isolates were oxidase and catalase positive. Fourteen *campylobacter spp* isolates were recovered from the five mentioned slaughterhouses as in table 1.

Table (1) Antibiotic susceptibility of fourteen *campylobacter spp* isolated from different localities in Khartoum State

Location	No of isolates	No. sensitive (%)	No. moderate (%)	No. resistant (%)	Total sensitivity test (%)
Gebel Awlia 1	2	4/14(28.6)	3/14 (21.4)	7/14 (50)	14/98 (14.4)
Bahri Alkadaro	3	5/21(23.8)	6/21(28.6)	10/21(47.6)	21/98 (21.4)
Gebel Awlia 2	3	3/21(14.3)	5/21(23.8)	13/21(61.9)	21/98(21.4)
Bahri Alsagai	3	1/21(4.8)	5/21(23.8)	15/21(71.4)	21/98(21.4)
SharqAlneel	3	9/21(42.9)	4/21(19)	8/21(38.1)	21/98(21.4)
Total	14	22/98(22.4)	23/98(23.5)	53/98(54.1)	98

Antibiotic susceptibility Test:

Sensitivity of each isolates against seven antibiotics namely Neomycin, Nitrofurantoin, Nalidixic acid, Gentamycin, Streptomycin, Tetracycline and Erythromycin ranged between 0-71.4%. Moderate responses ranged between 0-57.1% while resistant varied between 14.3%-85.7% as in figure1.

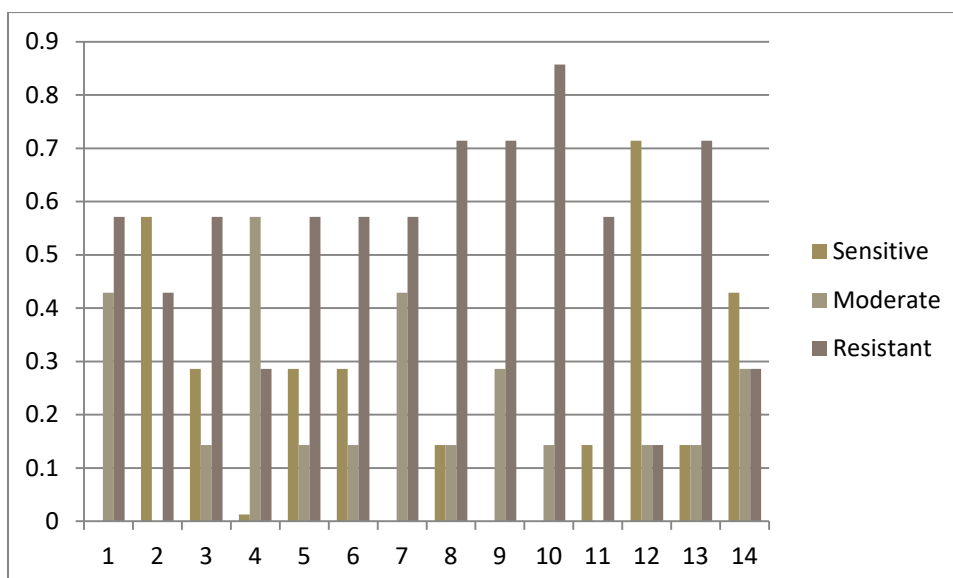


Figure (1).Antibiotic sensitivity profile of *Campylobacter* Isolates against the seven antibiotics

Nitrofurantoin was found as highly sensitive (64.3%) among antibiotics examined and tetracycline (7.1%) as less sensitive against campylobacter detected. Tetracycline revealed 64.3% high moderate whereas Erythromycin recorded 7.1%.

It was also noticed that Erythromycin, Gentamycin, Streptomycin and Neomycin verified the following resistant against isolates recovered 92.9%, 71.4%, 71.4%, 64.3% respectively as in figure 2.

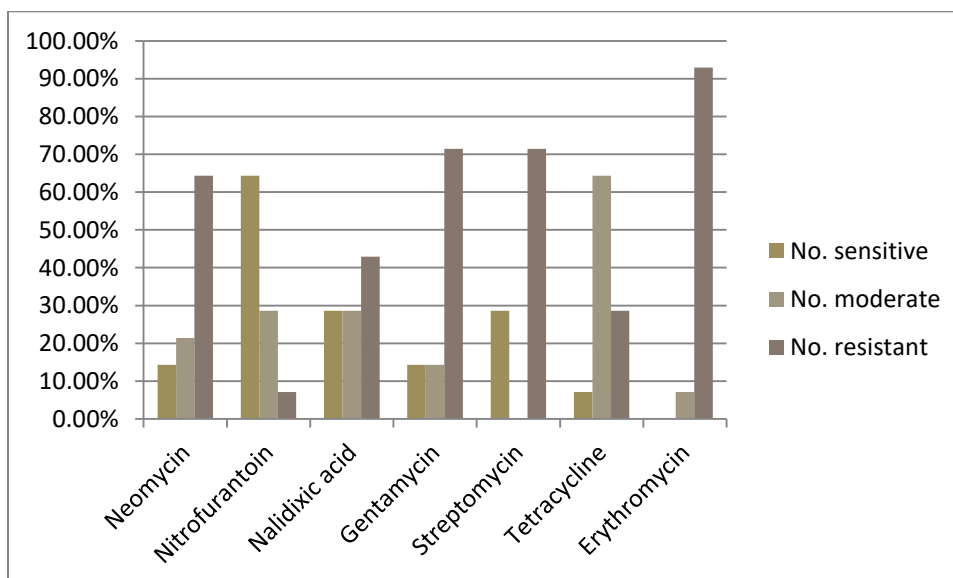


Figure (2).Sensitivity patterns of the seven antibiotics

In the current study multidrug resistant (MDR) against five, four, three and two antibiotics were reported. Ten isolates were found resistant to Gentamycin; Streptomycin and Erythromycin. Ten isolates were resistant against Streptomycin and Erythromycin. Nine isolates resistant against Neomycin and Erythromycin (figure 3).

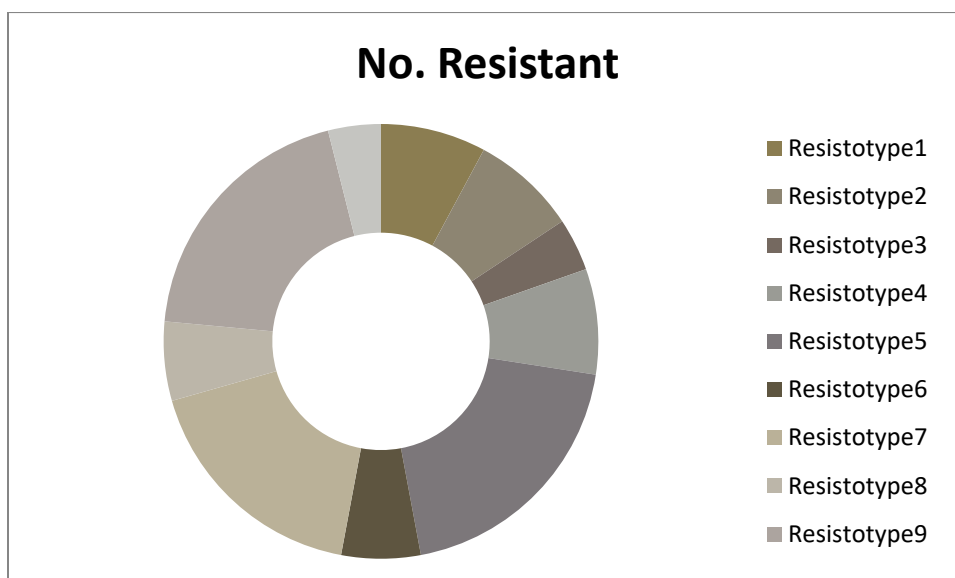


Figure (3) Multi-drug resistant of Campylobacter isolates

Resistotype 1: Neo Nalid Gent Strept Erythro, Resistotype 2: Neo Gent Strept Erythro, Resistotype 3: Nalid Gent Strept Erythro, Resistotype 4: Neo Gent Strepto, Resistotype 5: Gent Strepto Erythro, Resistotype 6: Nalid Erythro Resistotype 7: Neo Erythro, Resistotype 8: Tetra Erythro, Resistotype 9: Strepto Erythro, Resistotype 10: Strepto Tetra

Total sensitivity tests according to location was illustrated in table (1). Percentage of resistance ranges between 38.1 in SharqAlneel and 71.4 in Bahri Alsagai while total resistance was found to be 54.1. Sensitivity to the mentioned antibiotics was high (42.9%) in SharqAlneel. Moderate responses revealed by the antibiotics were nearly the same.

Discussion:

Poultry meat is considered the most important source of human Campylobacteriosis and the role of poultry as a reservoir for transmission to humans has been recognized. Around 20%–30% of human infections are linked to the manipulation, preparation and consumption of broiler meat, while 50%–80% may be attributed to the chicken reservoir as a whole (Elisabetta *et al*, 2019).

Campylobacter spp. represent human health risks when poultry is inadequately cooked or is cross-contaminated after cooking (MARCOS, 2000). Cross-contamination can occur at each phase in the process of bringing the product to the consumer, beginning at the farm level and continuing through processing (MARCOS, 2000). Slaughtering and processing steps such as scalding, picking, and chilling may be a source of cross-contamination (Arturo,2020). Additionally, meat can become contaminated with pathogens from intestinal contents, skin, or feathers (Arturo, 2020).

High degree of sensitivity to Erythromycin, resistant 72.7% to tetracycline from thirty three *Campylobacter* isolates from Arabian Gulf was reported (Abiola Senok *et al*, 2007). They also reported MDR in 6 poultry isolates in comparison with 92.9% Erythromycin resistant and 10 isolates as maximum MDR in the current study.

Resistant to Erythromycin 7%, Tetracycline 71%, Streptomycin 21%, Gentamicin 7% and Nalidixic acid 71% was reported in Vitnam (Petter, 2010) compared to 92.9%, 28.6%,71.4%,71.4% and 42.9% respectively in this study.

Therapeutic antimicrobials of choice in human patients suffering from life threatening campylobacteriosis are initially the macrolides and thereafter the fluoroquinolones and Gentamicin (Farad and Amir hossein, 2020).Resistance to these 2 classes of antibiotics in zoonotic *Campylobacter* species can increase hospitalization rates and the cost of therapy, and decrease the survival rate of patients (Farad and Amir hossein, 2020).

A study in South Africa revealed there was no resistance in the *Campylobacter* spp. isolated from birds in Gauteng to Gentamicin, neomycin that disagreed with what was found here (Jonker and Picard, 2010).

In the current study, multidrug resistant (defined as resistance to four or more different classes of antibiotics) is found in ten strains as max, in contrast to four strains detected by (Jonker and Picard, 2010).despite no multiple resistance has been also reported in a

number of countries—four European Union countries, Northern Ireland and Sweden (Bywater *et al*, 2004, Oza *et al*, 2003, Jeanette *et al*, 2007). The reasons of these results may be due to the fact that urbanization and increase consumption of poultry meat besides time interval of earlier studies.

Another study reported (14.8%-19.2%) Tetracycline, Erythromycin (11.1%) and no resistant showed by Nalidixic acid from poultry in the South-East Queensland region (Jeanette *et al*, 2007). Higher levels of tetracycline resistance have been reported from four European Union countries (35.4%) and the USA (43%) (Bywater *et al*, 2004, Gupta *et al*, 2004). In Sweden, where tetracycline has not been added to chicken feed since 1986 (Jeanette *et al*, 2007). The level of tetracycline-resistant *Campylobacter* has been reported to be 1% (Ronner *et al*, 2004, Jeanette *et al*, 2007)

A study of 62 broiler strains in Brazil revealed resistant to Neomycin (50%), Tetracycline (43%) whereas a lower percentage of strains was resistant to Erythromycin (10%), all strains were sensitive to Gentamycin (Suzete *et al*, 2008).

highest rate of resistance (75 %) Nalidixic acid, while Gentamycin was the most active (96%) antibiotic against studied isolates followed erythromycin (95%) and streptomycin (94%) in chicken and beef meat in Iran (Hosseini *et al*, 2014).

Unlike this study, total resistance of *Campylobacter* isolates to Nalidixic acid and total sensitive to erythromycin in Indian poultry (Neelam *et al*, 2018).

As multidrug resistant was detected here; constant awareness for *Campylobacter* spp. of public health significance should be maintained through the use of surveillance and the rapid reporting of trends. Previous study (Elisabetta *et al*, 2018) reported 81.45%, 3.76%, 75.6%, 22.96% in comparison to 42.9%, 71.4%, 28.6% and 92.9% of Nalidixic, Gentamycin, Tetracycline and Erythromycin in the current study.

The regional differences in the prevalence of AMR among the *Campylobacter* isolates in the present study could be the result of the manner in which antimicrobials are used in veterinary medicine. There are differences in production and veterinary practices between regions with regard to drug prescription, sales, or marketing since those matters are governed by provincial laws (Ousmane *et al*, 2019) and resistance profiles for chickens reflected the drugs administered on the farm (Idris *et al*, 2006). Environmental factors that are specific to certain regions could also be a source of resistant *Campylobacter*. In conclusions, the high rate of contamination in chicken meat in Khartoum alarms a significant public health concern. Most of the isolates were resistant; therefore, there is a possible risk of human infection with *Campylobacter* spp. via consumption of these products.

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