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Prevalence and antimicrobial resistance patterns of *Campylobacter* spp. isolated from retail meat in Lahore, Pakistan

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ABSTRACT

Campylobacter spp. is a leading cause of gastroenteritis in humans. Contaminated food of animal origin is considered to be the common source. Some of these bacteria are multi-drug resistant, which results in treatment complications. Indiscriminate use of antimicrobial drugs has been suggested to be largely responsible for resistance in zoonotic pathogens including *Campylobacter*. This study was conducted to determine the prevalence and antimicrobial resistance pattern of *Campylobacter* isolated from meat of three different food animal species sold at retail shops in Lahore, Pakistan. A total of 125 *Campylobacter* were isolated and tested for antimicrobial resistance against nine commonly used antibiotics in veterinary and human medicine. The highest resistance was observed against enrofloxacin (79.2%) followed by tylosin (77.6%), ciprofloxacin and amoxicillin (71.2% each), colistin (69.6%), neomycin (32.8%), nalidixic acid (31.2%), gentamicin (25.6%) and doxycycline (8.8%). Most of the isolates (90.4%) were resistant to more than two antibiotics and were considered as multi-drug resistant bacteria. The results indicate that antibiotic resistant bacteria are prevalent in animal meat in Pakistan probably due to uncontrolled use of antibiotics in food animals, thus posing a threat to public health.

1. Introduction

Most cases of *Campylobacter* infection in humans are self-limiting and do not require antibiotic therapy. However, severe cases such as those in immune-compromised patients do need to be treated with antibiotics (Engberg, Aarestrup, Taylor, Gerner-Smidt, & Nachamkin, 2001; Gibreel et al., 2004) such as fluoroquinolones and macrolides. For bacteremia caused by *Campylobacter*, aminoglycosides are often used (Alfredson & Korolik, 2007; Corcoran, Quinn, Cotter, Whyte, & Fanning, 2006; Lin et al., 2007; Moore et al., 2006; Payot et al., 2006). Unfortunately, the presence of antibiotic resistance in bacterial pathogens is a serious public health concern throughout the world (Han, Jang, Choo, Heu, & Ryu, 2007; Hawkey & Jones, 2009; Isenbarger et al., 2002). People infected with antibiotic resistant strains of *Campylobacter* are ill for a longer period of time and are more likely to be hospitalized (Gupta et al., 2004). The success rate of treatment against *Campylobacter* infection is decreasing due to an increase in antibiotic resistance (Lehtopolku et al., 2010). Unfortunately, information on antibiotic resistance in *Campylobacter* of animal origin in developing countries is not available (Osano & Arimi, 1999).

Irrational use of antibiotics for the treatment and control of infectious diseases in veterinary medicine is considered a key cause of development of antibiotic resistance in foodborne pathogens (Hoszowski & Wasyl, 2005). Most of the antibiotics used in human and animal medicine are similar and hence the use of antibiotics in animals poses a potentially serious risk to public health (Alfredson & Korolik, 2007; Hariharan, Sharma, Chikweto, Matthew, & DeAllie, 2009; Luangtongkum et al., 2009). Recently, the prevalence of

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antibiotic resistance in foodborne pathogens has increased and has become a complex issue (Možina, Kurinčič, Klančnik, & Mavri, 2011).

Antibiotics are often used as growth promoters in food animals. In developing countries, large amounts of various antibiotics are used in domestic poultry for the control of infectious agents and for growth promotion. This may help select resistant strains and their subsequent transmission to humans via contaminated food (Hoszowski & Wasyl, 2005). In Pakistan, no data are available on the presence and antimicrobial properties of *Campylobacter* in humans and food animals. The aim of this study was to determine the prevalence and antimicrobial resistance patterns of *Campylobacter* in various meat sources (beef, mutton, and chicken) in Lahore, Pakistan.

2. Materials and methods

2.1. Sampling

A total of 600 meat samples (200 each of beef, mutton, and chicken) were collected from retail meat shops from ten administrative divisions of Lahore district in Pakistan from September 2014 to February 2015. The popularity of meat consumption is relative high in Fall and Winter months i.e., September to February in Lahore Pakistan. From each division, 20 samples each of beef, mutton and chicken were collected. The samples were placed in an ice box, transported to the laboratory, and subjected to microbial analysis within 24 h of collection.

2.2. Isolation and identification of Campylobacter

Isolation of *Campylobacter* was carried out according to the international organization for standardization ISO 10272-1:2006 (Moran, Scates, & Madden, 2009). Meat samples were placed in separate bags and homogenized in a stomacher for 2 min with buffered peptone water at 1/10 ratio of w/v. An aliquot (1 mL) of this homogenate was transferred to a tube containing 9 mL of Bolton broth for enrichment. The inoculated Bolton broth was incubated at 42 °C for 48 h under microaerophilic conditions using Campy Gas sachet (Gaspak EZ Campy Container BBL 260680) in an anaerobic jar. An aliquot from the enriched broth was streaked on plates of mCCDA agar (CM 0739 Oxoid, England) containing cefoperazone and amphotericin B (SR0155 Oxoid, England) followed by incubation at 42 °C for 48 h under microaerophilic conditions. Suspected colonies were lifted from plates and identified as *Campylobacter* using Gram staining, motility, oxidase test, and latex agglutination (F46 Microgen, UK). These colonies were further streaked on fresh mCCDA plates for purification and DNA extraction. The purified isolates were also placed in 20% glycerol and stored at-80 °C for future use.

2.3. Speciation of Campylobacter

DNA was extracted from purified isolates using "QIAamp DNA Mini Kit" (Qiagen, cat# 51306, USA) according to manufacturer's instructions. The extracted DNA was stored at −20 °C until used. Multiplex PCR was carried out for confirmation and speciation of *Campylobacter*. Three sets of primers were used to identify *Campylobacter* spp: *C. jejuni,* and *C. coli* by targeting *16SrRNA*, *mapA* and *cueE* gene, respectively (Denis et al., 1999; Gonzalez, Grant, Richardson, Park, & Collins, 1997; Linton, Lawson, Owen, & Stanley, 1997; Stucki, Frey, Nicolet, & Burnens, 1995). PCR amplification reaction was performed in 25 μL mixture in a thermal cycler "T100" (BioRad USA). The PCR conditions for 35 cycles were: denaturation at 94 °C for 1 min, annealing at 48 °C for 1 min and extension at 72 °C for 1 min. The PCR products were visualized under UV light following by gel electrophoresis in 1.2% agarose gel.

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility tests on 125 isolates were performed by the disc diffusion method (Bauer et al., 1966). Briefly, three to five well-isolated colonies were selected from the culture plate. The colonies were suspended in normal saline solution followed by adjustment of turbidity to 0.5 McFarland standard. A sterile cotton swab was dipped into the suspension and streaked on the entire surface of a Mueller–Hinton agar plate (Oxoid, England) containing 5% sheep blood. The inoculum was allowed to dry for 5 min followed by application of antibiotic discs and incubation at 42 °C for 48 h under microaerophilic conditions. Stock cultures of *C. jejuni* (ATCC 33560) and *C. coli* (ATCC 33559) were used as reference strains. The diameters of the zones of inhibition were measured with a calliper and interpreted as recommended by Clinical and Laboratory Standards Institute Guidelines (CLSI, 2006). A total of nine antibiotics commonly used in veterinary and human practices were tested e.g., amoxicillin (10 μg), ciprofloxacin (5 μg), colistin (10 μg), doxycycline (30 μg), enrofloxacin (5 μg), gentamicin (10 μg), nalidixic acid (30 μg), neomycin (30 μg) and tylosin (30 μg).

2.5. Statistical analysis

Data were entered into a Microsoft Excel sheet for analysis and were analysed to obtain the numbers and percent of resistant and susceptible microorganisms using SPSS 20.0 statistical software.

3. Results

A total of 125 *Campylobacter* were isolated from the three meat sources of which 82 were *C. jejuni* and 43 were *C. coli*. Of the 200 beef samples, 31 (15.5%) were positive for *Campylobacter* while this number was 36 (18%) and 58 (29%) for mutton and chicken, respectively (Table 1). When tested for antimicrobial susceptibility, the highest resistance in all 125 isolates of *Campylobacter* spp. was against enrofloxacin (79.2%) followed by tylosin (77.6%), amoxicillin (71.2%), ciprofloxacin (71.2%), and colistin (69.6%). Most of the isolates (113 of 125 or 90.4%) were resistant to multiple antibiotics. The *C. jejuni* isolates (n = 82) were highly resistant to enrofloxacin and tylosin (78%) followed by amoxicillin (72%), ciprofloxacin (68.3%), and colistin (67%) (Fig. 1). The rate of resistance against these five antibiotics was similar in *C. coli* isolates (n = 43) too. The least resistance was against doxycycline (8.8% in *Campylobacter* spp.).

The overall resistance in *Campylobacter* isolates (n = 31) from beef origin was the highest against ciprofloxacin 83.9% (26/31) followed by enrofloxacin 77.4% (24/31) and colistin 74.2% (23/31) (Fig. 2). Resistance against doxycycline, nalidixic acid and neomycin was low. None of the *C. jejuni* (n = 19) isolates from beef showed any resistance to doxycycline while 2 of 12 (16.7%) of *C. coli* isolates were resistant to this antibiotic (Table 2). The resistance of *C. coli* (n = 12) to enrofloxacin, ciprofloxacin, and colistin was similar to that in *C. jejuni*. None of the *C. coli* isolates from beef was resistant to nalidixic acid.

Of the 36 isolates from mutton, 31 (86%) were resistant to tylosin followed by enrofloxcin (72.2%) and ciprofloxacin and colistin (66.7% each). Low resistance was observed against doxycycline 11% (4/36) and gentamicin 22% (8/36) (Fig. 2). Of the 36 mutton isolates, 25 and 11 were confirmed as *C. jejuni* and *C. coli*, respectively. In *C. jejuni*, the highest resistance was against tylosine (21/25 or 84%) followed by enrofloxacin (76%) and amoxicillin (72%). Only 8% (2/25) and 20% (5/25) of *C. jejuni* isolates were