Molecular Epidemiology of Thermophilic Campylobacter Infection in Poultry, Cattle and Humans in Plateau State, Nigeria

BY

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CERTIFICATION

We certify that Dr Ngulukun, Sati Samuel carried out this research work in the Department of Veterinary Public Health and Preventive Medicine at the University of Nigeria, Nsukka. The work presented herein is original and has not been previously reported anywhere else.

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DEDICATION

I dedicate this work to Almighty God and to my lovely wife, Jocelyn and children; Benan, Kennan and Dengnan for their unalloyed support during this PhD programme.

ABSTRACT

Occurrence of *Campylobacter* species was investigated in poultry, cattle and humans in Plateau state. Cloacal swabs, rectal swabs and faeces from apparently healthy broilers, cattle and humans in selected farms and Hospitals respectively were cultured for *Campylobacter* organisms on modified charcoal cefoperazone deoxycholate agar (MCCDA). Characterization of *Campylobacter* isolates was done by using standard biochemical tests and further confirmed by polymerase chain reaction (PCR). Multilocus sequence typing was done by amplifying and sequencing 7 house keeping genes. Out of the overall 1, 012 samples tested, 228 (22.2%) were positive for *Campylobacter* species. One hundred and seventy five (76.8%) were identified as *C. jejuni* while 53 (23.2%) were *C. coli*. Out of the 360 samples from poultry tested, 129 (35.8%) were positive for *Campylobacter* species, with 105 (81.4%) as *C. jejuni* and 24 (18.6%) as *C. coli*. Of the 352 samples from cattle, 65 (18.5%) were positive with 52 (80%) as *C.jejuni* and 13 (20%) as *C. coli*. Similarly, of the 300 samples from humans tested, 34 (11.3%) were positive with 18 (52.9%) as *C. jejuni* while 16 (47.1%) were *C. coli*. The results of the study also showed that the prevalence was significantly higher in calves (25%) than in adults (12.2%) ($P<0.05$). Similarly, the prevalence was significantly higher ($P < 0.05$) in children (15.8%) than adults (7.1%).

Susceptibility to 10 antimicrobial agents was evaluated using the disc diffusion technique. Of the 145 *Campylobacter* isolates from all sources tested, 115(79.3%) were resistant to one or more of the ten antimicrobial agents. Overall, the highest resistance was observed to nalidixic acid (60.7%), followed by tetracycline (57.2%), ciprofloxacin (53.1%), sulphamethoxazole/trimethoprim (46.2%) and erythromycin (41.4%). The low resistance was observed to azithromycin (27.6%), streptomycin (13.1%), gentamicin (6.9%) and chloramphenicol (4.8%). All the isolates were susceptible to clindamycin. Considering the overall resistance by species, *C. coli* was generally more resistant than *C. jejuni* to most of the antimicrobial agents. Overall, 81 (55.9%) of the isolates were multiresistant with nalidixic acid as the most frequently occurring antimicrobial agent, being found in 25 of the 29 multiresistant patterns.

Out of the 125 *Campylobacter* isolates tested by PCR, 112 (89.6%) were correctly confirmed as *C. jejuni* and *C. coli*. Like the biochemical tests, none of the isolate was confirmed as *C. lari.* Out of the 60 isolates sequenced by MLST, 36 were *C. jejuni* while 24 were *C. coli.* The number of

unique alleles at each locus varied from 5 for the *asp* and *unc* locus to 8 for the *gly* locus for the *C. jejuni* isolates while the number of unique alleles at each locus for the *C. coli* isolates varied from 2 for the *gln* locus to 5 for the *gly* locus. A high degree of variability was observed at locus *tkt*, which was caused by a unique allele *tkt*-3 characteristic of *C. jejuni*, but present in 2 of our *C. coli* isolates. Out of the 43 sequence types identified, 23 (53.5%) were novel. The remaining 20 (46.5%) had been previously reported. From the 36 *Campylobacter jejuni* isolates sequenced, a total of 21 sequence types were identified with 9 (42.9%) being new. The new sequence types were ST4036, ST4037, ST4038, ST4039, ST4040, ST4041, ST4064, ST4066 and ST4220. The most common sequence type was ST1932 which comprised of 6 isolates (28.6%), followed by ST1036 and ST607 with 3 isolates (14.3%) each and ST3554, ST460, ST4041, ST523, ST354 and ST4064 with 2 isolates (9.5%) each. From the 24 *Campylobacter coli* isolates sequenced, a total of 22 sequence types were identified with 14 (63.6%) being novel. The 43 STs were grouped in to 8 previously defined clonal complexes (CC). The clonal complexes were CC-460, CC-607, CC-353, CC-354, CC-658, CC-574, CC-22 and CC-828. Nine (20.9%) STs found in the isolates were unassigned. CC-828 (n = 16; 26.7%) was the most prevalent followed by CC-460 (n = 14; 23.3%) and CC 353 (n = 5; 10%). CC 828, CC460 and CC353 overlap among the poultry, cattle and human isolates. CC-607 was associated with poultry and human isolates only while CC-354 and CC-574 were associated with poultry isolates only. CC-658 was associated with poultry and cattle isolates only while CC-22 was found in the human isolates only.

The results of this study revealed that *Campylobacter* species is prevalent in poultry, cattle and humans in Plateau state. The study also revealed that most of the isolates were resistant to most of the antimicrobial agents used with *C. coli* being more resistant than *C. jejuni.* The *Campylobacter* strains showed high level of genetic diversity with *C. jejuni* isolates being more diverse than *C.coli*. Genetic exchange between *C.jejuni* and *C. coli* was also determined in this study. This study also revealed that certain STs and CCs are associated with source of isolation which is an important epidemiological finding in tracing back sources of infection.

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