# Serogrouping and antibiotic sensitivities of Campylobacter species isolated from Nigerian indigenous breed of pigs

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**Abstract**

Faecal samples of Nigerian Indigenous breeds of pigs around Ife were cultured for *Campylobacter species*. The isolated and identified species were serotyped by agglutination technique on the basis of soluble heat stable antigen, using antisera prepared by Rabbit immunization. All the isolates from diarrhoic pigs were tested for sensitivity to eleven antimicrobial agents by the agar diffusion method.

Fifty seven (57) isolates were recovered from diarrhoeic pigs, while 10 isolates were got from non-diarrhoeic pigs. These isolates were identified as *C.jejuni* (18); *C. coli* (38); C. faecalis (6) while 5 isolates could not be characterised.

From the serological studies, it was found that 14 of the 18 *C. jejuni* isolates belong to serogroup I; while the remaining 4 isolates belong to serogroup II. There was heterogeneiety of other isolates. This probably indicates the importance of their serological investigations and shows that agglutination test can be effectively applied for studies in a herd of pigs in local areas.

Of the eleven antimicrobial agents tested, Septrin, tetracycline, chloramphenicol, Ampicillin, Gentamicin, Furalzolidine, and Erythromycin were active against most strains in that order. The Celphacloridine, Limcomycin, sulphatriad and Metronidazric were relatively inactive.

## Introduction:

Isolation of *Campylobacters species* from man and domestic animals at Ile-Ife and its environ has been reported by Olubunmi and Adeniran, (1986). Serological studies of different strains of Campylobacter have been done by various authors. A serotyping scheme for *Campylobacter jejuni* has been developed based on slide agglutination of live bacteria with antigen prepared with formalised whole cell antigen, (Lior et al, 1982). Butzler and Skirrow, (1979) showed that serological tests with autologous and heterologous isolates with patients sera could be used to antigenically type the Campylobacters from animals and human sources. Indirect haemagglutination tests has been used and found to be sensitive but laborious, (Bokkenheus-er, 1972). Also Elisa test has been used but found to be laborious and expensive. Therefore biotyping in conjunction with serotyping can provide additional epidemiological information about *Campylobacter species.*

Few Antibiotic susceptibility studies have been done on related Campylobacters. Plastridge *et al* (1964) demonstrated that they were resistant to bacitracins and polymixins. Sensitivities have been determined on human isolates to twelve antibiotics; (Butzler *et al* 1974). Vanhoot et al (1978) tested another set of 95 clinical isolates from Brussels to 29 antimicrobial agents, by the use of an agar difusion method on muller — Hilton medium.

Antibiotics are generally used for the treatment of infections in pigs and they are often incorporated in their feeds as feed additives to promote growth. The indiscriminate use of antibiotics could lead to emergence of resistant strains of microorganisms including *Campylobacter species,* particularly in Nigeria where few, if any, sensitivity test is done before treatment is effected.

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Attention is gradually being shifted to the development of the indigenous breeds of livestock in Nigeria because of the present economic climate which had made importation of animals a difficult, if not, impossible task. As part of the effort in this direction therefore, the aims of this study include — to examine for the presence of Campylobacters in the Nigerian indigenous breeds of pigs; to characterise the isolates so obtained; to serogroup the characterised isolates and also to determine the antibiotic sensitivity and resistant pattern of the isolates.

**Materials and Methods**

One hundred and fifty faecal samples were collected from indigenous breeds of pigs on the University Teaching and Research Farm at Ile-Ife and also from small holding units around Ile-Ife. Samples were collected from both diarrhoeic and non-diarrhoeic pigs. All samples were collected with sterile swabs, broken into stuart's transport medium and transported to the Laboratory.

Samples were cultured on Butzler's Selective Medium Oxford Formulation (Oxoid Ltd), with Oxoid enrichment supplement added together with sheep blood — cultures were examined after 24, 48 and 72 hours of incubation under microaerophilic condition and at 37°c. The organisms were identified and characterised by the methods described by Veron and Chatelain (1973); Skirrow and Benjamin (1980); Harvey (1980), and as describe by Olubunmi and Adeniran (1986).

The method described by Penner and Hennessy (1980) were used to serotype the isolates; that is; haemagglutination technique for serotyping on the basis of heat stable antigen. The antigen was prepared from growth of the bacteria on blood agar plates (Oxoid No 2 blood agar base, Oxoid Ltd) with 7% sheep blood; incubated at 37°c for 48 hours, under microaerophilic conditions. The confluent growth was harvested in tryptone soya broth (TSB Oxoid Ltd). The solution obtained was incubated for 18 hours after which it was boiled for 1 hour. This was used to inoculate five Rabbits ranging from 2.7 to 3kg in weight intravenously, using the ear veins. The inoculation was done five times at 3 days intervals for 2 weeks. The doses used for the inoculation were 0.5ml; 1ml; 2ml; 3ml and 4ml. After 7 to 10 days of the last injection, blood was taken by cardiac puncture. The blood was allowed to clot and the sera were separated and stored at — 20°c as antisera. These antisera were later used to serogroup the Campylobacter isolates got from the pigs by slide agglutination using live bacteria cells.

The isolates were tested for antibiotic sensitivity using the technique described by Lunton (1976). The Oxoid multo disk consisting of eleven antibiotics as shown in table 1; were asceptically placed on Agar surface on which the organism had been inoculated. Zones of inhibition were measured manually with a slide rule and oxoid zone reader (McGlie and Finch, 1975).

TABLE 1: SYMBOLS AND CONCENTRATIONS OF ANTIBIOTICS USED

| Symbol | Antibiotics | Concentrations |
|---|---|---|
| $OT_{50}$ | Oxytetracycline | 50 microgram (Mcg) |
| $E_{10}$ | Erythromycin | 10 Mcg |
| $C_{50}$ | Chloramphenicol | 50 Mcg |
| $CN_{10}$ | Gentamicin | 10 Mcg |
| $DA_5$ | Metranidazole | 5 Mcg |
| $PN_{25}$ | Ampicillin | 25 Mcg |
| $SXT_{25}$ | Sulfamethazole/Trimethogram Contrimoxadole | 25 Mcg |
| $FR_{10}$ | Furalzolidine | 10 Mcg |
| $S_{300}$ | Sulphatriad | 300 Mcg |
| $CR_{25}$ | Celphaloridine | 25 Mcg |
| $L_{10}$ | Lincomycin | 10 Mcg |

## Results

Fifty seven (57) isolates of *Campylobacter species* were isolated from diarrhoeic pigs while 10 isolates were recovered from non-diarrhoeic pigs from 150 samples examined. The isolates were identified as *C.jejuni* (18); *C.coli;* (38); *C.fecalis* (6) while the remaining 5 isolates could not be characterised. All the isolates *(C.jejuni; C.coli* and *C.fecalis)* gave a strong slide agglutination with the antisera irrespective of the species used in preparing the antisera. This indicates heterogeneity of the species.

Fourteen (14) of the eighteen (18) *C.jejuni* isolates in this study was found to belong to serogroup I; while the remaining four (4) isolates was found to belong to serogroup II.

The results of in vitro antibiotic resistance patterns of isolates are as shown in Table 2. Septrin, tetracycline, Cloramphenicol; Ampicillin, Gentamicin, Furalzolidine and Erythromycin were very active agents against all the strains. But with each antibiotic a few resistant strains were found except with the septrin. The celphaloridine; Lincomycin and Metronidazole were relatively inactive with the celphacloridine only active against occasional strain. In general the metronidazole showed little activity against all the strains.

**TABLE 2: ANTIBIOTIC RESISTANT PATTERN OF CAMPYLOBACTER ISOLATES FROM LOCAL BREEDS OF PIGS.**

| Antibiotics | Symbol | Total No of Isolates | No fully (s) sensitive | No fully Resistant (R) |
|---|---|---|---|---|
| Tetracycline | $OT_{50}$ | 57 | 54 (94.7%) | 3 (5.3%) |
| Erythromycin | $E_{10}$ | 57 | 45 (78.9%) | 10 (17.5%) |
| Chloramphenicol | $C_{50}$ | 57 | 54 (94.7%) | 3 (5.3%) |
| Gentamicin | $CN_{10}$ | 57 | 54 (94.7%) | 3 (5.3%) |
| Metronidazole | $DA_5$ | 57 | 6 (10.5%) | 51 (89.5%) |
| Ampicillin | $PN_{25}$ | 57 | 54 (94.7%) | 3 (5.3%) |
| Septrin | $SXT_{25}$ | 57 | 57 (100%) | 0 (0%) |
| Furalzolidone | $FR_{10}$ | 57 | 51 (89.5%) | 6 (10.5%) |
| Sulphatriad | $S_{300}$ | 57 | 37 (64.9%) | 20 (35.1%) |
| Celphacloridine | $CR_{25}$ | 57 | 30 (52.6%) | 27 (47.4%) |
| Lincomycin | $L_{10}$ | 57 | 15 (26.3%) | 42 (73.7%) |

(S) — Fully sensitive, (R) Fully resistant
Classified according to zones of inhibition recommended by Oxoid (1978)

## Discussion

There are various reports in literature that show *Campylobacter species* as common occurrence in animals. The isolation rate found in this study in similar to the report of other workers from other parts of the world, using exortic breeds of pigs; (Olubunmi, 1982). The incidence of these organisms might be world wide after all and it could be important factor to consider while looking into the improvement of production of the Nigerian Indigenous breeds of pigs.

Infection with *Campylobacter jejuni* is usually characterised by a good antibody response. The serological response accompanying *C.jejuni* and *C.coli* infection has previously been investigated to a limited extent by complement fixation by Butzler (1973), agglutination of formalised suspension, Watson *et al* (1979) and immunofluorescence. Both authors confirmed that serological tests with antologous and heterologous isolates of Campylobacters could be used to type the Campylobacters from different animal and human sources. Also Kousen (1980) had demonstrated the apparent antigenic heterogenicity of Campylobacters. This indicates the importance of their serological grouping for clinical and epidemiological investigation.

High agglutination titre have been observed with the homologous organism and has been noted to last for many months, Jones *et al.* (1980).

The public health aspect of Campylobacter infections is better understood by the use of serological studies. It has been suggested that a symptomatic carriers of *C.jejuni* may exist among poultry attendants, who apparently got infected from chickens; (Skirrow 1977).

The present study has shown that using rabbits immunized antisera, some of the Campylobacter isolated from local breeds of pigs could be conveniently serogrouped as the result shows that two serogroups are seen among the observed animal strains.

The poor performance of the indigenous breeds of pigs might be due among other causes, to both clinical and sub-clinical infections with these organisms. Any effort directed towards improving the productivity of local breeds of pigs must therefore include an indepth investigation of the level of clinical and subclinical infections with *Campylobacter species*. Serological studies could be a useful and quick method of recognizing the subclinical infections.

The activity of 11 antimicrobial agents was tested against the 57 isolates of Campylobacters isolated from the indigenous breeds of pigs in this study. Although some isolates were resistant to Erythromycin (17.5%), Chloramphenicol (5.3%), Gentamicin (5.3%), Ampicillin (5.3%) and Furalzolidine (10.5%) resistance was more noticeable with metronidazole (89.5%), Cephaloridine (47.4%) and Lincomycin (73.7%).

These results correlate very well with the observation of Chow *et al* (1978).

Plastridge *et al* (1964) has demonstrated that *C.jejuni* showed resistance to Ampicillin while Vanhoof *et al* (1978) demonstrated that Furalzolidine and Gentamicin show excellent activity and that the tetracyclines and erythromycin except against a few resistant strains were also active. The results of the present study were similar to the findings of these previous workers, except for ampicillin which has a sensitivity of 94.7% in this study.

Resistance of these relatively not well recognized organisms in this environment to Erythromycin (17.5%) tetracycline (5.3%) and Ampicillin (5.3%) may be due to the free use of these antibiotics particularly tetracyclines as feed additives to promote growth or as chemoprophylactic agents to prevent and treat outbreak of diseases; Ojo (1974).

Septrin, Gentamicin, Ampicillin, Tetracycline and Chloramphenicol may be of immense use in treatment of Campylobacter infections as they are more active therapeutic agents.

Resistance to Erythromycin in this study may be due to the use of Tylocin in pigs.

The 100% sensitivity of the isolates to co-trimaxazole (Septrin) seems to be an interesting finding. This can be explained from the fact that, the drug has not been abused in the use of livestock.

The observation of Severin (1978) that Septrin has the same activity as sulphamethazole does not apply to this case as the sulphatriad used in this study showed 35.1% resistance of the isolates.

Resistance was more noticeable with Metronidazole (89.5%). Celphaloridine (47.7%); sulphatriad (35.7%) and lincomycin (73.7%) (Table 2). These results correlate very well with the observation of Chow *et al.* (1978) who observed high resistance to the celphalosporius. The high resistance of lincomycin in this study can however not be explained.

It can be concluded from this study that Campylobacter species are present in Nigerian Indigenous breeds of pigs; and that using rabbits immunized antisera, *Campylobacter jejuni/C.coli* isolated from these animals could be conveniently

serogrouped, as the result shows that two serogroups are commonly shared among the observed animal strains. A scheme of typing Campylobacters as to be able to define the epidemiology of Campylobacter enteritis and immune response to this infection needs to be developed locally.

From the results discussed above, it can be seen that it is not desirable that controlled trials should be carried out to define the efficacy of antibiotics such as furalzolidone and others in the treatment of Campylobacter enteritis. Similarly, resistance of Campylobacters to antibiotics should be systematically monitored since plasmid medicated resistance has been identified in some strains (Taylor *et al* 1980).

Therefore, antibiotic resistance should be correlated with the typing scheme that is developed.

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