

Antibiotic Resistance in *Campylobacter Jejuni/Coli* from Human and Animal Sources

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Abstract

Thirty strains of *campylobacter jejuni/coli* from human, pig, poultry and cow sources were biotyped using the scheme suggested by Skirrow and Benjamin (1980). Susceptibility to five antibiotics was measured under two conditions for each antibiotic (viz, variation of inoculum size or incubation atmosphere). Strains were also tested for beta-lactamase production using a starch paper technique. The inoculum size did not have an effect on the minimum inhibitory concentration (MICs) to cephaloridine and ampicillin and there was no relationship between their MICs and beta-lactamase production, though 87% of strains showed beta-lactamase activity. There was a rise in MIC to gentamicin under the "nitrogen atmosphere", but the MIC to erythromycin tended to be lower and tetracycline were found the most effective with MICs <1 ug/mi in 97% of the strains. Erythromycin and ampicillin were active in 87% of strains at a concentration of 2 ug/mI and <4 ug/mi respectively. Cephaloridine showed the highest range of MIC values viz. 32- 36 ug/mi (JPMA 33:91 ,1983).

Introduction

The *Campylobacter jejuni/coli* group of Veron and Chatelain (1973) is being recognised as a common cause of bacterial diarrhoea in man. Although well recognised as a pathogen by veterinary surgeons, under the early name of *Vibrio*, *Campylobacter* were largely unknown in the field of human medicine until the early 1970's. Using a special technique, Dekeyser et al. (1972) isolated a "related vibrio" from patients With diarrhoea. With the development and application of a selective culture medium, isolation of *C.jejuni/coli* from the faeces of enteritis patients has been a frequent occurrence in many areas of the world. *Campylobacter enteritis* may result from animal contacts or ingestion of contaminated food and water.

Isolation of *C.jejuni/foetus* from stools is not in itself an indication for antibiotic treatment, unless the patient is in acute distress. I however, the organisms sometimes enter the blood stream and they must be eradicated with antimicrobial agents. The organisms have also been shown to cause septic arthritis, spontaneous bacterial peritonitis, lung abscess cellulitis, urinary tract infection, cholecystitis and central nervous system infections. Susceptibility of *C.jejuni/coli* to various antimicrobial agent have also been studied in detail (Karmali et al.,-1981).

In this study five of the commonly used antibiotics were used to measure the minimum inhibitory concentrations (MICs) to different *campylobacter* biotypes, isolated from human and animal sources. The effect on the MIC of varying some of the conditions of the test and the frequency of betalactamase positive strains was also studied.

Material and Methods

bacterial strains

All the thirty *Campylobacter* strains were isolated at the Public Health Laboratory, Withington I lospital, Manchester (UK) or sent there from other laboratories for identification and typing. Eleven strains were of human origin, five isolated from cows, three from poultry and eleven from pigs.

After identification by standard procedures, these strains were stored in glycerol broth at 20°C until tested.

Control strains

The following control strains were used: C.jejuni NCTC 11168 C.foetus NCTC 5850 biotype 1

C.coli NCTC11353 Standard NCTC10418 E.coli

Staphy- NCTC 6571 lococcuc aureus

Culture media and antibiotics

The medium used was mueller-Hinton agar (Difco), with 0.5% additional agar (Oxoid agar No.3). Test compounds and sources were:

cephaloridine (Glaxo), gentamicin (Roussel), tetracycline (Lederle), ampicillin (Beecham) and erythromycin (Abbot).

Standard solutions of these drugs were prepared and subjected to two fold serial dilution prior to incorporation of the latter in mueller Hinton agar.

Susceptibility testing

The inoculum was derived from a 24-hour culture of c. jejuni/coli grown on blood agar plates and emulsified in nutrient broth to attain a final dilution of approximately 10⁶ organisms per ml. With a multi-inoculator 2 to 3 ul of the diluted bacterial suspension was applied to the surface of the antibiotic agar plates. These were incubated for 48 hrs at 37⁰C in two separate gas jars containing 9% oxygen, 4.5% CO₂ and 86.5% nitrogen (Nitrogen atmosphere) and 7.5% oxygen, 9.5% CO₂ and 83% hydrogen (Hydrogen atmosphere). MICs were recorded as the lowest antibiotic concentration that gave no visible growth. The susceptibility of strains to cephaloridine and ampicillin was also studied by using 1/100 dilution of the standard bacterial suspensions (i.e.10⁶ organisms per ml).

Detection of beta-lactamase production

An iodometric method (Holt, 1981) was used for the detection of beta-lactamase. Both benzylpenicillin and cephaloridine were used at a dilution of 10000 ug/mi. Staphylococcus Aureus and Pseudomonas sp. were used as beta-lactamase positive controls against penicillin and cephaloridine respectively.

Results

Table I
Source and Biotypes of the 30 Campylobacter Strains

Source	C.Coli	C.Jejuni biotype 1	C.jejuni biotype 2
Human	4	5	2
Pigs	11	—	
Poultry	--	1	2
Cows	2	2	1

Table I summarises the results of biotyping of the 30 strains.

These data show that beta-lactam antibiotics had low activity against the tested Campylobacter strains.

The susceptibility of strains to cephaloridine and ampicillin was not significantly influenced by inoculum size

As far as the effect of incubation atmosphere on MICs is concerned, there was no detectable influence on Tetracycline MICs, only one strain showed a two-fold rise in the nitrogen atmosphere. However, the MIC values of erythromycin and Gentamicin did differ in the two atmospheres. The change in the Erythromycin MIC was a two-fold fall under the nitrogen atmosphere only three of the gentamicin MICs showed no change where as the remainder were higher when done in the nitrogen-atmosphere.

Table II Percentages of All The 30 Strains Inhibited at Various Concentrations of The Five Antibiotics.

Drug	% strains inhibited at various concentrations (Microgram/ml)											
	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
Cephaloridine	-	-	-	-	-	-	-	-	10	-	87	3
Ampicillin	-	-	-	3	14	70	3	3	7	-	-	-
Erythromycin	-	3	40	10	34	10	3	-	-	-	-	-
Gentamicin	13	44	34	9	-	-	-	-	-	-	-	-
Tetracycline	40	51	3	3	3	-	-	-	-	-	-	-

The production of beta-lactamase was tested with all the strains by using two substrates Viz penicillin and cephaloridine. Beta-lactamase production was detected in 26 out of 30 strains.

Discussion

The MICs of the 30 strains to three antibiotics were measured under two atmospheres and only erythromycin and gentamicin MICs showed some change under the two atmospheres. The change in the erythromycin MIC was 2-fold and was within the range of experimental error, though there was a tendency for the MICs to be lower in the nitrogen-atmosphere. Only three of the gentamicin MICs showed no change and the remainder were higher when done in the nitrogen atmosphere which suggests that the different conditions of growth affected the MIC to gentamicin.

The method used for the detection of beta-lactamase will only detect extra-cellular beta-lactamase and no relationship could also be established between beta-lactamase production and the beta-lactam antibiotics MIC values. A few strains did not produce any enzyme although the MIC values were very high. These results make it unlikely that beta-lactamase production is the sole mechanism of *Campylobacter* resistance to these antibiotics. Drug resistance could also be due to alteration in cell wall structure, resulting in non-penetration of the drug.

The data presented in this study shows high MICs to cephaloridine and ampicillin which agrees with the reports by other workers (Chow et al., 1978; Butzler et al., 1974; Karmali et al., 1981). The range of erythromycin MICs was 4-8 microgram/ml in four strains, which is not as high as has been reported by different workers. However, the rest of the strains showed MICs to erythromycin between 0.25 microgram per ml to 2 microgram per ml which show effectiveness of erythromycin in most of the cases as stated in various reports (Butzler et al., 1974).

All the strains were inhibited by gentamicin at 1 microgram per ml or less which is in general agreement with other data (Butzler et al., 1974; Karmali et al., 1981). Only one strain with an MIC level

of 100 microgram per ml has been reported so far (Butzler et al. ;1974). Tetracycline MIC level was 1 microgram/mi or less in 97% of the strains which supports the work of other investigators (Butzler et al.,1974; Chow et al, 1980).

High erythromycin MICs in the case of two strains from poultry and one from a pig, alongwith a high MIC to ampicillin and cephaloridine among animal strains correlates with the report by Van: hoof et al. (1981). They have also suggested the possibility that animal strain might be a potential epidemiological reservoir for the resistant strains, but a more extensive study would be needed to provide satisfactory evidence for this.

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