JULIFLORINE SUSCEPTIBILITY OF ANIMAL AND HUMAN ISOLATES OF CAMPYLOBACTER

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Aqeel Ahmad, Shahana Urooj Kazmi, Khurshid Ali Khan (Department of Microbiology, University of Karachi, Karachi -32,Pakistan.)
Viquar Uddin Ahmad, Sabiha Qazi (HEJ Institute of Chemistry, University of Karachi, Karachi -32,Pakistan.)

Abstract

Juliflorine, the main alkaloid isolated from Prosopis juliflora was tested for its antibacterial activity against both the Gram positive and Gram negative bacteria. Filter paper disks impregnated with juliflorine were placed on streaked agar medium. Zones of growth inhibition for Campylobacter spp occurred at 10 pg per disk. Other enteropathogens (including the species of Salmonella, Shigella, Vibrio, Eseherichia, Proteus, Pseudomonas, Klebsiella and Yersinia) were resistant to at least 30 pg per disk, with the exception of a strain of Aeromonas hydrophilia, which showed some inhibition at 30 pg per disk. Most of the 25 Campylobacter strains, which were isolated from human clinical and animal sources, showed zones of inhibition greater than 10mm with 10 pg of juliflorine (JPMA 38: 20 , 1988).

INTRODUCFION

Camp ylobacter jejuni is now recognized world wide as a major cause of bacterial diarrhea in man. The development of selective plating medium[1] and the use of micro-aerophiic (5% 02 , 10% CO2 and 85% N2) incubation has greatly improved the recovery rates of C. jejuni from clinical stool specimens. In general C. je/uni enteritis is a self-limiting disease, but sometimes the micro-organism provoke a more severe and prolonged illness. Complications, associated with C. fejuni enteritis, include reiter's syndrome[2], reactive arthritis[3] and Guillain Barre syndrome[4,5]. Seriously ill and septicemic patients have to be treated with appropriate antibiotics. The role of antibacterial agents in the therapy of diarrhea is somewhat controversial[6] although many of them are accepted as standard treatments. Due to the toxicity and increasing drug resistance among different enteropathogens, chemotherapy for most intestinal infections is very often considered as ineffective and even disadvantageous. An alternative source of therapeutic substances are the medicinal plants. Siddiqui and Murthi[7] reported in 1948 than aqueous and alcoholic extracts of Prosopis juliflora, a shrub that grows abundantly in Sind and Punjab provinces of Pakistan . [8] have antibacterial activity. The isolation of juliflorine, the main alkaloid of P. juliflora was first achieved by Ahmed et al.9 In an attempt to study the anti¬bacterial activity of juliflorine against 22 distinct bacterial species, we discovered a diagnostic characteristic useful in the presumptive identifi¬cation of Campylobacter spp.

MATERIAL AND METHODS

In vitro juliflorine susceptibility assay:— From a stock solution of julif7orine dihydrochloride (10 mg/ml in sterile distilled water) 20 p1 dilution containing 1, 5, 10, 20, 30 and 50 pg of juliflorine were prepared and applied to sterile 6 mm filter paper disks (Schleicher and Schuell, Inc., Keene, N.H.). The disks were dried at room temperature and stored at 4°C until use. One loopful of approximately 10^6 — 10^7 CPU (colony forming units) was streaked on to the surface of Mueller Hinton agar (oxoid) plates and the disks were aseptically applied and pressed into the agar surface. The plates of C. jejuni were incubated at 42°C under a microaerobic atmosphere for 48 hours and zones of inhibition were

measured. Penicillin, streptomycin, erythromycin, ampicihin and tetracycline were also used for comparative studies. Plates streaked with aerobic organisms were incubated at 37°C after applying appropriate disks.

RESULTS

Campylobacter strains were found to be highly susceptible to very low level of juhiflorine. Most of the strains used gave a zone of inhibition of more than 10 mm with the 10 pg disk. A total of 25 strains of C jejuni, C. coil and C laridis, three other species of Campy lobacter and 11 enteric bacteria commonly found in clinical stool samples were screened for juhiflorine susceptibility with impregnated disks. No significant differences were observed between isolates from clinical sources (n=9) and from meat soUrces (nl6) (Table I).

TABLE I

Zones of Inhibition of Gram Negative Bacteria.

Culture used	Source	Donor	Zones of inhibition in mm Juliflorine	
			30 µg	50 µg
Salmonella Schottmuelleri	Human	a	00	00
Shigella dysenteriae	Human	a	00	00
Shigella sonnei	Human	a	00	10
Proteus vulgaris	Human	a	00	09
Klebsiella oxytoca	Human	a	00	08
Escherichia coli	Human	a	00	10
Pseudomonas aeruginosa	Human	a	00	00
Vibrio cholera	Human	b	00	08
Vibrio parahaemolyticus	Human	b(2)	00	00
Yersinia enterocolitica	Human	c(2)	00	00
Aeromonas hydrophilia	Human	d(2)	11	22
Campylobacter jejuni	Human	e(2) f(11), g(2), h(1), i(1)	28	36
Campylobacter laridis	Human	j(1)	30	35
Campylobacter fetus subsp. intestinalis	Human	f(1)	27	32
Campylobacter jejuni	Chicken	k(8)	22	30
Campylobacter jejuni	Pork	k(3)	25	33
Campylobacter jejuni	Lamb	k(3)	24	29
Campylobacter fetus subsp. fetus	Sheep	f(1)	21	31
Campylobacter fetus subsp. venerealis	Cow	f(1)	21	30

a = K.A. Khan, University of Karachi, Karachi–32, Pakistan.
b = M. Voll, University of Maryland, College Park,MD, U.S.A.
c = T. Cook, University of Maryland, College Park,MD, U.S.A.
d = S. Joseph, University of Maryland, College Park,MD, U.S.A.
e = Navel Medical Research Institute, Bathesda, MD, U.S.A.
f = American type culture collection, Rockville, MD, U.S.A.
g = R.Kazmi, Childrens Hospital, Washington,U.S.A.
h = C. Buchrski, George town University Hospital, Washington,U.S.A.
i = M. Doyle, Food Research Institute, Madison Wisconsin, U.S.A.
j = B.S. Robertson, University of Maryland, College Park,MD, U.S.A.
k = N.J.Stern, Meat Science Research Lab. Bettsville,MD, U.S.A.

The average zones of inhibition were 34 mm and 30.6mm respectively, when the isolates were subjected to 50 pg disk of juliflorine. Non — C. fe/uni and Non — C. coil were also highly susceptible to 10 pg of juliflorine including C laridis which, like others[10] was found resistant to tetracycline and nalidixic acid and, in contrast, all other enteropathogens tested were resistant to more than 30 pg per disk with the exception of one strain of Aenomonas hydrophiiia which showed susceptibility at 20 pg disk. Most of the enteric bacteria tolerated 50 pg per disk while some responded slightly (Table 1). When juliflorine susceptibility of Campy lo¬bacter strains wasP compared with other antibiotics listed in Table II,

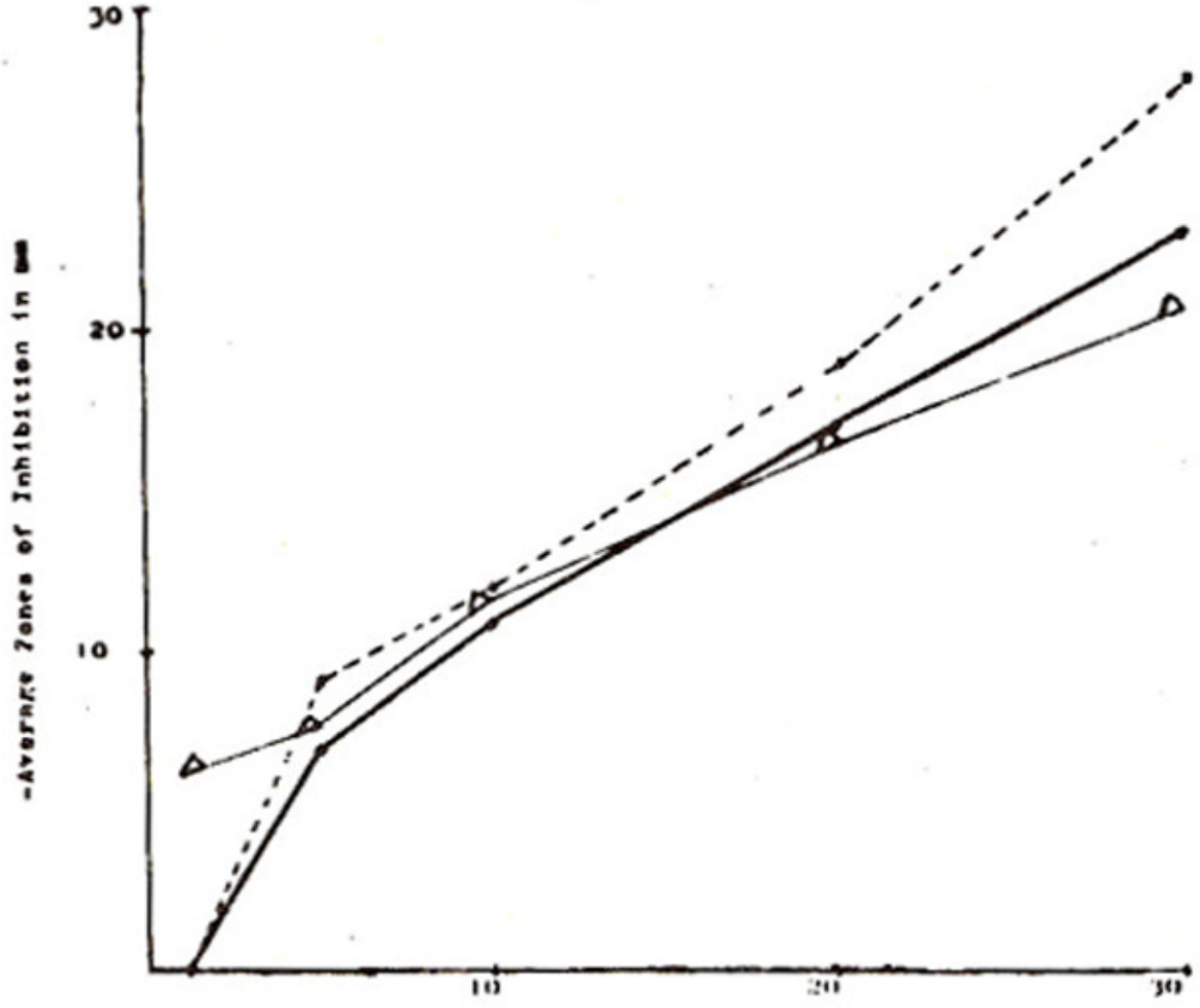
TABLE II

Comparison of Antibacterial Activity of Juliflorine with Antibiotics.

Culture used	Conc in μg	Zones of inhibition in mm					
		Juliflorine	Ampicillin	Erythro-mycin	Penicillin	Streptomy-cin	Tetracycline
Streptococcus pneumoniae	1	10	00	00	00	00	00
	5	12	00	00	00	08	00
	10	15	00	08	00	11	10
	20	17	00	10	10	18	13
	30	21	12	16	14	22	17
Streptococcus viridans	1	00	00	00	00	00	00
	5	00	00	00	00	00	00
	10	00	00	00	00	09	09
	20	08	00	00	08	11	12
	30	10	00	00	13	13	15
Streptococcus lactis	1	09	00	00	00	08	00
	5	12	10	07	10	11	09
	10	16	12	09	13	17	12
	20	18	16	12	17	21	17
	30	24	19	15	21	28	22
Staphylococcus epidermidis	1	00	00	00	00	10	10
	5	11	00	00	10	12	12
	10	15	11	00	13	18	16
	20	22	13	10	15	21	20
	30	27	18	12	20	27	26
Sarcina lutea	1	10	00	08	10	08	10
	5	12	10	10	12	12	13
	10	14	12	13	15	16	18
	20	18	15	15	19	21	23
	30	23	17	20	23	26	27
Bacillus megaterium	1	00	10	00	13	08	09
	5	00	13	07	17	10	15
	10	10	17	10	20	16	18
	20	16	20	17	23	20	20
	30	20	26	20	28	26	24
Campylobacter Jejuni	1	00	00	00	00	00	00
	5	09	00	00	00	00	00
	10	12	00	00	00	12	00
	20	19	00	00	08	17	09
	30	28	00	00	11	28	11

majority of the strains were resistant to erythromycin and ampicillin (30 ug/disk). Tetracycline and penicillin produced some inhibition giving a zone of 12 mm around a disk of 30 ug.C. jejuni were found to be equally susceptible to juliflorine and streptomycin. A 30 ug disk of both the juliflorine and streptomycin produced a 28 mm zone of inhibition. Against Gram positive bacteria, juliflorine was

found to be significantly effective against Streptococcus pneumoniae, S. lactis, Staphylococcus epidermidin, Sarcina lutea, Bacillus megaterium while Streptococcus viridans responded slightly. The effectiveness of juliflorine against S. lactis, S. pneumoniae and S. epidermidis exceeded that of ampicillin, erythromycin, penicillin and tetracycline and matched or slightly less than streptomycin. S. viridans was found to be resistant to ampicillin and erythromycin but responded poorly to juliflorine. Penicillin, streptomycin and tetracycline are comparatively more effective. Both juliflorine and penicillin are more effective than ampicillin and erythromycin against S. lutea but less effective than streptomycin and tetrac¬ycline. Activity of juhflorine against B. mega¬terium was found identical with erythromycin but less than the other antibiotics used. Comparison is also made between Campy¬lobacter of man and animal sources and Gram positive and Gram negative bacteria (Figure 1).



– Concentration of Juliflorine in μg–

Figure 1. Comparative Antibacterial activity of Juliflorine.
– – – – *Campylobacter Jejuni* (Human Source)
———— *Campylobacter Jejuni* (Animal source)
Δ Gram positive *Gram Negative Bacteria

DISCUSSION

The antibacterial activity of juliflorine, an alkaloid isolated from a medicinal plant Prosopis fuliflora has been tested against 16 Grain negative and 6 Gram positive bacteria Campylobacter spp, Streptococcus lactis, S. pneumoniae, S. viridans, Staphylococcus epidermidis, Sarcina lutea and Bacillus megaterium were found to be highly susceptible to the alkaloid in low concentrations (10 pg per disk) as compared to antibiotic used (Table II). Enteritis due to C. jejuni is usually a mild disease, self-limiting in most cases. However, antibiotic therapy may be indicated in the severe cases, in patients with prolonged illness. The current drug of choice is probably erythromycin which is known for its low toxicity but the prevalence ot resistant strains may be a serious problem. Most of the strains we used in this study were found to be resistant at 30 pg erytluomycin per disk and showed some inhibition with same concentration of tetracycline and penicillin (11mm zone/30 pg disk). The inhibitory effect of juliflorine on Campylobacter growth is almost identical with streptomycin (28 mm zone / 30 pg disk). Ampicillin, sulfonamide and cotrimaxazole were found to be moderately active against Campylobacter spp. The use of tetracycline, chloramphenicol and some other drugs is probably limited because of the risk of serious side effects and their toxicity. Microbial resistance[11] due to indiscriminate use of antibiotics is a constant threat to the usefulness of almost any drug. We suggest that juliflorine, a naturally occurring plant alkaloid may be used as therapeutic drug to control chronic Campylobacter infections. Since juliflorine, effectively inhibited the growth of Gram positive bacteria it can also be used against infection with these bacteria. However, this new compound as well as other need to undergo carefully controlled clinical trials in order to evaluate their usefulness in daily medical practice. The juliflorine has already been evaluated for carcinogenicity in Ames Salmonella/Microsomal test system and it has been established that a dose of upto 500 ug/plate is nonmutagenic and hence non-carcinogenic[12]. Juliflorine susceptibility was found to be a very stable characteristic of Campylobacter spp and most of the enteric bacteria that cause diarrhoea or dysentry like infections were highly resistant to this alkaloid (Table 1). The presence of C. je/uni in stool or food sample can be arrested by the inhibition of growth around a 10 pg juliflorine disk. Juliflorine susceptibility of Carnpylobacter spp may also prove useful as a diagnostic or differential characteristic between Campylobacter spp and other potential enteropathogens.

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