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YERSINIA ENTEROCOLITICA: VIRULENCE MARKERS AND ANTIBIOGRAM

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SUMMARY

Thirty four strains of *Yersinia enterocolitica* belonging to six sero-/biovars isolated from apparently healthy animals were screened for virulence markers and antimicrobial susceptibility test. Out of 34 isolates of *Yersinia enterocolitica*, 21 (81.8%) were positive for production of heat stable enterotoxin, 3 isolates (8.8%) positive for mouse lethality test and 1 isolate (2.9%) for guinea pig conjunctivitis. All isolates were surprisingly negative for plasmids analysis and autoagglutination test (FU Berlin). Antibiogram of isolates showed that chloramphenicol, colistin, and tetracycline were the most effective amongst antimicrobials against isolates. All were resistant to ampicillin, carbenicillin, methicillin and penicillin G by disc diffusion method.

INTRODUCTION

Yersinia enterocolitica- now classified as a member of the Family Enterobacteriaceae - was recognized as a distinct species in 1964. It has been isolated from man and animals, and from some human foods (Morris and Feeley, 1976). *Yersinia enterocolitica* is capable of causing a variety of diseases both in animals and man. These include gastroenteritis, septicaemia, acute polyarthritis, erythema nodosum, acute mesentric lymphadenitis and terminal ileitis closely resembling appendicitis (Mittal and tizard, 1981).

Most human pathogenic strains belong to serovars O: 3, O:8 and O:9 and biovars 2,3 and 4 (WHO Scientific Working Group, 1980). The pathogenicity of other serovars of *Yersinia enterocolitica* isolated from animals, birds, food, water and environment was a contraversial issue (Swaminathan et al., 1982).

Schiemann and Devenish (1982) found that the invasiveness of *Yersinia enterocolitica* was restricted to certain serovars or biovars.*Yersinia enterocolitica* virulence is a complex phenomenon. Cornels et al. (1987) recorded a number of distinct chromosomal and plasmid

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gene sequences result in the overall elaboration of the pathogenic or virulent phenotypes. Delor and Cornelis (1992) observed that the enterotoxin (Yst) was a major factor involved in the *Yersinia enterocolitica* associated diarrboea in the young rabbits.

Markova et al. (1993) observed that the susceptibility to antibiotics of *Yersinia enterocolitica* grown at 37°C had increased than when grown at 25°C. The suscepticility to kanamycin, cephalothin, tetracyclin and chloramphenicol of *Yersinia enterocolitica* was also influenced by growth medium and gas composition.

There is no published information on the virulence markers and antibiogram of *Yersinia enterocolitica* strains isolated from different animal species in Egypt. The present study was undertaken to remedy this omission.

MATERIAL AND METHODS

Bacterial strains:

The 34 strains of *Yersinia enterocolitica* used this study were provided by Tanios, A.I., Anim Health research Institute. All had been original isolated from apparently healthy cows, buffalo sheep and pigs almost sent for slaughter Basattin abattoir in Cairo. These isolates we identified by biochemical reactions (Bercovie and Mollaret, 1984), biotyping and serotypin (Wauters et al., 1987). All had been related to sero-/biovar O6/1A, 2 each of O8/1A and O9/ and O10 1A and one isolate sero-/biovar O8/1B (Table 1).

Reference strain of Yersinia enterocolitica:

Reference *Yersinia enterocolitica* serovars O:3 was kindly supplied by Dr. Szita Josef, National Institute of Hygiene, Budapest, Hungary.

Table (1) : Origin , sources and types of *Yersinia enterocolitica* used .

Animal species	Specimens	No. of isolates	Sero-/biovars					
			5/ 1A	6/ 1A	8/ 1A	O10/ 1A	O8/ 1B	O9/ 2
Cows	Rectal/colon contents	5	5	-	-	-	-	-
Buffaloes	Rectal/colon contents	6	3	-	1	1	-	1
Sheep	Rectal/colon contents	6	4	2	-	-	-	-
Pigs	Rectal/colon contents	6	3	-	-	1	1	1
	Oral / throat swabs	11	9	1	1	-	-	-
Total	34	34	24	3	2	2	1	2

Media for virulence assays:

Medium for preparation of enterotoxin: (Pai and Morse, 1978).

Brain heart infusion broth (Oxoid, CM 255), tryptone soya agar (Oxoid, CM 131) and Plate count agar (Biomerieux, 51831).

Media for antimicrobial susceptibility test:

Muller Hinton medium (Difco, 0252-01) and tryptic soya broth (Difco, 037-01).

Laboratory animal:

Infant mice (2-4 day old mice for enterotoxin assay), adult mice (6-8 week old mice weighting 20-25 g. for mouse lethality test) and adult guinea pigs (6-7 week old guinea pigs for invasiveness assay).

Antimicrobial susceptibility discs:

The folowing antimicrobial dics (Difco) were used: ampicillin (10 mcg), carbenicillin (100 mcg), cephalothin (30 mcg), chloramphenicol (30 mcg), colistin (10 mcg), erythromycin (15 mcg), gentamicin (10 mcg), kanamycin (30 mcg), methicillin (5 mcg), nalidixic acid (30 mcg), neomycin (30 mcg), nitrogurantoin (300 mcg), penicillin G (10 u), streptomycin (10 mcg), tetracycline (30 mcg) and trimethoprim/sulfamethoxazole (1.25/23.75).

Pathogenicity tests:

All *Yersinia enterocolitica* isolates as well as a reference strain (positive control) were tested by infant mouse test (Pai and Morse, 1978), mouse lethality test (Kay et al., 1983) and Sereney test (Schiemann and Devenish, 1982).

All isolates were kept in sterile screw capped bottles containing semi-solid 0.5% agar media and sent to Prof. Dr. Sc. Horsch F. and Dr. Nattermann, H. Institute fur Mikrobiologie und Tierseuchen, Standort Mitte, Fachbereich Veterinarmedizin, Frei Universitat, Berlin, Germany for detection of plasmid (kado and Liu, 1981) and autoagglutination (Laird and Cavanaugh, 1980).

The antimicrobial susceptibility testing was performed to *Yersinia enterocolitica* isolates according to the disc and agar diffusion emthod (Bauer et al., 1966).

RESULTS

Regarding the relationship of sero-/biovars to virulence of *Yersinia enterocolitica* (Table 2) it was clear that all sero-/biovars O5/1A, O6/1A and O10/1A isolates were negative in mouse lethality and guinea pig conjunctivitis tests. 13 of 24 sero-/biovar O5/1A isolates, 2 of 3 sero-/biovar O6/1A, 1 of 2 sero-/biovar Ol1/1A and all of the sero-/biovar O8/1A, O8/1B and O9/2 produced heat stable enterotoxin Sero-/biovar O8/1B isolate was lehtal to mice and produced enterotoxin in guinea pigs.

Reviewing the relationship between source of isolation and virulence of *Yersinia enterocolitica* (Table 3) indicated that virulent strains were more prevalent in porcine and buffalo isolates than cows and sheep.

Generally, out of a total of 34 isolates, 21 (61.8%) produced heat stable enterotoxin, 2 isolates (8.8 %) were lethal to mice and 1 isolate (2.9%) produced guinea pig conjunctivitis.
Surprisingly, all isolates were negative for plasmid and autoagglutination tests (FU Berlin).

The antimicrobial susceptibility of 34 *Yersinia enterocolitica* isolated from animals is presented in (Table 4), it was found that all isolates were susceptible to chloramphenicol, colistin and tetracycline isolates were susceptible to chloramphenicol, colistin and tetracyc... Moreover, most isolates were susceptible... nitrofurantoin and trimethoprim sulfamethoxazole. On the other hand, all Yer... *enterocolitica* isolates were resistant to ampici... carbenicillin, erythromycin, methicillin a... penicillin G. However, most isolates we... resistant to cephalothin. *Yersinia enterocoli...* showed susceptibility to streptomycin, nalidi... acid, kanamycin, neomycin and gentamicin.

The results indicate that *Yersinia enterocolitic...* varied in their susceptibility to antimicrobia... agents. This variation was observred not only among the different sero-/biovars but also among the various isolates of the same sero-/biovar (Table 5).

Table (2) : sero-/biovars groupig and virulence of *Yersinia enterocolitica*.

sero-/biovars	No. tested	No. positive for *		
		ST	MI	GPC
O5/1A	24	13(54.2 %)	0	0
O6/1A	3	2 (66.7 %)	0	0
O8/1A	2	2 (100 %)	0	0
O10/1A	2	1 (50 %)	0	0
O8/1B	1	1 (100 %)	1 (100 %)	1 (100 %)
O9/2	2	2 (100 %)	2 (100 %)	0
Total	34	21(61.8 %)	3(8.9 %)	1 (2.9 %)

* ST = production of heat stable enterotoxin .
ML = mouse lethality test .
GPC = guinea pig conjunctivitis .
( ) = percent of positive .

Table (3) : Source of isolation and virulence of *Yersinia enterocolitica* .

Source of isolation	No. tested	No. positive for *			All negative
		ST	ML	GPC	
Cows	5	3 (60 %)	0	0	2
Buffaloes	6	4 (66.7 %)	1 (16.7 %)	0	1
Sheep	6	3 (50 %)	0	0	3
Pigs	17	11(64.7%)	2 (11.8 %)	1 (5.9 %)	3
Total	34	21(61.8%)	3(8.8 %)	1 (2.9 %)	9

* See above footnotes .

Table (4) : Antimicrobial susceptibility of 34 *Yersinia enterocolitica* strains isolated from animals .

Antimicrobial agents	Susceptibility *					
	R.		In.		S.	
	No.	%	No.	%	No.	%
Ampicillin	34	100	-	-	-	-
Carbenicillin	34	100	-	-	-	-
Cephalothin	30	88.2	4	11.8	-	-
Chloramphenicol	-	-	-	-	34	100
Colistin	-	-	-	-	34	100
Erythromycin	34	100	-	-	-	-
Gentamicin	11	32.4	9	26.5	14	41.2
Kanamycin	9	26.5	10	29.4	15	44.1
Methicillin	34	100	-	-	-	-
Nalidixic acid	6	17.6	7	20.6	21	61.8
Neomycin	10	29.4	11	32.4	13	28.2
Nitrofurantoin	-	-	-	-	34	100
Penicillin G	34	100	-	-	-	-
Streptomycin	5	14.7	7	20.6	22	64.7
Tetracycline	-	-	-	-	34	100
Trimethoprim / sulfamethoxazole	3	8.8	8	23.5	23	67.6

* R= susceptibility In = intermediate S = susceptible

Table (5) : Antibiogram of different sero-/biovars of *Yersinia enterocolitica*

Antimicrobial agents	*Yersinia enterocolitica* sero-/biovars																	
	O5/1A 24 isolates			O6/1A 3 isolates			O8/1A 2 isolates			O10/1A 2 isolates			O8/1B 1 isolate			O9/2 2 isolates		
	R.	In	S.	R.	In	S.	R.	In	S.	R.	In	S.	R.	In	S.	R.	In	S.
Ampicillin	24	-	-	3	-	-	2	-	-	2	-	-	1	-	-	2	-	-
Carbenicillin	24	-	-	3	-	-	2	-	-	2	-	-	1	-	-	2	-	-
Cephalothin	22	2	-	2	1	-	2	-	-	2	-	-	-	1	-	2	-	-
Chloramphenicol	-	-	24	-	-	3	-	-	2	-	-	2	-	-	1	-	-	2
Colistin	-	-	24	-	-	3	-	-	2	-	-	2	-	-	1	-	-	2
Erythromycin	24	-	-	3	-	-	2	-	-	2	-	-	1	-	-	2	-	-
Gentamicin	8	4	12	1	2	-	1	1	-	-	1	1	1	-	-	-	1	1
Kanamycin	6	8	10	-	1	2	2	-	-	-	1	1	1	-	-	-	-	2
Methicillin	24	-	-	3	-	-	2	-	-	2	-	-	1	-	-	2	-	-
Nalidixic acid	1	5	18	1	2	-	2	-	-	2	-	-	-	-	1	-	-	2
Neomycin	6	7	11	2	1	-	1	1	-	-	1	1	1	-	-	-	1	1
Nitrofurantoin	-	2	22	-	-	3	-	-	2	-	-	2	-	-	1	-	-	2
Penicillin G	24	-	-	3	-	-	2	-	-	2	-	-	1	-	-	2	-	-
Streptomycin	-	5	19	3	-	-	2	-	-	-	1	1	-	1	-	-	-	2
Tetracycline	-	-	24	-	-	3	-	-	2	-	-	2	-	-	1	-	-	2
Trimethoprim / sulfamethoxazole	2	4	18	1	2	-	-	1	1	-	-	2	-	-	1	-	1	1

* See above footnotes .

DISCUSSION

Increased awareness of *Yersinia enterocolitica* in human and animals has stimulated interest about characteristics of this bacterium. As regards to the relationship of sero-/biovars to virulence of *Yersinia enterocolitica* in this study (Table 2), it is shown that each of the sero-/biovar combinations was positive in one or more of virulence assays. This conforms with these of (Cornelis et al., 1987; Wauters et al., 1987 and Robins-Browne et al., 1989) who found sero-/biovars O8/IB, O9/2 and O8 and O8/1A were more pathogenic than other sero-/biovars.

The data obtained indicated that most strains isolated even though from apparently healthy animals were positive in one or more virulent test assays suggesting that they might have been virulent. However, it is unwise - based on data of this work to conclude or relate the results of virulence assays to virulence in animals or humans. This assumption conforms with conclusions drawn in a series of publications that pointed out pathogenicity differences among Yersinias, confirmed the compelx nature of virulence in *Yersinia enterocolitica*, and confirmed that no single current assay was correlated with virulence of *Yersinia enterocolitica* (Kay et al., 1993).

The relationship between source of isolation and virulence of *Yersinia enterocolitica* (Table 3) indicated that virulent strains were more prevalent in porcine and buffalo isolates than cows and sheep Although most isolates of *Yersinia enterocolitica* isolated from pigs were considered pathogenic (Doyle et al., 1981), some auth found that most strains of *Yersinia enterocoli* isolated from pigs are of environmental ori and are very seldom involved in human infec (Hunter et al., 1983; Harmon et al., 1984 a Okoroafor et al., 1988).

Adesiyun et al.(1986) indicated that cattle a pigs have the potential to transmit virulent strai of *Yersinia enterocolitica* to human beings Nigeria. Moreover, Slee and Skilbeck, (199 found that infection with *Yersinia enterocoliti* persisted for up to 29 weeks in sheep an suggested that sheep are a maintenace host for thi organism in Australia.

In this study, all isolates were surprisingly negative for plasmid and autoagglutination test (FU Berlin). Although there are close relation between the presence of 44 Megadalton plasmid and calcium dependency or autoagglutination to virulence of *Yersinia enterocolitica* (Kaneko and Maruyama, 1986 and robins-Browne et al. et al., 1989), plasmid is easily lost due to subculture in laboratory from originally virulent isolates of *Yersinia enterocolitica* (Cornelis et al.).

Table (4) shows the autimicrobial susceptibility of 34 of *Yersinia enterocolitica* isolated from animals. These results were nearly similar to those obtained by (WHO Scientific Working Group, (1980; Baker and Farner, 1982 and Okoroafor et al., 1988).

Although the differences in behaviour of most sero-/biovars (Table 5) to different antimicrobial agents seemed to be insignificant, clear difference

was observed to susceptibility of the aminoglucosides. The antimicrobial susceptibility test result is comparable with the results obtained from Adesiyun et al., (1992) and Markova et al., (1993). The emergency of resistance of *Yersinia enterocolitica* could by attributed to continuous and haphazard use of some antibiotics in veterinary practice.

To our knowledge, this work is considered the first record in virulence features of *Yersinia enterocolitica* isolates in Egypt.

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