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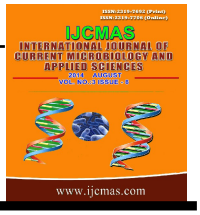

Original Research Article

Antimicrobial resistance profile of *Yersinia enterocolitica* and *Yersinia intermedia* isolates from retail pork

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Keywords

Yersinia enterocolitica, *Yersinia intermedia*, Antimicrobial resistance, Pork

A B S T R A C T

The present study was conducted to evaluate the resistance rate against antimicrobial agents of *Yersinia enterocolitica* and *Yersinia intermedia* isolates obtained from pork samples collected from different retail markets in Thrissur and Eranakulam, in Kerala. Isolates were subjected to antimicrobial resistance testing by the disc diffusion method. *Yersinia enterocolitica* isolate showed resistance to Ampicillin, Naidixic acid and Tetracycline. *Yersinia intermedia* was sensitive to all the antibiotic tested. In Yersinia, antimicrobial resistance rates are stated to be rare. Hence, antimicrobial resistance in the above-mentioned bacteria is now considered as a major public health issue.

Introduction

Antimicrobial resistance has always been a major concern for nosocomial infections in hospital environments. Since drug resistance in zoonotic pathogens has affected the therapeutical intervention in humans, antimicrobial resistance in food-borne pathogens has become a public health issue. The use of anti-infectives for therapeutic, prophylactic and for growth promotion has raised questions about the development of resistant microbes in food animals followed by a possible transfer to humans via the food chain. In the present study, we analyzed the antimicrobial resistance profile of Yersinia isolates obtained from retail pork samples.

Yersinia enterocolitica is known as a psychotropic waterborne and foodborne enteropathogen. This microorganism can grow to large numbers at refrigeration temperatures, so meat, chicken, milk, cheese contaminated with that organism could become a significant health risk for consumers (Simonova et al., 2008).

Bacteria belonging to *Yersinia intermedia*, have been isolated from the environment, various animals, food and sometimes, healthy and sick humans, mainly from their stools (Martin et al., 2009). Although strains of *Y. intermedia* were usually considered non-pathogenic for human, it has been suggested that they may become host adapted for humans as opportunistic pathogens and be clinically significant in future (Atobla *et al*., 2012).

Yersinia enterocolitica is primarily a gastrointestinal tract pathogen has become a major cause of diarrhoea in most of the industrialised world (Bolton et al., 2013). It causes a broad range of diseases from acute bowel disease to extra intestinal manifestation such as reactive arthritis, uveitis and sepsis (Bottone, 1999).

Antimicrobial treatment of human yersiniosis is in most cases not necessarily indicated. However, systemic and extraintestinal infections and enterocolitis in immune-compromised patients require antibiotic therapy (Mayrhofer et al. 2004).

In Yersinia antimicrobial resistance rates are stated to be rare. So the antimicrobial resistance of *Y. enterocolitica* is now considering as a major public health issue. The aim of this study is to investigate the antimicrobial susceptibility pattern of one isolate each of *Yersinia entrocolitica* and other *Yersinia species* isolated from retail pork samples.

Materials and Methods

Yersinia isolates were examined for their antimicrobial drug susceptibility/resistance pattern by disc diffusion method, against different antibiotics using antibiotic discs (HiMedia). The isolates were tested against Ampicillin (2µg), Cefoperazone (75 µg), Ceftriaxone (30µg), Cefotaxime (30µg), Chloramphenicol (10µg), Ciprofloxacin (5µg), Colistin (10 µg), Enrofloxacin (5µg) Gentamicin (30µg), Nalidixic Acid (30µg), Sulphafurazone (300 µg), Streptomycin (10µg), and Tetracycline (30µg). The turbidity standard solution was prepared by adding 0.5 ml of 0.048 M $BaCl_2$ to 99.5 ml of 0.36 N H_2SO_4 (one per cent w/v). This solution was equal to half the density of No.1 Mac Farland standard solution. This solution was taken into glass tube, sealed tightly and kept in the dark, at room temperature for further use. The tube was vigorously agitated just before each use.

Inoculation

Three to four isolated colonies were selected from a pure culture and transferred into sterile nutrient broth and incubated at 37^0C, overnight. The turbidity of culture was adjusted using solution having half the density of Mac Farland standard No.1. The swab was dipped into standardised inoculum and excess inoculum was removed from the swab by rotating it several times with a firm pressure on the inside wall of the test tube, above the fluid level. The Mueller Hinton agar (HiMedia) plates were inoculated by swabbing over its entire surface, within 15 min. after adjusting the density of inoculum. The swabbing procedure was repeated two more times, rotating the plates approximately 60^0 at each time, to ensure an even distribution of inoculum. The inoculum was allowed to dry for 15 min.

Application of Antibiotic Discs:

The discs were applied to the surface of the inoculated agar with a sterile forceps. With the tip of the forceps, each disc was gently pressed down to ensure complete contact with the agar surface. The inoculated plate was inverted and incubated at 37 ^{0}C for 18 h., within 15 min. after the application of the discs. At the end of the incubation period, the plates were examined and the diameter of the zones of complete inhibition was measured to the nearest whole millimeter with a scale held on the back of the Petri-plate, which was illuminated with a reflected light. The clinical breakpoints for *Y. enterocolitica* susceptibility testing were defined according to the Clinical and Laboratory Standard Institute (CLSI, 2010) and the isolates were grouped as sensitive,

intermediary sensitive and resistant, against each antibiotic.

Results and Discussion

The antibiotic sensitivity pattern of *Yersinia* isolates from pork is represented in table -1. The isolate of *Y. enterocolitica* obtained from pork was sensitive to Cefoperazone, Chloramphenicol, Colistine, Enrofloxacin, Gentamicin, Streptomycin and Sulphurazone. The isolate was resistant to Ampicillin, Naidixic acid and Tetracycline. Intermediate response was shown towards Cefotaxime, Ceftriaxone and Ciprofloxacin. *Yersinia intermedia* isolate was sensitive to Cefoperazone, Cefotaxime, Chloramphenicol, Colistine, Enrofloxacin, Gentamicin, Streptomycin, and Sulphafurazone. Intermediate response was shown towards Ampicillin, Ceftriaxone, Ciprofloxacin, Nalidixic acid and Tetracycline.

The emergence of drug resistant bacteria and special antimicrobial resistance patterns has raised increasing concern among the public health. Antibiotic susceptibility tests are used to find out the antibiotic resistance profile of different bacteria. In the present study, the susceptibility test was performed for two isolates of Yersinia by standard disc diffusion method using 13 commonly used antibiotics.

Antibiogram of the *Y. enterocolitica* isolate revealed that, it was sensitive to Cefoperazone, Chloramphenicol, Colistine, Enrofloxacin, Gentamicin, Streptomycin and Sulphurazone. The isolate has shown resistant to Ampicillin, Naidixic acid and Tetracycline. *Yersinia intermedia* isolate was sensitive to Cefoperazone, Cefotaxime, Chloramphenicol, Colistine, Enrofloxacin, Gentamicin, Streptomycin, and Sulphafurazone.

Table.1 Antibiotic sensitivity pattern of Yersinia isolates from pork

SI No	Antibiotic (µg/ disc)	*Y. enterocolitica*	*Y. intermedia*
1	Ampicillin (10)	R	I
2	Cefoperazone (75)	S	S
3	Cefotaxime (30)	I	S
4	Ceftriaxone (30)	I	I
5	Chloramphenicol (10)	S	S
6	Ciprofloxacin (5)	I	I
7	Colistine (10)	S	S
8	Enrofloxacin (5)	S	S
9	Gentamicin (30)	S	S
10	Nalidixic acid (30)	R	I
11	Streptomycin (10)	S	S
12	Sulphafurazone (300)	S	S
13	Tetracycline (30)	R	I

R- resistant, I- intermediate , S- sensitive

Resistance of *Y. enterocolitica* against Tetracyclin was reported by Simonova *et al*. (2008) which is in accordance with the present study. Pandove *et al*. (2012) observed that *Y. enterocolitica* isolates were resistant to Ampicillin, Nalidixic acid and Ciprofloxacin. In the present study also, the organism was showing resistance to Ampicillin and Nalidixic acid where intermediate sensitivity to Ciprofloxacin. Bolton *et al*. (2013) observed that all 10 isolates of *Y. enterocolitica* were resistant to Sulphonamides and Tetracycline. Whereas resistance to Tetracycline and susceptibility to Sulphurazone was observed in this study. Resistance to Tetracycline was observed by many researchers (Mayrhofer *et al*. 2004; Kot and Rainko, 2009; Bolton *et al*., 2013) which are in agreement with the present study. A high tendancy of antibiotic resistance was observed among *Y.enterocolitica* when Yazdi *et al.* (2011) screened 60 isolates of *Y. enterocolitica* and reported that 56 per cent

isolates were resistant to two or more antibiotics tested. All the *Y. intermedia* isolates screened by Yazdi *et al.* (2011) were sensitive to Chloramphenicol, Ciprofloxacin, Cefotaxime and Gentamicin. In the present study, also *Y. intermedia* isolate was showing sensitivity towards Chloramphenicol, Cefotaxime and Gentamicin whereas intermediate response to Ciprofloxacin.

A considerable variation can be observed in the antibiotic resistance profile of the organisms in general. The indiscriminate use of antibiotics may lead to the development of resistance to most currently used antibiotics and their resistance gene can be transferred to other pathogenic organisms present in gastrointestinal tract (Thong and Modarressi, 2011). This process may have undesirable clinical implications within human and livestock population having contact with such resistant pathogens.

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