Study of the Behavior of Some *Yersinia enterocolitica* Strains Susceptible to Disinfectants and Antibiotics Isolated from Swine

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Abstract
The species from *Yersinia* genus are widespread in nature, they could be isolated from warm-blooded and cold-blooded animals, from foods, water and soil. Among genus species, *Yersinia enterocolitica* is most frequently isolated from human and animals. The resistance of *Yersinia enterocolitica* is similar to *Yersinia pseudotuberculosis*. Moreover, it resists to refrigeration temperature (+4°C). It is susceptible to streptomycin, chloramphenicol, tetracyclines, polymyxin B and colistin sulfate. Also, *Yersinia enterocolitica* is easily inactivated by sodium nitrite and sodium nitrate added in foodstuffs. It still shows some resistance to these salts, in solution, and could tolerate a 5% NaCl concentration in culture media. Toora shows that adding a 5% NaCl in food could lead to a growing rate reduction. In our research we study the behavior of *Yersinia enterocolitica* strains isolated from swine feces, on different isolation and identification media, chlorine tolerance, but also the behavior against eleven anti-infectious substances (nalidixic acid, furazolidone, erythromycin, tetracycline, gentamicin, streptomycin, ampicillin, kanamycin, cefalotin, trimethoprim and enrofloxacin).

Keywords: antibiotics, disinfectants, susceptible, swine, *Yersinia enterocolitica*.

Introduction

The determination of metabolic properties of *Yersinia enterocolitica* specie has a distinctive importance, being a base procedure for identification. Commonly, *Yersinia enterocolitica* is moderate sensible to the action of environmental factors, including antibiotics, physical and chemical factors [1,2,3]. As it is well known, the main way to limit losses in *Yersinia enterocolitica* infections is therapy based on antibiotics, due to lack of means of active immunoprophylaxis (vaccines) or passive immunoprophylaxis (specific sera). It is sensible to streptomycin, chloramphenicol, tetracycline, polymyxin B, colistin sulfate etc. [4, 5].

Yersinia enterocolitica is easily inactivated by the sodium nitrate and sodium nitrite added in foodstuffs. It still shows some resistance to these salts, in solution, and can also tolerate a sodium chloride concentration up to 5% in culture media [6].

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2. Materials and methods

The researches were conducted on 17 strains of Yersinia enterocolitica isolated from swine. In order to study the tolerance of Yersinia enterocolitica strains to some chlorine variations, and to some antibiotics, these were reinoculated in nutrient broth and nutrient agar. The strains were initially morphological and cultural examined, as morphological expression of germs belonging to Yersinia genus own a very important guidance role in the species identification process, but have also an almost pathognomonic value in diagnostic.

3. Results and discussion

Chlorine tolerance

Chlorine tolerance study was conducted on five Yersinia enterocolitica strains. The strains were grown on nutrient agar slant, the developed colonies were taken by washing with sterile distilled water. The cells suspension was diluted at a concentration of 10^3, 10^4, 10^5 şi 10^6 cells / ml for each strain. From each strain dilution 1 ml was taken, the volume was passed on distiled water, and were added different chlorine doses, depending on the density of cells/ml (Table 1).

Table 1. Chlorine doses used

CFU/ ml	Chlorine dose
10^3	4 ppm
	1 ppm
	0,3 ppm
10^4	8 ppm
	2 ppm
	0,7 ppm
10^5	20 ppm
	7 ppm
	2,5 ppm
10^6	35 ppm
	12 ppm
	4 ppm
10^7	50 ppm
	25 ppm
	7,5 ppm

Table 2. Tolerance of Yersinia enterocolitica strains at different chlorine concentrations

CFU/ml	Chlorine dose/ml	Inactivation time (minutes)
10^3	4 ppm	1
	1 ppm	10
	0,3 ppm	20
10^4	8 ppm	1
	2 ppm	10
	0,7 ppm	30
10^5	20 ppm	1
	7 ppm	10
	2,5 ppm	30
10^6	35 ppm	1
	12 ppm	10
	4 ppm	30
10^7	50 ppm	1
	25 ppm	10
	7,5 ppm	30

The obtained results reveal that the inactivation time depends both on the chlorine dose and the bacterial cells load (Table 2). Thus, Yersinia enterocolitica strains in a dilution of 10^3 cells / ml were inactivated under chlorine action in one minute at a concentration of 4 ppm / ml, for a dilution of 10^7 cells / ml the inactivation occurs only at a 50 ppm / ml chlorine concentration. It was also found that with increasing contact time, regardless of the cells / ml load, the chlorine dose needed for inactivation decreases (Figure 1). Study regarding the sensitivity of some Yersinia enterocolitica strains to some anti-infective substances.

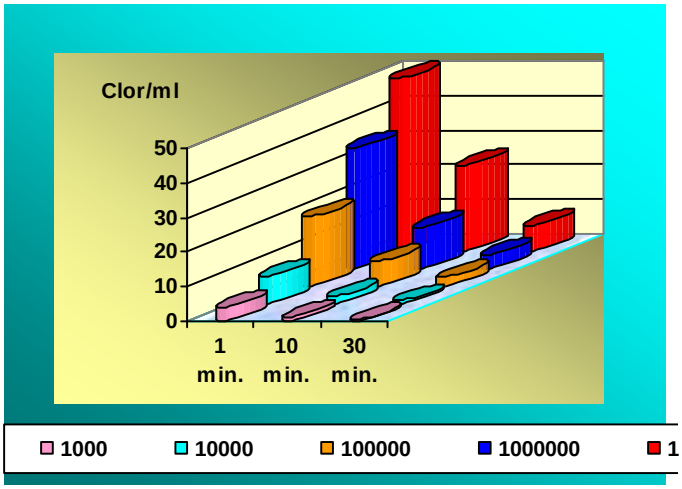

Figure 1. Relationship between exposure time and chlorine dose

As it is well known, the main way to limit losses in Yersinia enterocolitica infections is therapy based on antibiotics, due to lack of means of active immunoprophylaxis (vaccines) or passive immunoprophylaxis (specific sera).

The researches were conducted on twelve Yersinia enterocolitica strains isolated from swine and have been tested regarding their behavior against eleven anti-infectious substances (nalidixic acid, furazolidone, erythromycin, tetracycline, gentamicin, streptomycin, ampicillin, kanamycin, cefalotin, trimethoprim and enrofloxacin).

The antibiotic sensitivity determination was made by antibiogram test, based on germs growth inhibition in contact with various doses of antibiotics on adequate media.

The diffusion method was used as work protocol, using tablets with standard content of substance. The culture medium (Mueller – Hinton agar) was distributed in a quantity of 16 ml per Petri dish. After keeping the medium for 15 minutes in thermostat in order to remove excess moisture and for drying the medium surface, the plates were flooded with bacterial suspension (standardized to 10^7 cells / ml) and left in contact for five minutes, then the suspension excess was removed.

The inoculated medium was reinserted in thermostat, with the caps of the Petri dishes opened, for 25 minutes, for drying. The micro-tablets were distributed in such a manner to have at least 30 mm between them and the antibiotic symbol had to be on the upper side, in order to can read the results. The results were collected after 72 hours incubation at 37°C, by measuring the inhibition zone diameter. The collected data emphasize a different behavior of Yersinia enterocolitica strains from one antibiotic to another. The most active substances were enrofloxacin, gentamicin, kanamycin and nalidixic acid. The Yersinia enterocolitica strains tested were resistant to ampicillin, cefalotin and erythromycin. We consider that the resistance against ampicillin was determined by the ability of Yersinia enterocolitica strains to secrete penicillinase (Table 3). It was also found that four of the 12 studied strains showed resistance to tetracycline. The different resistance of Yersinia enterocolitica strains to tetracycline has been reported by Funk et al.,in the U.S.

Table 3. Susceptibility testing for *Y. enterocolitica* strains (inhibition zone diameter expressed in mm)

Strain Nr.	Substance										
	NA	**F**	**E**	**T**	**A**	**G**	**S**	**K**	**Cefl**	**Tr**	**En**
1	14	13	10	14	7	12	14	12	8	14	16
2	16	14	8	16	7	13	15	14	6	15	16
3	16	14	9	10	10	15	17	15	9	16	17
4	17	15	10	9	10	14	18	16	9	14	18
5	18	15	9	14	9	14	18	14	8	15	18
6	14	13	9	12	8	15	17	13	10	16	16
7	15	14	8	7	6	13	16	14	9	15	17
8	15	13	7	15	9	15	16	15	7	14	17
9	16	15	9	8	8	16	17	14	7	15	16
10	14	14	8	14	9	14	18	15	8	15	18
11	16	12	8	16	7	12	16	16	6	14	17
12	17	13	6	12	7	14	17	15	8	14	18
Average	**14,5**	**15**	**9,18**	**13,3**	**8,81**	**15,1**	**16,6**	**15,7**	**9,90**	**16,0**	**17**

Legend: NA = nalidixic acid; G= gentamicin; T = tetracycline; E = erythromycin; F = furazolidone; S = streptomycin; K= kanamycin; Tr = trimethoprim; Cefl = cefalotin; A = ampicillin; En = enrofloxacin

It has to be mentioned that the inhibitory action of considered antibiotics was different from one strain to another. Thus, streptomycin determined an inhibition zone with a diameter between 14 and 18 mm, for kanamycin the zone diameter was between 12 and 16 mm, while for enrofloxacin the diameter was between 16 and 18 mm.

The most uniformly inhibitory action against Yersinia enterocolitica strains was recorder for anti-infective substances like: nalidixic acid, furazolidone, gentamicin and trimethoprim.

4. Conclusions

- Yersinia enterocolitica strains multiply on usual growth media, differently, based on incubation temperature. For characteristic colonies selection incubation for 48 hours, at 37°C, is recommended.
- The chlorine bactericidal action was dependant by the cells / ml concentration. At 10^3 CFU / ml the Yersinia enterocolitica strains were inactivated in one minute at a chlorine dose of 4 ppm / ml, for 10^7 CFU / ml was necessary a chlorine concentration of 50 ppm / ml.
- Yersinia enterocolitica strains tested were resistant to ampicillin, cefalotin and erythromycin and were sensitive to gentamicin, kanamycin, nalidixic acid and furazolidone.
- The behavior of Yersinia enterocolitica specie to tetracycline was different, depending on the strain.

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