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The Effect of Several Antibiotics on *Lactococcus garvieae* Isolated from Jordanian Dairy Products

Nizar Issa Alrabadi

Department Food Science and Nutrition,
Faculty of Agricultural Sciences, Jarash University, Jordan

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ABSTRACT

This study investigated the antibiotic resistance profile of the *L. garvieae* isolated strains from Jordanian dairy products. Disk diffusion method was used. In particular, different antibiotics were tested against *L. garvieae*; the antibiotic disks were Trimethoprim (5 µg), Clindamycin (2 µg), Nitrofurantoin (300 µg), Erythromycin (15 µg), Ampicillin (15 µg), Trimethoprim/Sulfamethoxazole (1.25/23.75 µg Polymyxin B (300U) and Tetracycline (30 µg). The results indicated high and statistically significant effects of Trimethoprim, Nitrofurantoin and Trimethoprim/Sulfamthoxazole on *L. garvieae*. Trimethoprim had the greatest antimicrobial effect on *L. garvieae* strains. All of the strains were sensitive to this antibiotic. Trimethoprim showed 20 mm inhibition zone in some strains. On the other hand, there were no antibiotic effects of Clindamycin and Polymyxin B on *L. garvieae.* Slight and statistically insignificant effects were found of other tested antibiotics. The study recommended using Trimethoprim antibiotic against *L. garvieae* because it inhibited its growths in all isolated cultures.

Keywords: Jordanian Dairy Products, Antibiotics, *Lactococcus Garvieae*

1. INTRODUCTION

The genus *Lactococcus* is composed of seven species: *L. garvieae*, *L. fujiensis L. piscium*, *L. chungangensis*, *L. plantarum*, *L. raffinolactis* and *L. lactis*, (Cai *et al*., 2011). *L. garvieae* is the only kind that is classified as pathogenic (Facklam and Elliott, 1995; Vendrell *et al*., 2006). Thus, it is identified as an emerging zoonotic pathogen and the etiological agent of the lactococcosis (an emergent disease) in fish. Recently, *L. garvieae* has been the main cause of diseases affecting rainbow trout of fish in many countries around the world. Ghittino and Prearo (1992) argued that approximately 50% of total trout European production was lost due to diseases caused by this bacterium. Thus, the clinical and veterinary treatment of it is vital to study.

L. garvieae was first isolated from cases of bovine mastitis (Collins *et al.* 1983), thereafter, from clinical samples of human blood, urine and skin (Vinh *et al*., 2006; Wang *et al*., 2007) and different kinds of food products such as fermented milk (El-Baradei *et al*., 2008) cheeses (Alegria *et al*., 2009; El-Baradei *et al*., 2007) and meat products (Aquilanti *et al*., 2007). Although, the pathogenic mechanisms of *L. garvieae* are poorly understood, this study investigated its sensitivity to different types of antibiotics. In fact, this study is important to determine whether the antibiotic resistance profile of the *L. garvieae* isolated from different Jordanian dairy products agree with the strains from different areas of the world. Antibiotic treatment of mastitis leads to significant increase in milk quantity and quality, lower somatic cell count and is likely associated with a reduction in prevalence of clinical mastitis among herds, which is economically beneficial (Oliver *et al*., 2003). Bacterial identification and susceptibility tests are important for selecting the appropriate antimicrobial agent affecting certain diseases (Gentilini *et al*., 2002). Examples of recent studies that conducted such tests are

Murti and Kumar (2011) and Tirumalai and Prakash (2012). The rationale for this study is to determine the antibiotic which inhabits the growth of *L. garvieae* because it is becoming a risky bacterium that threatens the lives of humans and animals.

2. MATERIALS AND METHODS

2.1. Isolated and Standard Strains

Strains were isolated from Rayeb (fermented milk) which made from different types of milk including cow, sheep and goat milk. The standard strains were *L. garvieae* ATCC 43921 and ATCC 49156. They were obtained from the American Type Culture Collection (ATCC).

2.2. Isolation, Purification and Identification of *L. Garvieae*

Serial dilutions from samples (11 g) were emulsified in 99 mL of sterile 2% (w/v) tri-sodium citrate solution and homogenized using an Ultra-Turrax mechanical blender. Serial dilutions were prepared in sterile 1% (w/v) peptone water, plated on M17 agar and incubated at 32°C for 48 h. Then, they were purified by streaking four times on the same medium and temperature and tested by classical methods (gram staining, catalase test, growth at different concentrations of NaCl and different pH levels). Finally, the genomic DNA of the isolated strains was extracted using Sigma's GenElute Bacterial Genomic Kit, then it was identified through specific Polymerase Chain Reaction (PCR) test and specifically using the primers, (TTTGAGAGTTTGATCCTGG) and (AAGTAATTTTCCACTCTACTT) (Pu *et al*., 2002). Ten of them gave positive results.

2.3. Antimicrobial Susceptibility Test

The isolated and standard strains of *L. garvieae* were tested for their resistance to some antibiotics using disk diffusion method (Kirby *et al*., 1966); in this test, 2 mL of an overnight culture of *L. garvieae* were added to 20 mL of M17 agar medium and left to solidify. The antibiotic disks (Difco) were in contact with the agar, the plates were incubated at 32°C for 24 h and the antagonistic activity was evaluated by observing a clear zone of growth inhibition. The antibiotic disks were Trimethoprim (5 µg), Clindamycin (2 µg), Nitrofurantoin (300 µg), Erythromycin (15 µg), Ampicillin (15 µg), Trimethoprim/Sulfamethoxazole (1.25 µg/23.75 µg Polymyxin B (300U) and Tetracycline (30 µg). Duplicate of each antibiotic for each isolated strains were prepared and the average reading of each antibiotic was calculated. The inhibition zones for each antibiotic were determined in accordance with the Shryock and NCCLS (2002). Isolated strains were categorized as susceptible (sensitive) and resistant. T-test was used to determine the statistical significance of the inhibition zone. It was estimated through SPSS statistical package.

3. RESULTS

As shown in **Fig. 1**, ten isolated strains gave positive results in the PCR test and identified as *L. garvieae.* The antibiotic resistance profiles of *L. garvieae* isolated strains against eight antimicrobial agents are presented in **Table 1** and **Fig. 2**. Our results indicated that Trimethoprim (5 µg), Nitrofurantoin (300 µg) and Trimethoprim/Sulfamethoxazole (1.25/23.75 µg) had a great antibiotic action against all *L. garvieae* isolates. These results were statistically significant in all isolates and standard cultures. Furthermore, Trimethoprim (5 µg) had the greatest antimicrobial effect on *L. garvieae* isolates. Thus, it showed 20 mm inhibition zone in some isolates. On the other hand, Clindamycin (2 µg) and Polymyxin B (300U) had no effect on different *L. garvieae* isolates. 50% of *L. garvieae* isolates exhibited sensitivity to Erythromycin (15 µg); 58% of the isolates exhibited sensitivity to Ampicillin (15 µg) while only 42% of them exhibited sensitivity to Tetracycline (30 µg). However, all the reported values of these three antibiotics were statistically insignificant.

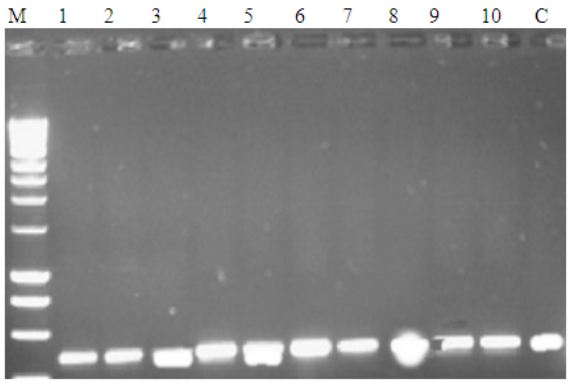

Fig. 1. Identification of L. garvieae by specific PCR assay 482pb M: Smartladder, Line: 1-10 isolated cultures, C: positive control

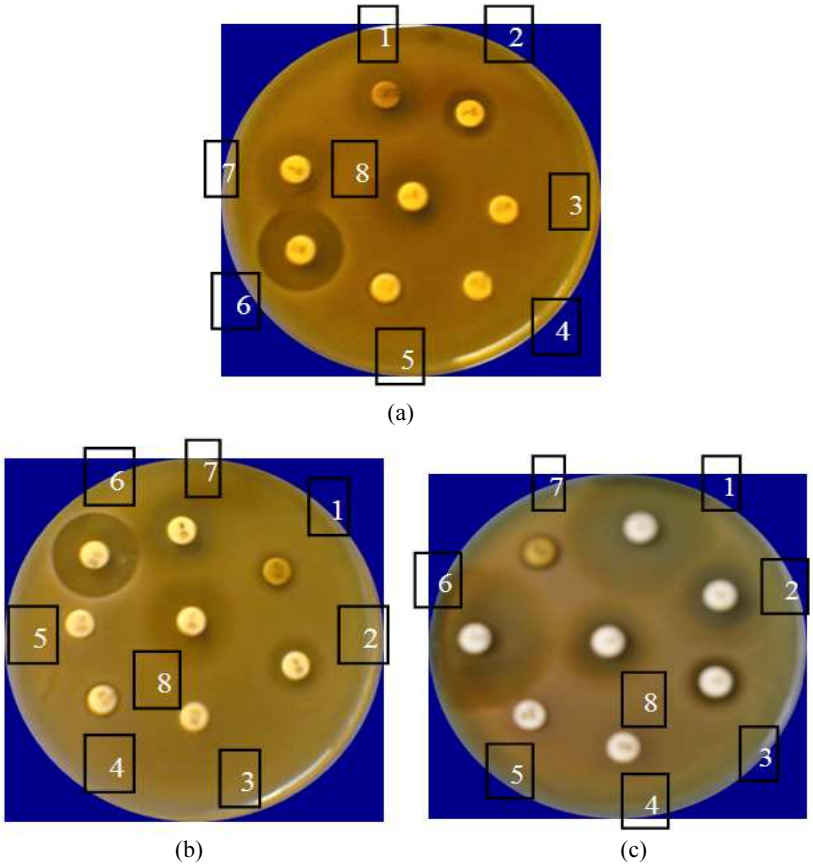

Fig. 2. Effect of different antibiotic on L.garvieae a, b, c denote randomly selected medias inculcated by *L. garvieae* and treated by different antibiotics. 1: Trimethoprim/Sulfamethoxazole, 2: Nitrofurantoin, 3: Erythromycin, 4: Polymyxin, 5: Clindamycin, 6: Trimethoprim, 7: Ampicillin, 8: Tetracycline

Table 1. Effect of different antibiotic on *L.garvieae*

		Antibiotic and its concentration							
Strain number	Strain source	T(5 µg)	CD(2 µg)	NF (300 µg)	E (15 µg)	AM (10 µg)	T/S (1.25 µg/ 23.75 µg)	PB (300U)	TE (30 µg)
Inhabitation Zone (mm)									
1	Cow	$+16^{**}$	-0^{NS}	$+12^{*}$	$+9^{NS}$	$+9^{NS}$	$+14^{**}$	-0^{NS}	$+8^{NS}$
2	Cow	$+14^{**}$	-0^{NS}	$+11^{*}$	$+8^{NS}$	$+9^{NS}$	$+13^{*}$	-0^{NS}	-0^{NS}
3	Cow	$+15^{**}$	-0^{NS}	$+10^{*}$	$+6^{NS}$	$+9^{NS}$	$+11^{*}$	-0^{NS}	-0^{NS}
4	Sheep	$+18^{**}$	-0^{NS}	$+14^{**}$	-0^{NS}	$+8^{NS}$	$+13^{*}$	-0^{NS}	-0^{NS}
5	Sheep	$+13^{**}$	-0^{NS}	$+10^{*}$	-0^{NS}	$+7^{NS}$	$+12^{*}$	-0^{NS}	$+7^{NS}$
6	Sheep	$+17^{**}$	-0^{NS}	$+11^{*}$	$+7^{NS}$	-0^{NS}	$+12^{*}$	-0^{NS}	$+8^{NS}$
7	Sheep	$+18^{**}$	-0^{NS}	$+10^{*}$	$+7^{NS}$	-0^{NS}	$+14^{**}$	-0^{NS}	$+8^{NS}$
8	Goat	$+19^{**}$	-0^{NS}	$+11^{*}$	-0^{NS}	$+7^{NS}$	$+12^{*}$	-0^{NS}	-0^{NS}
9	Goat	$+16^{**}$	-0^{NS}	$+13^{*}$	$+7^{NS}$	$+7^{NS}$	$+14^{**}$	-0^{NS}	-0^{NS}
10	Goat	$+20^{**}$	-0^{NS}	$+11^{*}$	-0^{NS}	-0^{NS}	$+13^{*}$	-0^{NS}	$+7^{NS}$
11	ATCC 43921	$+15^{**}$	-0^{NS}	$+12^{*}$	-0^{NS}	-0^{NS}	$+14^{**}$	-0^{NS}	-0^{NS}
12	ATCC 49156	$+14^{**}$	-0^{NS}	$+11^{*}$	-0^{NS}	-0^{NS}	$+13^{*}$	-0^{NS}	-0^{NS}
% of sensitive *L.garvieae* strains		100%	0%	100%	50%	58%	100%	0%	42%

4. DISCUSSION

The results indicated that Trimethoprim was having best antimicrobial activity against *L. garvieae* because it inhibited its growths in all isolated strains. On the other hand, the study recommended not using Clindamycin, Polymyxin B, Erythromycin, Ampicillin and Tetracycline because they showed slight or no effect at all against *L. garvieae*. Comparing to other studies which used similar concentrations of antibiotics, our results were consistent with (Elliot and Facklam., 1996; Zlotkin *et al*., 1998), in that all the *L. garvieae* strains were resistant to clindamycin antibiotic and compatible with Raissy and Ansari (2011) that not all *L. garvieae* strains were sensitive to Ampicillin. Additionally, our results agreed with Baeck *et al*., 2006 that the isolated strains of *L. garvieae* were sensitive to Nitrofurantoin. However, we contradicted Tanrikul and Gultepe (2011) who found that *L.garvieae* is sensitive to Ampicillin. Understanding the resistance profile of *L. garvieae* is crucial given its severe pathogenic effects. Hence, this study explored the most effective drug against it.

5. CONCLUSION

Bacterial susceptibility tests are vital for selecting the most effective antibiotic eliminating certain diseases. This study investigated the effect of several antibiotics on *L. garvieae* isolated from Jordanian dairy products. Our findings indicated that Trimethoprim, Nitrofurantoin and Trimethoprim/Sulfamethoxazole had high and statistically significant antimicrobial effect on *L. garvieae*, however, Trimethoprim showed the greatest effect.

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