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Full Length Research Paper

Safety of Lactic Starter Cultures used in Algerian Dairy Industry Case Study: Antibiotic Resistance

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ABSTRACT

The manufacture of fermented dairy products requires the incorporation of large amounts of imported lactic starter cultures. The robustness of ferments to fluctuations in operational parameters of industrial processes is a quality that is prized by industrial operators. Therefore, the most effective strains under hostile industrial conditions tend to be selected in the screening operations of industrial strains. Strains demonstrating resistance to antibiotics are an example. The use of antibiotics in animal production is considered as the main cause of selection for antibiotic resistance in bacteria that can ebentually be found in food. Several scientific studies support the hypothesis of a link between the use of antibiotics in primary production and the emergence of antibiotic resistance in human pathogens, with food as an important means of transmitting.

We have evaluated the susceptibility of imported samples of lactic starter cultures to a panel of antibiotics used in veterinary medicine in Algeria. Our results showed a good proliferation of the majority of strains of lactic acid bacteria in the presence of the tested antibiotics, including Sulfaprime S with concentrations up to 350 µg/ml. This could pose a potential danger to consumer health with risk of transfer resistance genes to human intestinal flora or in the worst case to pathogens.

Key words: Safety, lactic starter cultures, antibiotic resistance, Algerian dairy industry

1. INTRODUCTION

Algeria is a country heavily dependent on food imports. Therefore it is particularly vulnerable to risks associated with microorganisms for human consumption, including lactic acid bacteria. Among these risks, the emergence of antibiotic resistance, especially in the pathogenic flora, continues to attract an increasing interest in the scientific community.

However, this risk could be more important in the flora considered benign because the genetic elements responsible for resistance to antibiotics, particularly those containing mobile elements can move rapidly in human and animal populations.

The case of lactic acid bacteria is particularly interesting because of their status GRAS (Generally Recognized As Safe) (EFSA, 2008). In effect, pathogens resistant to multiple antibiotics can carry resistance to commensal germs of the human gut by horizontal transfer. In addition, the microorganisms present in large numbers in a food or in the human intestine exhibit greater probability of

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transfer of antibiotic resistance genes in comparison to the microorganisms present in smaller numbers. This is the case of bacteria which constitute lactic starter cultures which grow in large numbers during the fermentation of dairy products. In addition, sublethal conditions in which lactic bacteria survive in the human intestinal tract increase the chances of horizontal transmission via the combination of plasmids carrying antibiotic resistance genes in relation to the frequency observed between unstressed bacterial cells (AFSCA, 2012). Several studies support the hypothesis of a link between the use of antibiotics in primary food production and the presence of antimicrobial resistance in human pathogens, with food as an important means of transmitting.

2. MATERIALS AND METHODS

We evaluated the susceptibility of imported samples of lactic starter cultures to a panel of three antibiotics (Pen & Strep, S Sulfaprime and Ampicillin) chosen for their frequent use in Algerian dairy production. This approach is performed to verify the ability of starter to grow in the presence of antibiotics in order to better assess the risk of horizontal gene transfer of antibiotic resistance from the starter to the human intestinal flora.

Although antibiotic resistance is perceived as a criterion of technological quality lactic starters industry, the risk of transfer of resistance genes to human intestinal flora represents a potential danger to human health.

The realized method (Figure 1) is based on the European Directive CVMP (2000), the work carried out by D'Aimmo et al. (2007) as well as l'IDF 223 (2010).

The following figure illustrates the steps performed to test the antibiotic resistance of lactic starter cultures to the tested antibiotics. We also consulted a panel of scientific and industrial experts by Delphi method to assess the potential for local manufacture of indigenous lactic starters.

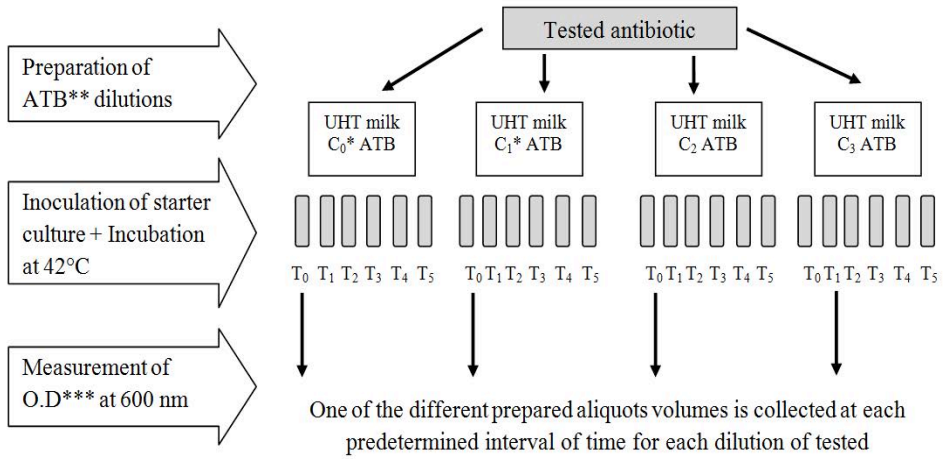


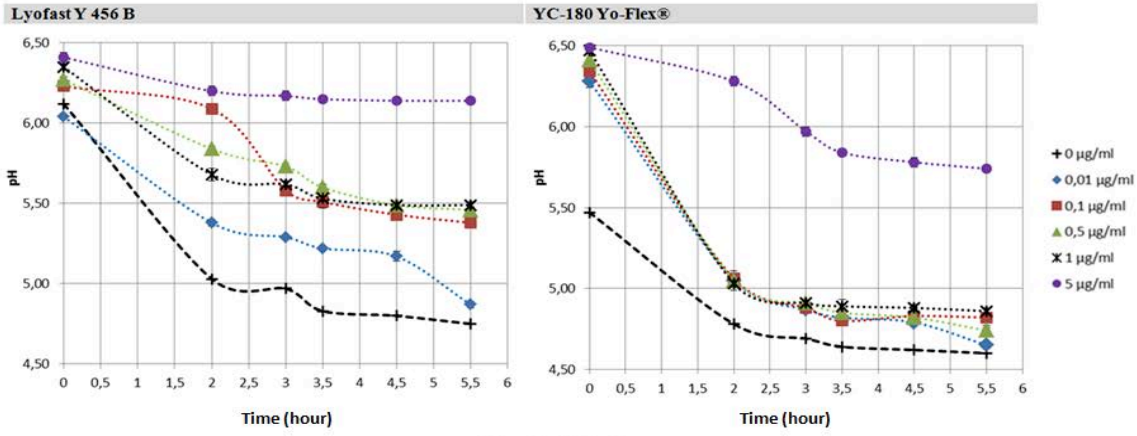
Fig. 1: Method used for testing the susceptibility of lactic starter cultures to various tested antibiotics. (***C0**** Antibiotic-free control; ***C1**** A given concentration of the antibiotic to be tested; ***ATB**** Tested antibiotic; ***O.D. ***** Optical density; ***T0=0:00H, T1=2:00H, T2=3:00H, T3=3:30H, T4=4:30H, T5=5:30H*).

3. RESULTS AND DISCUSSION

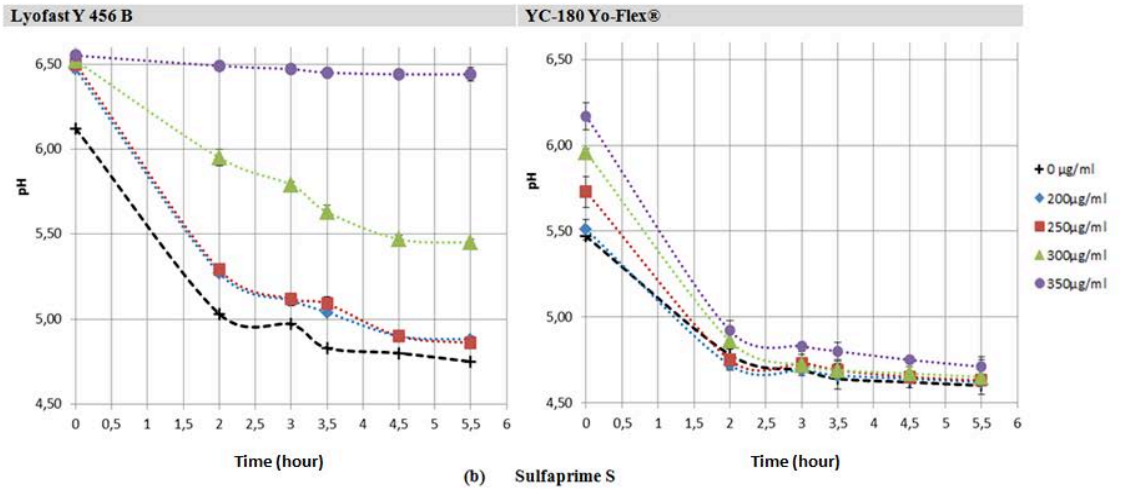
We have evaluated the effect of increasing concentrations of antibiotics Pen&Strep, Sulfaprime S and Ampicillin on the acidifying power (Figure 2) and growth kinetics (Figures 3 and 4) two categories of lactic starters: those used in

the manufacture of yoghurt (Lyofast Y 456 and YC-180 - Yo-Flex®) and ferments used in the manufacture of cheese (ALPHA 10, CHN-11).

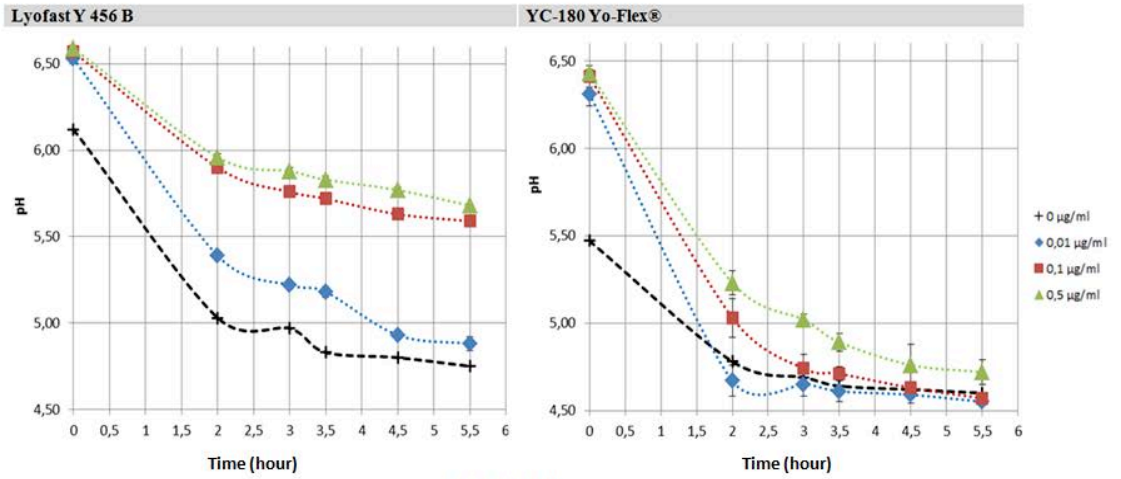
Our results showed a good proliferation of the majority of strains of lactic starters in the presence of the tested antibiotics (Figures 3 and 4), including Sulfaprime S with concentrations up to 350 µl/ml. This could pose a potential danger to the health of consumers with risk of transfer resistance genes to human intestinal flora or in the worst case to pathogens.



(a) Pen&Strep



(b) Sulfaprime S



(c) Ampicilline

Fig. 2: Kinetics of lactic acidification of yogurt using starter cultures ***(Lyofast Y 456 B and YC-180 Yo-Flex®)*** in the presence of increasing concentrations of antibiotics: (a) Pen & Strep, (b) S Sulfaprime and (c) Ampicillin

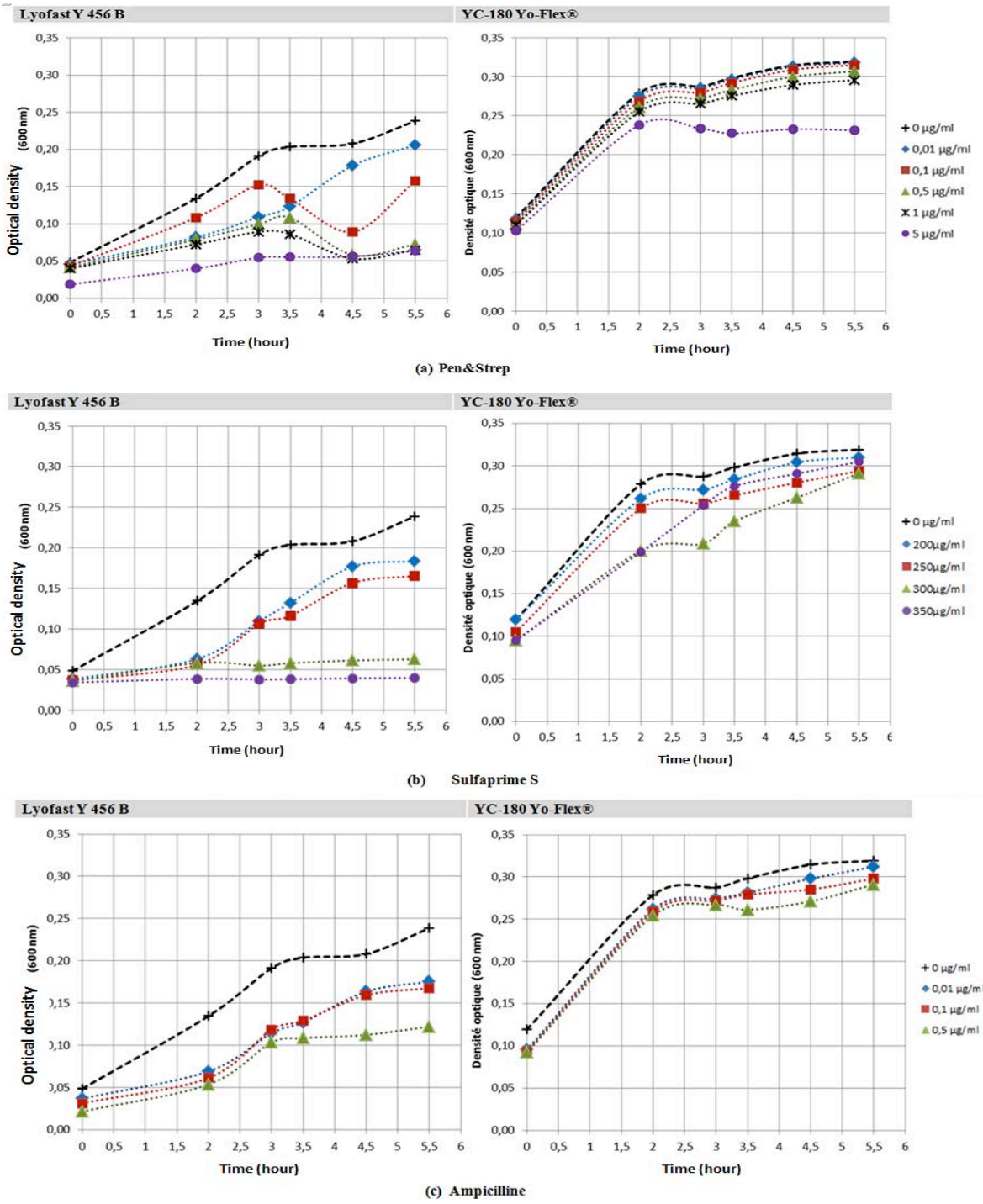


Fig. 3: Kinetics of cellular growth of lactic starters yogurt (Lyofast Y 456 B and YC-180 Yo-Flex®) in the presence of increasing concentrations of antibiotics: (a) Pen&Strep, (b) Sulfaprime S and (c) Ampicillin

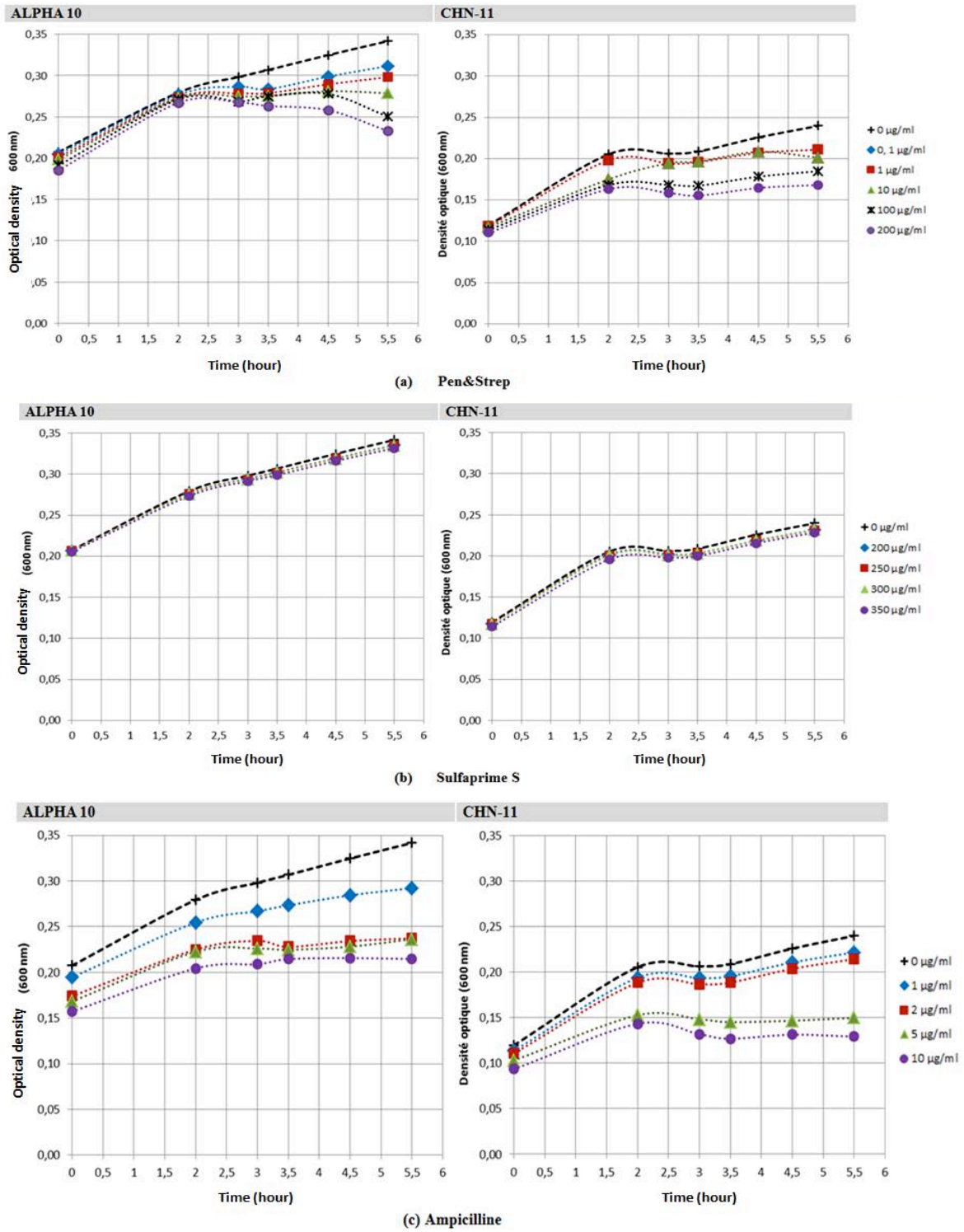


Fig. 4 : Kinetics of cellular growth of lactic starters used in cheese production (ALPHA 10 and CHN-11) in the presence of increasing concentrations of antibiotics: (a) Pen&Strep, (b) Sulfaprime S and (c) Ampicillin.

Table 1 summarizes the effect of different doses of tested antibiotics on the final pH value compared to the initial value obtained in the absence of antibiotics. We note that, unlike the ferment Lyofast Y 456 B, ferment YC-180 Yo-Flex® product final pH values comparable with those obtained in the absence of antibiotics and for all concentration ranges tested and antibiotics.

Table 1: Effect of different doses of tested antibiotics on the production of lactic acid by starters of yoghurt.

	Change in the final pH *											
Antibiotic	**Pen&Strep**					**Sulfaprime S**				**Ampicillin**		
Dose (µg/ ml)	**0,01**	**0,1**	**0,5**	**1**	**5**	**200**	**250**	**300**	**350**	**0,01**	**0,1**	**0,5**
YC-180 Yo-Flex®	+0,05	+0,22	+0,24	+0,26	+1,14	+0,02	+0,03	+0,05	+0,11	-0,05	-0,07	+0,12
Lyofast Y 456 B	+0,12	+0,63	+0,71	+0,74	+1,39	+0,13	+0,11	+0,70	+1,69	+0,13	+0,84	+0,93

***** Relative to final pH obtained in the absence of antibiotics *[= $pH_{ATB} - pH_{Control}$]*

Lactic acid bacteria behave differently in the presence of antibiotics, three scenarios are possible as summarized in Table 2:

Table 2: Susceptibility of lactic acid bacteria to antibiotics (D'Aimmo *et al.,* 2007).

Susceptible	**Moderately resistant**	**Resistant**
MIC* < 8 µg/ml	MIC ≥ 8 µg/ml	MIC > 32 µg/ml

**** MIC***: *Minimal Inhibitory Concentration*

We used the classification of Table 2 to assess the susceptibility of lactic starter cultures analyzed in our study relative to tested antibiotics (see Table 3). However, our classification is indicative since our results were obtained from mixtures of strains not from purified strains.

Table 3: Determination of MIC of different antibiotics towards lactic starters tested after 5.5 hours of fermentation.

Antibiotic	**Lactic ferments**	**MIC (µg/ml)**	**Resistance profile**
Pen&Strep	YC-180 Yo-Flex®	>5	Susceptible to **moderate resistance**
	Lyofast Y 456 B	0,1< $CMI_{50\%}$*<0,5	Sensible
	CHN-11	>200	***Resistant***
	ALPHA 10	>200	***Resistant***
Sulfaprime S	YC-180 Yo-Flex®	>350	***Resistant***
	Lyofast Y 456 B	250< $CMI_{50\%}$<300	***Resistant***
	CHN-11	>350	***Resistant***
	ALPHA 10	>350	***Resistant***
Ampicillin	YC-180 Yo-Flex®	>0,5	Susceptible
	Lyofast Y 456 B	0,5	Susceptible
	CHN-11	>10	***Resistant***
	ALPHA 10	>10	***Resistant***

****$MIC_{50\%}$** *: values which inhibit 50% of the strains belonging to the same species*

The results collected from our Delphi survey showed that manufacturing indigenous lactic starter cultures depend on the acquisition of technical know-how in particular in the field of industrial fermentation, but also the mastery of modern tools of molecular characterization and high-throughput screening to identify viable alternatives, both commercially and technologically the starter imported are perceived favorably by industrial operators because

of their reliability, despite the risks associated (bacteriophages, GMO, and antibiotic resistance).

4. CONCLUSION

The horizontal transfer of antibiotic resistance genes to the intestinal flora of the consumer through imported lactic starters incorporated in fermented dairy products poses a real threat to public health.

Our results demonstrate the existence of varying degrees of susceptibility or even resistance to antibiotics Pen & Strep Sulfaprime S and Ampicillin.

In the medium term, Algeria is able to produce its own lactic acid bacteria, particularly species commonly used. It is essential to implement a sustainable dynamic collaboration between the dairy industry and scientific research.

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