BACTERIOLOGICAL STUDY OF THE FARMED MUSSEL (*MYTILUS GALLOPROVINCIALIS*) AND ITS IMPACT ON PUBLIC HEALTH

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Abstract. The present study was carried out to assess the bacteriological quality of the cultured mussel "*Mytilus galloprovincialis*" and its impact on public health and to study the influence of the three physicochemical variation (environmental) factors: medium temperature (TS), pH and salinity (SL). The exploration of the bacteriological quality of the mussels "*Mytilus galloprovincialis*" was carried out by research and enumeration of Total flora (TF), Total coliform (TC), Fecal coliform (FC), *Escherichia coli* (EC), Fecal streptococci(FS) and *Staphylococcus aureus* (SA), using specific techniques and methods. Likewise, for environmental factors, they were evaluated by typical instruments. The results of the bacterial count revealed that the means of the isolated bacteria were 2.27 ± 0.24 log10 cells / 100ml, 3.34 ± 0.51 log10 cells / 100ml, 2.64 ± 0.72 log10 cells / 100ml, 0.94 ± 1.63 log10 cells / 100ml, 1.85 ± 1.61 log10 cells / 100ml and 0.63 ± 1.09 log10 cells / 100ml, respectively for TF, TC, FC, EC, FS and SA. The study of the influence of the three variation factors on the development of the bacterial indicators tested for mussels showed that the majority of the correlations between the six bacterial indicators and the physicochemical parameters are high (r> 0.5) except for four correlations which are weak (r <0.5), one between FS and pH (r = 0.4042; R^2 = 0.1633), the second between TF and SL (r = 0.2515; R^2 = 0.0632), the third between TC and SL (r = 0.3516; R^2 = 0.1236) and the fourth between FS and SL (r = 0.0249; R^2 = 0.0006). In conclusion, the consumption of the mussel "*Mytilus galloprovincialis*" can pose risks to public health. For this, the establishment of a quality policy, aimed at popularizing good hygiene practices.

Keywords: *Mytilus galloprovincialis*, Food hygiene, Food poisoning

INTRODUCTION

The coastal zone has long played an essential role in the development of human civilization. The use of ships for trade and transport has made this area a prime site for city development. As a natural habitat, this area is home to unique living resources and attracts attention for this simple reason (Kaddour, 2006).

Fish and seafood are the second source of animal protein behind meat (ICMSF, 2000). Mussels are undoubtedly the most effective means of converting the organic matter produced by the autotrophic marine organism located at the first level of the food chain (phytoplankton) into pleasant and rich human food. Without forgetting that the mussels are considered as sentinel species they are used as biological indicators of

anthropized ecosystems. They are in fact used as biological indicators in biomonitoring programs in order to measure the degree of environmental pollution (Benchikh, 2006).

Mytillus galloprovincialis is a species of Mediterranean origin. It is also present on the southwest coasts of the Black Sea, on the Spanish and Portuguese coasts and on the French and British Atlantic coasts (Cooperation tranfrontaliere ITALIETUNISIE, 2007-2013). In addition, the common mussel *Mytilus galloprovincialis* has aroused the interest of many biologists for the importance it has on the economic and malacological level (Aloui-Bejaouj, 1998).

Human activities, certain weather conditions (heavy rain) or other factors can cause pollution of seawater, which in turn contaminates shellfish. If these are consumed, they can quickly cause toxic effects, sometimes causing Toxi-Infections Alimentaires Collectives (TIAC) (Payen, 2007).

In addition, according to Lee *et al.* (2001), bivalve molluscs concentrate contaminants from the water column in which they live. These contaminations can then cause illness in humans when they consume shellfish. With regard to microbial contaminants, the risk is reinforced by the fact that these shellfish are often eaten raw (e.g., oysters) or relatively undercooked (e.g., mussels).

The main objective of this study was to explore the bacterial quality of *Mytilus galloprovincialis* cultured mussels caught by artisanal methods at the west coast of Bou Ismail,Algeria, based on the count of six bacterial indicators: the total flora (TF), total coliforms (TC), thermotolerant or fecal coliforms (FC), *Escherichia coli* (EC), fecal streptococci (FS) and *Staphylococcus aureus* (SA), on the one hand, and on the other hand First, to study the impact of certain risk factors linked to the living environment of mussels (seawater) on the bacterial concentration of mussels. This work aims to orient future prevention proposals towards contamination responsible for economic losses and to propose corrective measures to fight against these contaminations in order to preserve the health of the consumer.

MATERIAL AND METHODS

Sampling

Samples were taken from a single location, to study the influence of environmental parameters, for three months (February, March and April) on the west coast of Bou Ismail. Three important and most well-known physicochemical parameters were measured at the sample site, namely: temperature, pH and salinity. The mussels collected are adults and large. They were packaged in clearly identified single-use plastic bags. In addition, one liter of seawater was taken 30 cm deep in sterile and well identified bottles. These samples were immediately placed in a cooler and sent directly to the analysis laboratory under cold conditions. Their content was analyzed within 24 hours (Rodier *et al.,* 2005). Samples were generally taken within a constant hour of eight in the morning.

Bacteriological analysis

Preparation of the mold sample (homogenate)

Microbiological analyzes were carried out on the flesh and the intervalval fluid. The undamaged mussels were washed thoroughly under running water and brushed to remove external stains. They were then disinfected by rapid flaming with

heat. The opening of the mold was carried out by a sterile scalpel on the side of the byssus, the interval liquid and the flesh were collected in a sterile Stomacher type sachet. 100gm of flesh and intervalve fluid were weighed to determine coliforms and fecal Streptococci and 25gm for other germs. The volume of mold was diluted in two volumes of diluent tryptone salt solution and ground in a Stomacher (electronic time) for 60 seconds. This suspension then constitutes the mother dilution (MD) which is 1/3. It was left for 15-20 min to revive the bacteria. From the 1/3 mother suspension, two dilutions (1/30, 1/300) were prepared in 9 ml tubes of tryptone salt (Delarras, 2003).

Total flora count (TF)

From the 1/3 dilutions made, aseptically carry 1 ml in an empty Petri dish then add 15 ml of the PCA agar. The contents of the box are mixed by eight-shaped movements. Once solidified, a second layer of 5 ml of the medium is added in order to avoid the various contaminants. The dishes are incubated at 30 °C for 72 h. The colonies are counted on boxes that do not exceed 300 colonies (Larpent, 1997).

Enumeration of faecal germs using the probable number method

The method used for the determination of fecal contamination indicators (coliforms, fecal streptococci) is the method of fermentation in multiple tubes which is based on the seeding of a series of three tubes containing liquid media, then the determination of the number the more likely from positive tubes by referring to the table of three probability tubes from Mc Grady (Delarras, 2003; Rodier *et al.,* 2005).

Search for total coliforms (TC)

This test was carried out using bromocresol purple lactose broth (BCPL broth). All the tubes are fitted with Durham bells to detect the possible release of gas in the environment (more than 1/10 of the volume of the bell) (O.M.S/PNUE, 1995). This method uses two consecutive tests:

- Presumptive test: this test consists of seeding series of three tubes of the single concentration BCPL medium per 1 ml of sample (stock solution (1/3) and dilutions (1/30 and 1/300)). The media are incubated at 37 ° C for 24-48 h. Microbial growth with gas production in the Durham bell as well as a yellow change in the pH indicator are considered to be a presumptive reaction for the presence of total coliforms.
- Confirmatory test: confirmation of the presence of total coliforms is carried out on Schubert medium. It manifests as growth with gas production after incubation at 37 ° C for 24-48 h. The characteristic number is formed from the number of positive tubes in each series. The latter is reported in the table of the most probable number technique with three tubes specific to shellfish to obtain the number of total coliforms present in 100 g of flesh and intervalval fluid (Delarras, 2003).

Search for thermotolerant coliforms (TC) - *Escherichia coli* (EC)

From the positive BCPLs, the Schubert medium is inoculated. The latter is incubated for 24-48 h at 44 ° C. Growth with evolution of gas in the Durham bell is confirming the presence of thermotolerant coliforms (feces). The presence of *E. coli* is evidenced by the production of indole in the positive tubes of Schubert. This production results in the appearance of a red ring after the addition of Kovac's reagent.

The number of positive tubes is noted and then reported in the table of shell-specific most probable number in order to obtain the number of fecal coliforms and *E. coli* in 100 g of flesh and intervalve fluid (Delarras, 2003).

Enumeration of fecal or thermotolerant Streptococci *"Enterococcus"* (SF) by the Most Likely Number method

Enterococci (fecal streptococci) are counted in liquid medium by the three-tube most probable number technique (most likely number) (Delarras, 2003). This method is done in two steps.

- Presumptive test: this method is based on the inoculation of three simple concentration Rothe tubes with each of the stock solutions and dilutions. This medium contains sodium azide as a selective agent (Larpent, 1997; Delarras, 2003). After an incubation of 24 h at 37 ° C, the tubes presenting a disorder are presumptive of the presence of fecal Streptococci.
- Confirmatory test: each positive tube is inoculated on Litsky medium which contains, in addition to sodium azide, a second selective agent: ethyl violet. After 24 h incubation at 37 ° C, the tubes with a cloud and possibly a purple tablet are considered positive (Larpent, 1997). The results are expressed as the number of Streptococci per 100 g of the flesh and the intervalval fluid, referring to the table of MPNs specific to shellfish (Delarras, 2003; Rodier *et al.,* 2005).

Search and enumeration of *Staphylococcus aureus* (SA)

For the detection of *Staphylococcus aureus*, an enrichment of 1 ml of the mother solution and dilutions in a Giolitti Cantoni broth supplemented with potassium tellurite.

Tubes with black haze after 24 h incubation at 37 ° C will be isolated on Chapman medium. After an incubation of 24-48 h at 37 ° C, the presence of golden colonies on Chapman medium (fermentation of mannitol) indicates the presence of pathogenic *Staphylococcus aureus* (Delarras, 2003).

Confirmation of the presence of pathogenic *Staphylococcus aureus* is carried out by two biochemical tests:

- Catalase test: the presence of catalase results in the appearance of a gas bubble in the presence of hydrogen peroxide.
- Coagulase test: generally, pathogenic *Staphylococcus aureus* has a coagulase. The search for the latter is done by removing the catalase positive colonies and putting them in contact with rabbit plasma. The presence of this enzyme results in the appearance of a coagulate (Larpent, 1997).

Statistical analyzes

A factor analysis of variance (ANOVA) associated with the Newman-Keuls means comparison test (SNK) were used to compare the means of bacterial count.

The Pearson correlation test (Pearson Correlation Matrix) was used to estimate the significant link between the different six bacterial indicators of the farmed mussel (*Mytilus galloprovincialis*).

The correlation coefficient (r) and determination coefficient (R^2) were calculated to estimate the link between the average bacterial concentrations counted from mussels and the sea with the mean values of temperature, pH and salinity recorded from the place of the levy.

Confidence intervals (CI) were calculated for the menstrual evolution of the concentration rate of each bacteria and the appreciation of the monthly variation of the three physicochemical parameters of seawater.

The calculations were made using XLSTAT 2009 software and Microsoft Office Excel® 2007 software after decimal logarithmic transformation of the results expressed in cells / 100ml (cfu / 100ml) to normalize the distribution. All results equal to 0 are rounded to 1 during logarithmic transformations.

RESULTS AND DISCUSSION

Results of fecal bacteria counts of mussels and seawater

Table 1 report the contamination rate of the mussels by the six floras according to the months of sampling. Overall in all positive cases, regardless of the nature of the bacteria, their high concentration was recorded in the month of April.

The majority of samples infected with the six bacterial indicators (TF, TC, FC, EC, FS and SA), except for EC, FS and SA during the month of February and EC and SA during the month of March. The concentrations vary from 2 to 2.46 $\log_{10}$ cells / 100ml for TF (with an average of 2.27 ± 0.24 $\log_{10}$ cells / 100ml), from 2.79 to 3.8 $\log_{10}$ cells / 100ml for TC (with an average of 3.34 ± 0.51 $\log_{10}$ cells / 100ml), from 2.05 to 3.44 $\log_{10}$ cells / 100ml for CF (with an average of 2.64 ± 0.72 $\log_{10}$ cells / 100ml), from 0 to 2.83 $\log_{10}$ cells / 100ml for EC (with an average of 0.94 ± 1.63 $\log_{10}$ cells / 100ml), from 0 to 2.82 $\log_{10}$ cells / 100ml for FS (with an average of 1.85 ± 1.61 $\log_{10}$ cells / 100ml) and 0 to 1.88 $\log_{10}$ cells / 100ml for SA (with an average of 0.63 ± 1.09 $\log_{10}$ cells / 100ml).

Statistical analysis of the comparison of the results obtained between the months of study reveals a significant difference ($p < 0.05$).

Table 1

Variation in the concentration of mussel bacteria according to the sampling periods ($\log_{10}$ cells / 100 ml)

Periods	TF [a'b']	TC [a']	FC [a'b']	EC [b'c']	FS [a'b']	SA [c']
February[b]	2.00	2.79	2.05	0	0	0
March [b]	2.34	3.44	2.44	0	2.74	0
April [a]	2.46	3.80	3.44	2.83	2.82	1.88
Total	6.8	10.03	7.93	2.83	5.56	1.88
Average	2.27±0.24	3.34±0.51	2.64±0.72	0.94±1.63	1.85±1.61	0.63±1.09
CI (95%)	[2.09 ; 2.45]	[3.02 ; 3.66]	[2.14 ; 3.14]	[-0.96 ; 2.84]	[0.51 ; 3.19]	[-0.93 ; 2.19]

TF: Total flora; TC: Total coliforms; FC: Thermotolerant coliforms (faeces); CE: Escherichia coli; FS: Fecal streptococci; SA: Staphylococcus aureus; CI (95%): co-financing interval (95%); SA: Statistical analyzes; Total P value (comparison between months) = 0.004 <0.05; For the column "period" the same letters (a, b) above the months signify a significant difference (p <0.05, SNK test); For the bacteria line the same letters (a ', b', c ') above the bacteria names mean a significant difference (p <0.05, SNK test).

Correlation study between bacterial indicators (TF, TC, FC, EC, FS and SA) of mussels ($\log_{10}$ cells / 100 ml)

The correlation matrices of the six bacterial floras measured during the study period for farmed mussels (*Mytilus galloprovincialis*) are shown in Table 2. The correlation gives the nature of the relationship between the bacterial indicators studied for mussels breeding.

In the majority of cases, with the exception of two cases of poor correlations, one between EC and FS (r = 0.521; p = 0.651) and the second between SF and SA (r = 0.521; p = 0.651), the remains are strong positive associations which have been observed between the rest of the bacterial indicators: TF and TC (r = 0.994; p = 0.067), TF and FC (r = 0.869; p = 0.329), TF and EC (r = 0.702; p = 0.505), TF and FS (r = 0.974; p = 0.146), TF and SA (r = 0.702; p = 0.505), TC and FC (r = 0.916; p = 0.263), TC and EC (r = 0.773; p = 0.438), TC and FS (r = 0.945; p = 0.213), TC and SA (r = 0.773; p = 0.438), FC and EC (r = 0.962; p = 0.175), FC and FS (r = 0.734; p = 0.475), FC and SA (r = 0.962; p = 0.175). At the end, another strong clearly significant correlation was noticed between EC and SA (r = 1.000; p <0.0001).

Table 2

P-value values and correlation matrix (Pearson) between the bacterial indicators (FT, CT, CF, EC, SF and SA) of mussels (log_{10} cells / 100 ml)

1.1. Correlation matrix (Pearson)						
	TF	TC	FC	EC	FS	SA
TF	1	0.994	0.869	0.702	0.974	0.702
TC	0.994	1	0.916	0.773	0.945	0.773
FC	0.869	0.916	1	0.962	0.734	0.962
EC	0.702	0.773	0.962	1	0.521	1.000
FS	0.974	0.945	0.734	0.521	1	0.521
SA	0.702	0.773	0.962	1.000	0.521	1
Values in bold are different from 0 at significance level alpha = 0.05						
1.2. p-values						
	TF	TC	FC	EC	FS	SA
TF	0	0.067	0.329	0.505	0.146	0.505
TC	0.067	0	0.263	0.438	0.213	0.438
FC	0.329	0.263	0	0.175	0.475	0.175
EC	0.505	0.438	0.175	0	0.651	< 0.0001
FS	0.146	0.213	0.475	0.651	0	0.651
SA	0.505	0.438	0.175	< 0.0001	0.651	0
Values in bold are different from 0 at significance level alpha = 0.05						

TF: Total flora; TC: Total coliforms; FC: Thermotolerant coliforms (faeces); EC: Escherichia coli; FS: Fecal streptococci; SA: Staphylococcus aureus.

Relationship between the bacterial indicators of the mussel and the three physicochemical parameters of the place of sampling

The temperature recorded in the sampling locations varied regularly during the study period, with a minimum of 18 ° C during the month of February and a maximum of 22 ° C during the month of April. The average water temperature in all months was 20 ± 2 ° C. In addition, the results of pH measurement of the place of sampling have shown that they vary between 8.13 during the month of March and 8.20 during the month of April with an average of 8.16 ± 0.04. At the same time, salinity was relatively stable during the study period. The values observed vary between 36.72 PSU during the month of March and 36.88 PSU during the month of April with an average of 36.80 ± 0.08 PSU (Table 3).

Table 3

Monthly variation of the three physicochemical parameters of seawater

Periods	Temperature (° C)	pH	Salinity (PSU)
February	18	8.14	36.80
March	20	8.13	36.72
April	22	8.20	36.88
Total	60	24.47	110.4
Average	20.00±2.00	8.16±0.04	36.80±0.08
CI (95%)	[19.49 ; 20.51]	[8.14 ; 8.18]	[36.79 ; 36.81]

pH: Hydrogen potential; CI (95%): co-financing interval(95%); °C: Degree Celsius; PSU *(Pratical* Salinity *Unit).*

Statistical analysis has shown that the six isolated floras of the mussels have a development with a high positive correlation (r > 0.5) with the three physicochemical indicators, eleven of which are very strong and three of which are poor (FC-SL, TC-pH and TF-pH), except for four weak correlations (r <0.5): the first between SF and pH (r = 0.4042; R^2 = 0.1633), the second between TF and SL (r = 0.2515; R^2 = 0.0632), the third between TC and SL (r = 0.3516; R^2 = 0.1236) and the fourth between FS and SL (r = 0.0249; R^2 = 0.0006) (Table 4).

Table 4

Correlation between the results of the counts of the six bacterial indicators (TF, TC, FC, EC, FS and SA in log_{10} cells / 100 ml) isolated from the molds and the three physicochemical parameters

Relationship between parameters	COC (r)	COD (R^2)
TF-TM	0.9639	0.9292
CT-TM	0.9865	0.9733
FC-TM	0.9694	0.9397
EC-TM	0.8660	0.7500
FS-TM	0.8782	0.7713
SA-TM	0.8660	0.7500
TF-pH	0.6015	0.3618
TC-pH	0.6820	0.4651
FC-pH	0.9180	0.8426
EC-pH	0.9912	0.9826
FS-pH	0.4042	0.1633
SA-pH	0.9912	0.9826
TF -SL	0.2515	0.0632
TC-SL	0.3516	0.1236
FC-SL	0.6974	0.4863
EC-SL	0.8660	0.7500
FS-SL	0.0249	0.0006
SA-SL	0.8660	0.7500

TF: Total flora; TC: Total coliforms; FC: Thermotolerant coliforms (faeces); EC: Escherichia coli; FS: Fecal streptococci; SA: Staphylococcus aureus; TM: Temperature, pH: Hydrogen potential; SL: Salinity; COC (r): Correlation coefficient (r); COD (R^2): Coefficient of determination (R^2)

Mussels are foods of marine animal origin that can be eaten raw or undercooked by humans. At the same time, Algerian regulations have set their microbiological criteria in order to consume a healthy product which does not constitute a risk to public health. Overall, our results showed significant concentrations of TC, FC, TF and FS. On the other hand, low concentrations were noted in the EC and the SA. This shows that contamination of the farmed mussel (*Mytilus galloprovincialis*) is mainly of fecal origin.

According to Henigman *et al.* (2019), extreme weather conditions, especially heavy rain, have a strong impact on marine pollution and a consequent increase in the value of *E. Coli* in the molds. In parallel, studies (Riou *et al.,* 2007) of the hydrodynamic model have shown the influence of rain on the microbiological contamination of shellfish; due to the higher water level, the influx of fecal contaminants into growing areas has increased. Small inflows, which are due to heavy rainfall, are expected to have a major impact, and large amounts of fecal pollutants are introduced into the sea, also due to overcrowded wastewater treatment plants. In particular, the local areas near these tributaries and the coast are overcrowded. The degree of contamination during these periods is also associated with the population density in the coastal zone (Glasoe and Christy, 2004).

In addition, according to Bougarroua (2019), the levels of contamination recorded in the Cala Iris zone are mainly due to the waste from reared animals resulting from grazing and poultry activities, as well as the waste resulting from port activities and ships in the region. Currently, a treatment plant is being created in this study area, which can improve the microbiological quality of mussels.

In parallel, Grimes *et al.* (1986), cited by Ignatova-Ivanova *et al.* pointed out that many researchers mistakenly int erpret the discharge of wastewater as the source of pathogens rather than a source of nutrients, which can stimulate the growth of indigenous pathogens.

Moreover, osmotic balance as well as cell renewal in mussels can be used as objective diagnostic criteria for their physiological state in the event of bacterial infection. This approach is of great importance in relation to the need to find and develop simple and effective methods for assessing the physiological states of marine organisms, in particular bivalve molluscs, in aquaculture and bio-monitoring studies (Parisi *et al.,* 2019).

Compared to the months of sampling, in all positive cases, the highest bacterial loads were recorded in the month of April. These results can be explained by the presence of favorable conditions for mussel metabolism, which leads to an increase in the rate of contamination. According to Bougarroua (2019), molluscs are par excellence filter-feeders. They are therefore sensitive to any form of pollution and can concentrate germs and microorganisms that can cause toxic-infections in the consumer. Protecting consumer health and ensuring the safety of shellfish growing areas are among the objectives of monitoring shellfish farming areas.

As an example, *Escherichia coli* O157: H7 or *E. coli* O157: H7 is a toxin-producing bacteria that causes intestinal disease in people and lasts for about a week (CFSPH, 2006).

The study of the relationship between the average number of cases of bacterial flora and the average values of temperature, pH and salinity has made it possible to

highlight eleven very high positive correlations and three are ordinary (FC-SL, TC-pH and TF-pH), on the one hand, and on the other hand, four weak positive correlations. This is well linked to the existence of physicochemical conditions favorable to the survival and development of the majority of the bacterial flora and to the good progress of the evolutionary cycle of these bacteria.

According to Boscolo Papo (2014), cited by Battistini et al., mussels are generally located in highly anthropized areas, although these guarantee a good supply of nutrients for mussel farming, could represent a problem for the introduction of pollutants which can weaken the immune system of mussels.

Finally, it is interesting to know the prevalence of bacteria in mussels fished in the Algerian coasts, because they cause expensive economic losses of the farmed mussel (*Mytilus galloprovincialis*) and constitute a major risk for the health of consumers. In addition, there is a need for further application of improved control and prevention measures. Preventive treatments are recommended to be applied regularly to reduce bacterial contamination and eradicate mussel disease thereafter.

In addition, it will be very useful to carry out systematic screening in all mussel (*Mytilus galloprovincialis*) farms by general and specific laboratory examinations for confirmation which will help to better fight against bacterial flora and preserve the health of consumers thereafter.

CONCLUSIONS

It should be noted that the quality control of mussels and seawater is of great importance for the prevention of the health of consumers, however, all toxins produced by bacterial indicators that can pose risks to human health, especially when consuming raw and undercooked foodstuffs. In addition, it should be noted that the bacteria studied are linked to certain physicochemical parameters of seawater such as temperature, pH and salinity. It is therefore necessary to set up a preventive program to eradicate these bacteria and reduce the risk of food poisoning, by slowing down the transmission of etiological agents. Awareness and popularization of the population and effective veterinary inspections at the fishery level are mandatory in order to minimize the maximum economic loss and preserve the health of consumers.

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