

Influence of rainfall on *E. coli* concentrations in clams: results of collaboration between competent health authority and producers' association in the province of Fermo (Italy)

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Abstract

The *Area Vasta di Fermo* (the competent health authority of the Marche Region), in collaboration with the local producers' Association conducted a series of studies on the presence of *E. coli* as an indicator of faecal contamination in the environment, in shellfish and in fishery waters, and on the factors that can affect their presence. These studies, carried out from 2008 to 2011, included an assessment of the currents along the coast, of the precipitations, and data from the monitoring of *E. coli* on shellfish harvested in the collection areas were examined. The results showed that in most cases, small concentrations of microorganisms in shellfish corresponded to little or no precipitations, while an increase in the levels of *E. coli* was preceded by more or less abundant rainfalls. The conclusions suggest that it is advisable to carry out a more detailed risk analysis which should take into account the above-mentioned factors. Furthermore, monitoring alone based on the determination of indicator organisms, especially when carried out as a single analysis or with a small number of *E. coli* determinations, does not provide a satisfactory indication of safety. The regional surveillance plans should be applied timely and rigorously, together with on-site investigations aimed at identifying changes that can affect the presence of *E. coli* in shellfish. Food business operators themselves could implement good manufacturing practices to verify whether the microbiological parameters are within the prescribed limits after rainfalls, especially if heavy.

Introduction

Edible bivalve molluscs may constitute a risk to food safety because, as filtering organisms, they are potential receptacles of pathogens, toxins and contaminants (Huss *et al.*, 2000), so that classifying and monitoring fishery waters (Latini, 2010) and the molluscs themselves is fundamental to ensure food safety in shellfish production. The fishing and marketing of molluscs should therefore comply with the chemical, biotoxicological and microbiological parameters established by the relevant European standards.

EC Regulation no. 854 of 29 April 2004 (European Commission, 2004b) states that member Countries must classify fishery waters and set up monitoring systems for fishery waters that are already classified, based on the regular monitoring of both water and shellfish.

Classification and monitoring are implemented differently from Region to Region, partly because of the different types of coasts, and partly due to differences in the shellfish collected, in terms of species and quantity, in the various parts of Italy. In the Marche Region the classification of fishery waters is regulated by the *Delibera della Giunta Regionale* no. 1300 of 3 August 2009 (Regione Marche, 2009), while monitoring is regulated by the *Decreto del Dirigente P.F. Veterinaria e Sicurezza Alimentare* no. 190/VSA of 13 November 2009 (Regione Marche, 2010a). The classification is done according to EC Regulation no. 854/2004 and no. 2073 of 15 November 2005 (European Commission, 2004b, 2005), which classify fishery waters in three categories according to the *E. coli* concentrations in molluscs (expressed as MPN/100 g): A zone (≤ 230), B zone (≤ 4600), C zone ($\leq 46,000$), respectively. Another fundamental aspect for the proper classification of fishery waters is the preliminary assessment of pollutants, their local circulation and their actual impact. This must be carried out by the competent authorities, while food business operators, in accordance with EC Regulation no. 853 of 29 April 2004 (European Commission, 2004a), must take into account all the relevant information, including environmental and weather conditions. The above-mentioned *Decreto del Dirigente no. 190/2009* (Regione Marche, 2010a) sets out the monitoring frequency and criteria and establishes partnerships between the competent authorities and food business operators through operational protocols.

The *Area Vasta di Fermo* (the competent health authority of the Marche Region), in collaboration with the producers' Association *Consorzio per la gestione della pesca dei molluschi bivalvi nel Compartimento marittimo di*

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San Benedetto del Tronto (Co.Vo.Pi.), conducted a series of studies on the presence of *E. coli* as an indicator of faecal contamination in the environment, in shellfish and in fishery waters, and on the factors that can affect their presence. These studies included an assessment of sea currents and tides, a census of the sources of human and animal pollution and the evaluation of data on organic pollutants released in the environment in various times of the year, as connected to seasonal variations, resident and animal populations in the catchment area, rainfall, wastewater treatment and orographic features of the area involved. This information was in part acquired from documentation made available by the various competent agencies (Competent Health Authority, Official Laboratories, Marche Region, Province of Fermo, Integrated Provincial Water Consortium) and in part derived from direct measurements made along the 26 km of shoreline under study. In order to identify potential sources of pollution, the documentation consulted included: technical reports of the wastewater treatment plants, biological monitoring, ecological and environmental classification of the water courses that empty into the sea along the coast under study, reports on the vulnerability to nitrate pollution of surface waters, data on livestock, maps with the locations of breeding farms in the area, data on wildlife and data on the monitoring of coastal seawaters.

The purpose of this study is to report on the data regarding the samples taken along the coast of the province of Fermo during the period 2008-2011 and the number of samplings resulting in *E. coli* values greater than 230 MPN/100 g, and to illustrate the possible relations between the increased concentrations of *E. coli* in the harvested shellfish and the levels of precipitation immediately prior to sampling.

Materials and Methods

The survey was conducted in accordance with the provisions of the *Delibera della Giunta Regionale* no. 1665 of 22 November 2010 (Regione Marche, 2010b) and in application of the Guide to Good Practice of the European Union Reference Laboratory on monitoring bacteriological contamination of bivalve molluscs, issued on 4 August 2010 (CEFAS, 2010).

E. coli monitoring was performed by the competent health authority from 2008 to 2011 in the production areas of natural beds of *Venus gallina* along the coast within the Area Vasta di Fermo. During the period from 2008 to 2010 seven zones were classified; according to the classification made in 2011 ten zones were classified (Figure 1), divided into subzones based on bathymetry (3-6 m and 6-9 m); the difference is due to the fact that three new zones were added which were previously under the competent health authority Area Vasta di San Benedetto del Tronto. In these subzones, the natural beds of *V. gallina* were sampled from aboard a fishing boat equipped with a special rake, and the samples were taken by travelling backwards along designated stretches of water. In each stretch, two strips parallel to the coastline were examined, at depths of 3-6m and 6-9m, respectively; the catch was selected by size using rotating screens mounted on the deck, and under-measure shellfish were returned to the sea. In each zone/subzone the frequency of microbiological testing was at least every two months.

The mollusc samples to be analysed, consisting of a single batch of at least 4 kg of commercial-size shellfish, were sent to the Official Laboratory responsible for the area accompa-

nied by the sampling report.

E. coli determinations were made using the official methods prescribed by the relevant standards (ISO TS1649-3). Historical data on daily precipitations during the period 2008-2011 was acquired from the Centro di Ecologia e Climatologia, Osservatorio Geofisico Sperimentale di Macerata.

The data regarding the official sampling for the detection of *E. coli* in molluscs, carried out from January 2008 to December 2011, were compared with the historical data on total daily rainfall during the three days prior to each sampling, as measured in two monitoring stations located at Civitanova Marche (for production zones between BM.1 and B17.2) and San Benedetto del Tronto (for zones A-B18 and AQ).

Results

Table 1 shows the number of samplings and of *E. coli* determinations in mollusc tissues exceeding the limit of 230 MPN/100 g (non conformities).

In 2008, the prescribed *E. coli* limits were exceeded 3 times, 2 of which in A zones and 1 in a B zone. The non-conformities were detected in samples taken during the months of October, November and December. In 2009, the limits were exceeded 29 times, 4 of which in A zones and 25 in B zones, in samplings made in March (12), April (2), September (1) and December (14). In 2010, 61 cases of exceeded limits were detected, 12 of which in A zones and 49 in B zones, observed in January (14), February (8), March (14), April (2), June (4), August (3), November (12) and December (4). In 2011, 33 cases were detected, 9 of which in

A zones and 24 in B zones, recorded in February (1), March (4), June (18), August (7) and December (3).

A comparison between the microbiological data and the analytical data on precipitations showed that in most cases small concentrations of *E. coli* in shellfish corresponded to small or no levels of precipitation, while an increase in the levels of *E. coli* was preceded by more or less abundant precipitations. An example of the comparison between *E. coli* concentrations and precipitations in A17.2/6-9 zone is shown in Figure 1. In some cases heavy rainfall was not followed by an increase in *E. coli* concentrations. In one case only (17.8.11) increased concentrations were found even in the absence of precipitations during the three preceding days.

The concurrence between heavy rainfall during the three days prior to collection and increases in *E. coli* levels was more evident in the following zones, shown in Figure 2: BM.1/3-6 m (immediately south of the river Chienti, which receives the outflows of a purification plant, a few natural drainage ditches and spillways from the municipal sewage network), B15/3-6 m (on the southern edge of the town of Porto Sant'Elpidio, where the river Tenna empties along with the Porto Sant'Elpidio purification plant and spillways from the municipal sewage network), B16.2/3-6 and 6-9 m (on the southern edge of the town of Porto San Giorgio, where the river Ete Vivo empties along with a few ditches and discharges from the purification plant of the town of Fermo), B17.1 and B17.2 (where a few ditches empty), B18 and A18 (facing the mouth of the river Aso, where a few purification plants empty), AQ/3-6 and 6-9 m (where a few ditches and discharges from a purification plant empty).

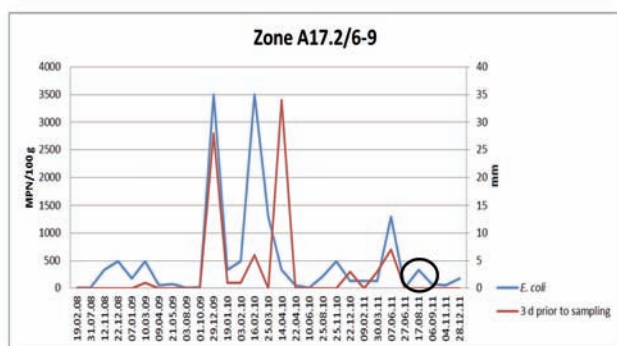


Figure 1. Comparison between *E. coli* concentrations (MPN/100 g) in shellfish in the production zone A17.2/6-9 and analytical data on precipitations (mm of rainfall) three days prior to sampling in the period 2008-2011.

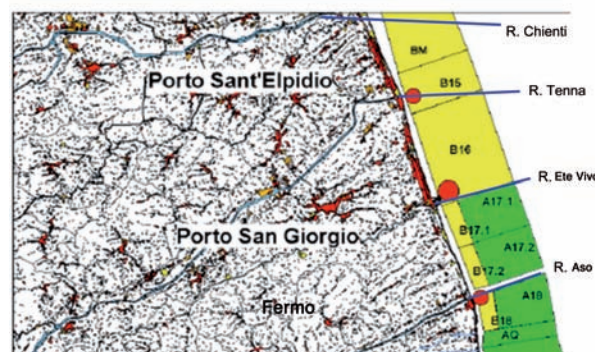


Figure 2. Classified production areas consisting of natural beds of *V. gallina* falling within the competence of Area Vasta di Fermo.

Discussion

Finding positive measurements in absence of precipitations during the three days prior to sampling may be due to the fact that the sampling period coincided with the peak of the tourist season (17.8.11), when the amount of wastewater treated by purification plants can be generally very large, exceed plant capacity and cause the spillage of above-normal pollution loads into the receiving waters.

A further consideration is related to the location of the areas where the concurrence between abundant rainfalls and increased levels of *E. coli* was more evident: in all likelihood, once drained into the sea, the rainwater and fluvial run-off are transported south by the local prevailing current which flows from north to south, according to available data on currents and tides (Cushman-Rosin *et al.*, 2001); in addition, since the regions upstream of these areas are densely inhabited, industrialized and highly touristic, the waters closer to the coast would be at greater risk of faecal contamination linked to municipal wastewater.

A further aspect to highlight is the relation between river regime and the seasons: the torrential nature of the rivers of the Marche Region greatly affects the amount of sediment carried to the sea, with large variations in flow rates between high-water and low-water periods. When a particularly abundant precipitation occurs after a prolonged drought, the river beds fill quickly and consistently and the waters carry away the pollutants that have

accumulated along the course of the river.

The socio-economic data of the three hydrographic areas in the province of Fermo (Chienti, Tenna/Ete Vivo, Aso/Tesino) show that, over the years, there has been a gradual abandonment of mountain areas and an increase in the population living in seaside towns, resulting in the development and expansion of industrial activities. Because of this, anthropogenic pollution is greater near the coast, especially in the areas of the Chienti and Tenna rivers. Together with these factors it must be taken into account the considerable pressure exerted on the territory of the coastal communities by tourism during the summer months, especially in the southernmost part of the province. Other important factors in evaluating the extent of pollution are the characteristics of the sewage and wastewater purification plants (design organic load, type of treatments applied, *etc.*). In the coastal towns the percentage of coverage of sewerage networks is about 80-90%. On the whole, the ten treatment plants, located in the area have capacities suitable for the amount of wastewater to be treated. In addition, expansion works are already planned for some of them, and a new sewage plant is scheduled to be built in the Lower Tenna area.

In case of excessive rainfall, spillway discharges may occur that completely bypass the purifier and discharge wastewater directly into the receiving bodies. The survey of water supplies, discharges, ditches and all other potential sources of pollution along the coast of the province of Fermo provided an overview of the

general situation regarding coastal pollution loads. The possible sources of pollution directly involved are represented by the four rivers that are met along the coast (Chienti, Tenna, Ete Vivo and Aso), which are intercepted with dams or sluices to generate hydroelectric power or for agricultural and industrial purposes. The large water withdrawals make the river courses more prone to flash floods, with frequent increases in the amounts of pollutants and sediment that reach the sea.

Although the waters of these rivers are in critical conditions, considering the data on the whole it can be noted that the number of samples with values of *E. coli* above 230 MPN/100 g detected in shellfish production zones located in front of river mouths was similar to the number detected in other zones (Table 1). This can also be attributed to the fact that the sediments (and any pollutants) carried to the sea are widely distributed by the sea currents. The effects of the tides and currents were also taken into consideration. The main surface current in this part of the Adriatic sea runs from north to south, but other minor, deeper currents have been identified whose directions are still not well known. The interplay between surface and deep currents, together with the action of the waves and the tides, produces considerable mixing of marine sediments, which represent the most important vehicle for all sorts of pollutants that, once carried to the sea, can reach considerable distances.

Table 1. Number of samplings and of non-conformities for *E. coli* in shellfish by zone/subzone and year.

| Zone | Subzone | 2008 | | 2009 | | 2010 | | 2011 | |
|--------|---------|-----------|---|-----------|---|-----------|---|-----------|---|
| | | Samplings | + | Samplings | + | Samplings | + | Samplings | + |
| BM.1 | 3-6 | 3 | | 5 | 2 | 6 | 3 | 6 | 2 |
| | 6-9 | 3 | | 6 | 3 | 6 | 3 | 6 | 1 |
| BM.2 | 3-6 | 3 | | 6 | 3 | 6 | 3 | 6 | 1 |
| | 6-9 | 3 | | 6 | 2 | 6 | 3 | 7 | 2 |
| B15 | 3-6 | 3 | | 5 | 2 | 6 | 3 | 6 | 1 |
| | 6-9 | 3 | | 6 | 3 | 6 | 4 | 6 | 1 |
| B16.1 | 3-6 | 2 | | 6 | 2 | 6 | 4 | 7 | 1 |
| | 6-9 | 2 | | 6 | 1 | 6 | 5 | 7 | 1 |
| B16.2 | 3-6 | 3 | | 5 | 1 | 7 | 5 | 6 | 1 |
| | 6-9 | 3 | | 5 | 2 | 6 | 3 | 6 | 1 |
| B17.1 | 3-6 | 3 | | 5 | 1 | 7 | 4 | 6 | 3 |
| A17.1 | 6-9 | 3 | | 5 | 1 | 8 | 5 | 8 | 3 |
| B17.2 | 3-6 | 3 | | 6 | 2 | 7 | 4 | 6 | 3 |
| A 17.2 | 6-9 | 4 | 2 | 7 | 2 | 10 | 6 | 8 | 2 |
| B18* | 3-6 | - | | - | - | | 8 | 2 | |
| A18* | 6-9 | - | | - | - | | 6 | 2 | |
| AQ* | 3-6 | - | | - | - | | 8 | 2 | |
| | 6-9 | - | | - | - | | 8 | 2 | |

+, number of non conformities (non conformity=values above 230 MPN/100 g). *Zone acquired by Area Vasta di Fermo since 2011.

Conclusions

In conclusion, while routine tests on batches received at dispatch centres do provide additional control on microbiological quality, they do not make up for a fully adequate monitoring and classification system that includes measurements that take into account additional risk factors, such as precipitations. To better assess the correlations between precipitations and increased values of *E. coli* in shellfish, it would be interesting to examine samples taken three days after the start of heavy rainfalls, since, as observed above, it should be possible to find an increase in bacterium levels in the shellfish. In that regard, food business operators themselves could include the following activities among good manufacturing practices: shellfish taken from A zones three days after the start of a rainfall, especially if heavy, should be sent to purification centres, while shellfish taken from B zones three days after the start of a rainfall should undergo additional testing to check whether the microbiological parameters are within the prescribed limits. However, monitoring alone, based on the determination of indicator organisms, allows a less than satisfactory risk assessment of contamination by any pathogenic bacteria and viruses that may be present; a single analysis, or a small number of determinations regarding *E. coli*, does not provide a fully reliable indication of healthiness.

In order to ensure the safety of bivalve molluscs it is essential that the regional surveillance plans be applied timely and rigorously, but equally important is the continuity of on-

site investigations looking to identify urban or environmental changes that can affect the presence of *E. coli* in shellfish (Latini, 2010).

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