Universität zu Lübeck

Determination of pathogens in chilli sauces of street taco stands in Dolores Hidalgo, Mexico

An internship review

24/04/2015

Summary

Background: The student

I studied my bachellor in Universidad de Guanajuato, in Guanajuato, Mexico, obtaining the grade of Bachelor of Science as Chemical Pharmacist and Biologist. This five-year bachelor programme allows the student to get the knowledge and the basis to work in any kind of laboratory involving chemistry, biochemistry, and biology, but focusing mainly into human medicine. Afterwards, I began to work in the clinical laboratory area until I got my own laboratory, until the opportunity of studying in Germany came along.

Having this strong clinical background makes me avoid that that can be known or perceived as "basic science", and it makes me try to take a look into more clinical, straight-to-the-point knowledge, which can instead develop a direct action as a response of a problem.

Bckground: The institution

Laboratorio Dolores Hidalgo is a clinical analyses laboratory that opened its doors in 2006, located in the city of Dolores Hidalgo, in the state of Guanajuato. It quickly got the position as one of the best and most important laboratories in the north of the state of Guanajuato, regardless its humble position and cheap prices. This laboratory is owned and directed by CPB Sp. Clinic Biochem. Ma. Elsa Zavala Rincón, graduated from Universidad Autónoma de Querétaro.

The laboratory has different areas: haematology, clinical biochemistry, parasitology, urinalysis, immunology, and microbiology. Special tests are sent to reference laboratories in Celaya (ULACE) and Mexico City (LASER).

Background: The project

Mexico is well known to be home of some important pathogens globally speaking, but due to its different landscapes, ecosystems and geological conditions, not all of them are endemic in the whole Mexican country (as an example, *Plasmodium* species are restricted to the south and some areas of the east and west coasts, due to its jungle environment). A recurrent problem in Central Mexico is the endemic condition of *Salmonella*, which can be assessed by the constant presence of antibodies against typhic and paratyphic antigens in patients' sera, meaning that people is constantly in contact with this pathogen. A deep analysis wasn't necessary due to the common habit of all the Mexicans while choosing fast food: everybody eats street tacos, all economic strata in the country; some eat them there or take them away. It's possible to find them on daytime and night time.

Analysing tacos is not supposed to be an easy task, due to the different ingredients involved: tortillas, different kinds of meat, raw vegetables (like onion and coriander), cooked vegetables (carrots, onions, among others), cheese (which is more related to brucellosis cases), and chilli sauces (most of them are prepared with cooked ingredients, but some of them can be prepared with raw ones as well). One ingredient had to be analysed just for this three-month internship, and due to its easy form to analyse it, chilli sauces were selected as the first target.

Experiment design

As stated before, there are daytime and night time tacos, and sometimes the kind of meat changes according to it. At the same time, due to cultural factors, these taco stands are located in the middle of the street, but there are taco locals too. A taco local in a place that has a bathroom and sometimes a kitchen, and people can sit down to enjoy their meal, but still, there is a stand located just in the main door of the place.

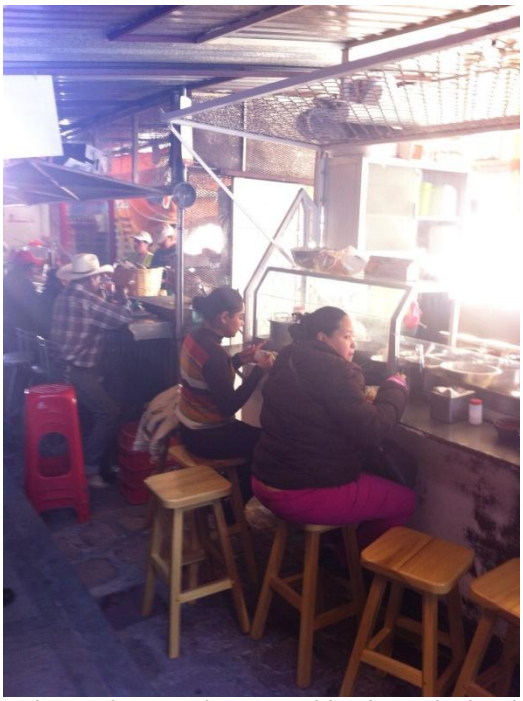

Figure 1 and 2: Daytime taco stands. People who work in popular markets tend to go to this places for lunch break.

Figure 3: A taco local is located just next to the red car meanwhile there is a construction site next to it. There is no protection of any kind to avoid food contamination.

One of the main goals of this experiment, besides the determination of Salmonella and some other pathogens, is the evaluation of hygiene measure based in the number of bacteria found in the chilli sauces as well as the determination of other pathogenic bacteria. In order to achieve these results, the interaction of two main factors among the "taquerías" (the taco stands/locals) were assessed: one, the time of the day where the tacos were sold (daytime or night time); and two, the place where they are sold (street stand or local).

One would expect that daytime tacos would have more bacteria compared to night time tacos, due to the increased movement in the city during daytime (people walking around, worse environmental conditions due to automobile traffic, and street animals like dogs or cats walking around). At the same time, one would expect that local tacos would be cleaner due to the facts that they have bathrooms (allowing cooks and costumers to wash their hands) and it's a more private environment.

It's important to point the dynamics of chilli sauces: they are not served from bottles as a person who hasn't been in Mexico would think; instead, they are served in small bowls with a spoon to put it directly into the tacos, so the sauces are not protected from the environment, being exposed to dust and bugs such as flies.

In order to make the analysis the next evaluation dynamics were designed:

	Street stand	Local
Daytime	Five taquerías	Five taquerías
Night time	Five taquerías	Five taquerías

Table 1: A total of twenty taquerías were examined, in order to evaluate the interaction among the time and the place where the service is offered and evaluate the hygiene measures.

As any other relevant scientific experiment design, a control is required. In this case, the controls were obtained randomly from places where the hygiene measures are supposedly better and controlled. In this case, we used ten sauces from different sources as controls: three sauces cooked in households, three sauces from health institutions, and four sauces from restaurants (the difference between a restaurant and a local, public-hygiene wise, is that at the locals, the cooks not always prepare the sauces there, since they can bringing them from their home, meanwhile in a restaurant, due to the exigencies stated by the Health Bureau, everything that is served there should be cooked inside the kitchen which has to accomplish certain set of requirements).

It's also important to notice that not all the taquerías serve the same amount of sauces: most of them offer two (most of the time green sauce [green chilli with green tomato] and red sauce [red chilli, with or without red tomato], but there are some of them that only offer one or until four.

At the end, 49 sauces (including the ten controls) where evaluated.

Experimental procedure

Regardless the time and location, every sauce was assessed by:

- Type of sauce: Red sauce and green sauce are usually cooked sauces, meanwhile avocado, pineapple, or “pico de gallo” (translated as “rooster’s beak”, containes tomato, onion, green chilli and coriander) are usually made of raw ingredients. This could lead to a significant difference among the way that the sauces are prepared.
- pH: In order to evaluate if there is a significant different among variations.
- Presence of parasites: Faust’s flotation test was performed to all the sauces. Contrary to the expected, all of them were negative.
- Presence of hyphae: Clinically speaking, yeasts are not considered as pathologic in Mexico (unless it is related to a respiratory condition), but hyphae-forming fungi are. Their presence was evaluated with Sabouraud-Dextrose agar. All of the sauces were negative.
- Bacterial examination: Only when these sauces come just out of the kitchen, they could be considered as sterile, but just when they make contact with air, they become a nutritive broth for bacteria. Sauces were evaluated in a pure form and in a 1:10 dilution with saline solution, under aerobic and anaerobic conditions incubated at 37°C for 48 hours.

The media that were used for the bacterial evaluation were the following:

- Blood agar, for an overall evaluation.
- Manitol-Salt agar, for staphylococci evaluation.
- McConkey agar, for gram negative evaluation.
- Columbia agar, for gram positive evaluation.
- XLD agar, in order to detect the presence of *Salmonella* and other bacteria such as *Shigella*.

Figure 3: An incomplete set of media for a journey of inoculation for five sauces, still, showing the six different media used for the differentiation of bacteria and fungi. Starting from the upper left corner, clockwise order: Blood agar, Dextrose-Sabouraud agar, XLD agar, Manitol-Salt agar, CNA Columbia agar, and two sets of McConkey agar. The inoculation was done with sterile plastic loops along the whole surface of the media, under aerobic and anaerobic conditions, with pure sample and a 1:10 dilution.

Once bacteria grew in the media, some of them could be already identified but some others need to be analysed with more techniques.

- Gram staining, in order to determine if they were Gram positive or negative, and cocci or bacilli, and at the same time, discard yeast.
- Growth in Bio-Rad UriSelect, a chromogenic agar that would allow narrowing the characteristics of the bacteria that couldn't be determined with conventional media. It will provide certain characteristics that could be easily evaluated with the help of a table provided in the kit.
- Oxidase test for the Gram negative bacteria.
- Siemens MicroScan assessment. This equipment will allow determining the bacteria that couldn't be assessed by the other methods. In order to determine Gram negative bacteria, the equipment will ask in advance if the assessed bacteria were oxidase positive or negative.

Staphylococcus aureus and *Staphylococcus epidermidis* were assessed for methiciline-resistance, an upcoming problem in both developed and developing countries. This evaluation was done qualitatively inoculating maximum ten random colonies per sample of these bacteria in nutritive broth and then, an evaluation of the resistance to Oxacylin was done. If they were resistant, they were considered as methicilin-resistant.

A similar process was done for Gram negative bacteria to evaluate the production of beta-lactamases, assessing resistance against Ceftriaxone. No positive result for beta-lactamases production was found in this experiment.

Results

Twenty taquerías were assessed, obtaining a total of 39 salsas, having a total of 49 if we consider the ten controls as well. In the study samples, 37 different bacteria were found. The most numerous ones were *Micrococcus* spp, *Bacillus* spp, and *Klebsiella pneumoniae.* The first two could be considered as saprophytic. Some coliform bacteria were found but in lesser quantities.

In the study group, only nine bacteria were found. There was a dominance of *Micrococcus* spp, *Klebsiella pneumoniae* and *Bacillus cereus.*

Regarding the hygiene evaluation, using the analysis of variance (ANOVA) statistical test, with a *p* value of 0,05, there was no significant difference among the four study groups, but there was regarding the difference between the groups and the controls. At this point, we can conclude that effectively, chilli sauces are easily contaminated by saprophytic bacteria, as well as those that could come from the cook. Study samples would have almost 100-fold more bacteria compared to the control group.

Regarding the original hypothesis, no *Salmonella* was detected in this study, but instead, an outbreak of *K. pneumoniae* indeed was. Almost all the sauces contained this bacterium. At some point around the 25% of the experiment, I thought it was due to contamination, being myself the source of it, so I tested myself in order to discard this possibility. The result was negative. At the same time, I made cultures of didistilled water to examine if the contamination came from the media or the equipment, which was new or sterile.

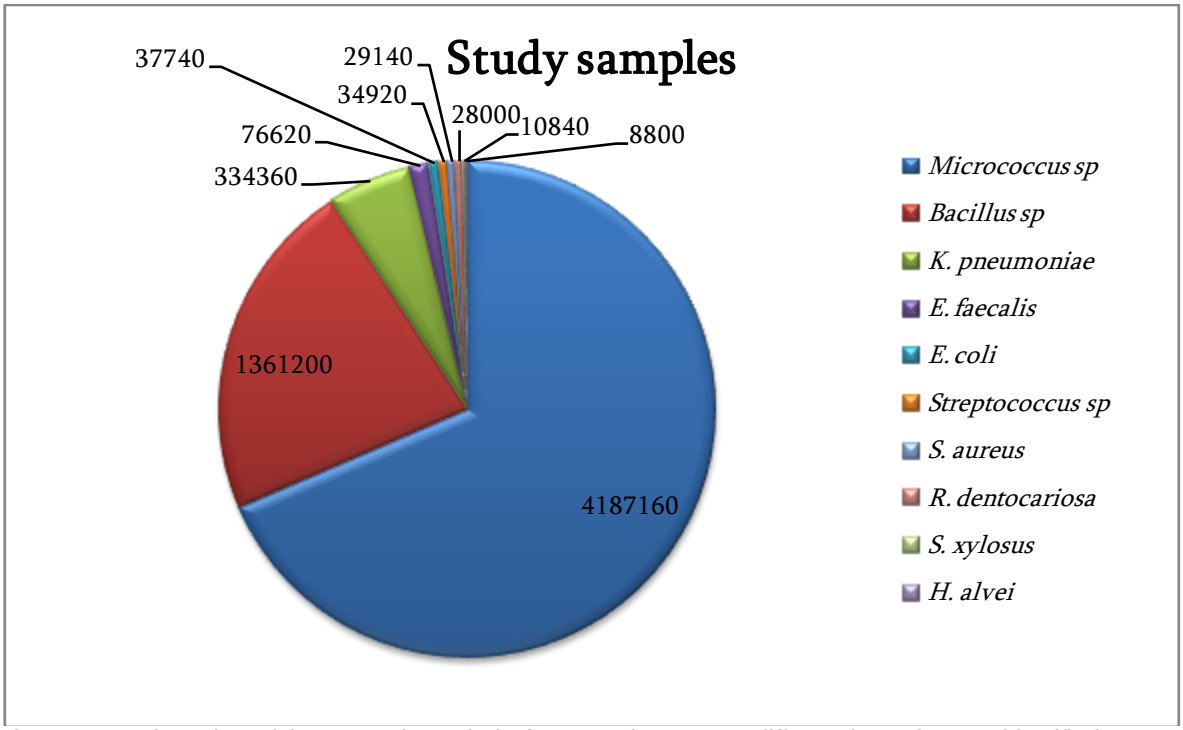

Figure 4: Total number of the top 10 bacteria in the control group. 37 different bacteria were identified among the 39 samples.

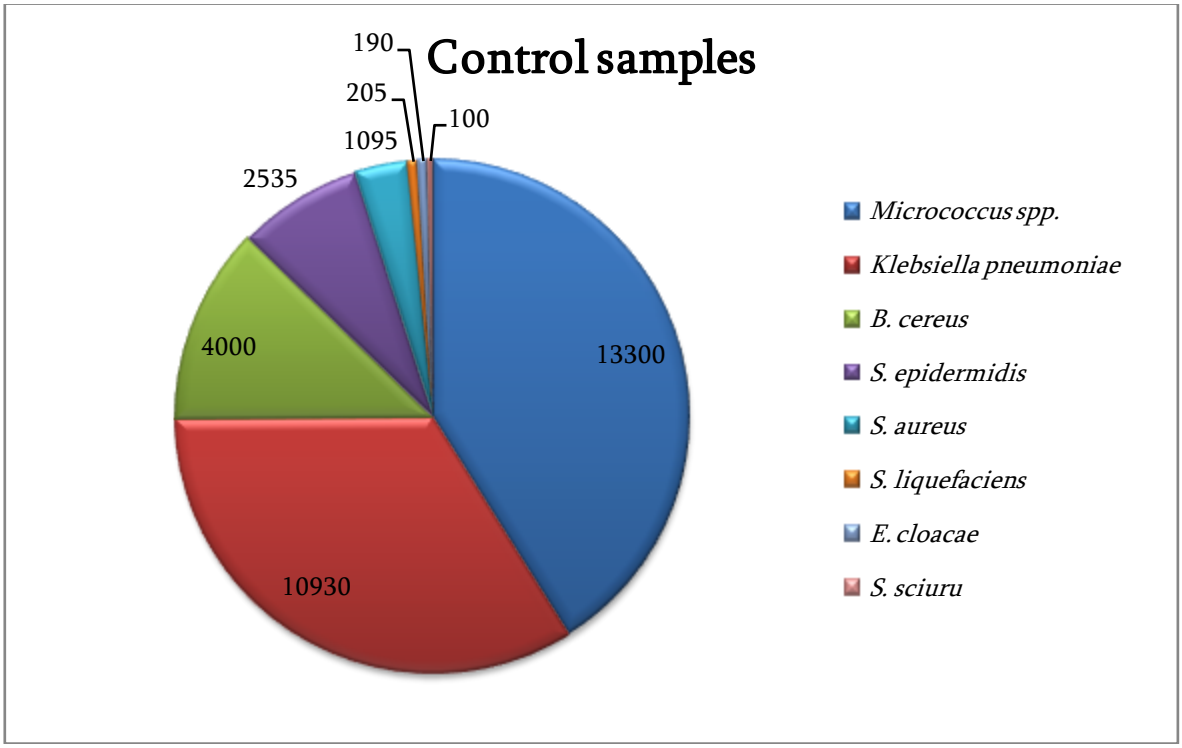

Figure 5: Nine different bacteria were identified in the control group, with more reduced numbers compared to the control group. Please, consider that the colours are totally unrelated to those in the control group. These charts are only used to compare bacteria numbers in one single group.

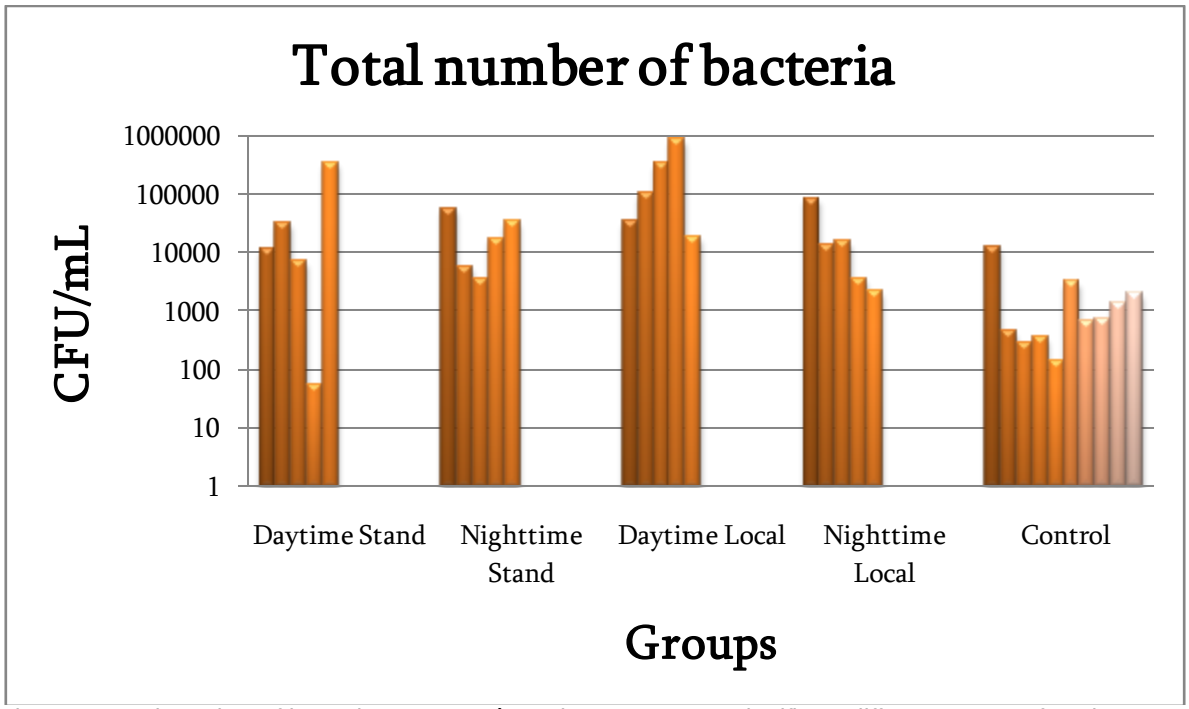

Figure 6: Total number of bacteria per taquería. Using ANOVA, no significant differences were found among the four groups, meanwhile there was between the study groups and the control group.

It needs to be noticed that *K. pneumoniae* was growing in Manitol-Salt agar, a medium restricted to staphylococci and some other few bacteria given its high levels of salt. It's well known that a lot of food is conserved with salt. Having salt-resistant Gram negative bacteria is not the best thing to happen in the food industry. So far, immunocompetent people are not affected by this strain of bacteria, but the fact that it's around is concerning regarding immunocompromised people, such as infants, old people and sick people.

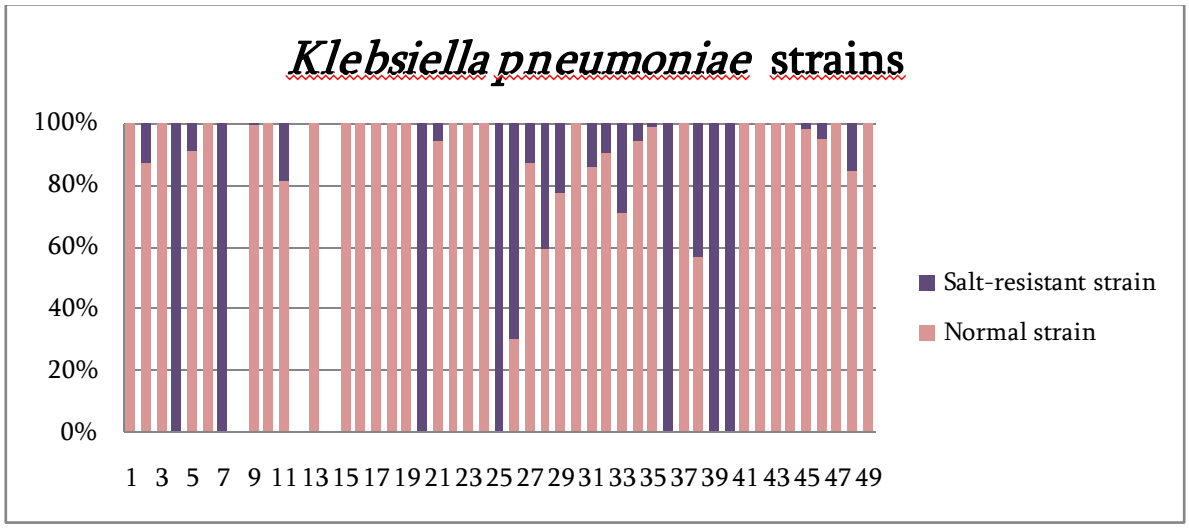

Figure 7: Presence of salt-resistant *K. pneumoniae*. The total numbers were counted in the McConkey and blood agar media, meanwhile the salt-resistant strains were counted in Manitol-Salt medium.

Figure 8: *Klebsiella pneumoniae* growing in McConkey agar. Regardless some other bacteria growing in the agar, we can see the predominance of this strain on the medium.

Figure 9: Salt-resistant *K. pneumoniae* growing in Manitol-Salt agar. Please notice that the colonies are even dripping from the media.

At the same time, it was important to notice the presence of methicilin-resistant staphylococci, both *S. aureus* and *S. epidermidis*, in study and control groups. This is more likely to be due to human transmission rather than any other factor.

	S. aureus	MRSA	*S. epidermidis*	MRSE
Daytime stand	9/10	3/9	6/9	0/6
Nighttime stand	5/8	0/5	2/8	0/2
Daytime local	5/7	1/5	2/7	1/2
Nighttime local	6/12	0/6	1/12	0/1
Control	4/10	0/4	5/10	2/5

Table 2: Qualitative analysis of MRSA and MRSE in the samples. In the first and third rows, we can see the number of sauces per group that were infected by the bacteria, meanwhile in the second and fourth rows, we see the number of MRS per infected sauce.

Conclusions and Outcome

After this experiment, we can conclude that we can find pathogens in these chilli sauce, but most of the times, they're not in infective doses. The main concern here was the outbreak of *K. pneumoniae*, since seems to be everywhere, even in the control group. The next step to take is to establish where it comes from. Does it come from the water? From the soil? Is it related to human transmission? Is the soil carried away into the sauces due to the strong winds in the city?

At the same time, the presence of MRSA and MRSE is a matter of concern due to the implications it can take if the consumers don't have a strong immunological system and are "colonised" by these bacteria, leading to future complications and even infection to other people.

At the end, it wasn't possible to detect *Salmonella* in these samples, since analysing the whole set of ingredient for taco preparation would be deserved for a thesis project, not for an internship, but still gave enough feedback to consider some other public health issues.

Fecalism was out of the question. Sauces were sterilised and inoculated with *K. pneumoniae* and *E. coli* to analyse their interaction and see if there was some kind of competence between them, since in the experimental sauces, just in few samples was detected *E. coli*. The main outcome of this small experiment was that the growths of these bacteria were totally independent among each other.

About the city

Dolores Hidalgo is denominated as the city where the Independence was declared in Mexico. Regardless its status and its historical importance, it's a small city holding 150000 inhabitants, considering those who live in the countryside as well. The city downtown is quite busy during daytime, but at night time, it's mostly quiet and easy.

Its more appealing attractions are:

- The Independence Museum, located in Miguel Hidalgo y Costilla's (the man known as "the Father of Independence") former house. In this museum, many objects and documents regarding the Independence war can be found.
- Ice cream in the main square. The ice-cream that is sold in Dolores Hidalgo is not properly known for being ordinary. Among the multiple stands selling ice cream surrounding the main square, hundreds of flavours can be found. Chocolate, vanilla, strawberry, lemon, pineapple... all of that sounds pretty ordinary, but still delicious. More exotic flavours can be found, such as rose petals, daisies, sweet corn, cheese, beer, tequila, pineapple-Malibu, Rum-Cola, etc. Probably that edible enough, even interesting, but there are some of them (which I haven't dared to try due to its nature) that are crazy enough, such as shrimp, octopus, mole (a Mexican sauce that has as base cacao, chilli and spices), pork skin, and more. A total experience for any kind of *gourmand*.
- Ceramics and pottery. In former times, Dolores Hidalgo was known to be the hometown of the miners that worked in the surroundings. During that time, the people who stayed in town used to work creating pottery. Nowadays is known as one of the best ceramics centres in Mexico. Young and well prepared designers decide to stay at home to optimise the pottery designs of the pottery enterprises that have been the family business for generations, offering classic and modern designs, for any kind of taste. Incredibly, prices are very cheap. A set of three escalated pots of very nice quality cost around ten euros, still very cheap for Mexican standards.

About the experience

The city, regardless being neighbour of San Miguel de Allende (voted as the best touristic city in the world in 2013) and Guanajuato (the capital of the Federal State, having one of the best universities of the country in certain fields, who accepts students from all over the world for internships, as well as being the home of one of the most multicultural festivals in the American continent), still maintains a XIX century air, both architectonically and culturally. People is still dealing with a inspiration-less mindset, many young people find no inspiration in studying, but doesn't seem convinced to get a nice job. People still rely in religion a lot, so at least once a week there is a religious festivity, leading to traffic jams and noisy fireworks (without any visual appeal).

Making aside these things, the city has an amazing offer of food options, especially in downtown. Nothing especially or typically regional, just the standard Mexican food cooked with nice ingredients and good flavour. I have to point that, of all the tacos that were bought to get the sauces, were eaten by me and I don't regret anything.

Usually, the locals don't speak good English, but since the tourism is increasing, a lot of they are starting to learn to offer their services properly, and certain percentage of young people are able to speak it.

Regardless of its old-fashioned status, Dolores Hidalgo seem to be home of many electronic music parties at Federal-State level. People from other cities tend to go to the city just to enjoy these parties that happen in bars, restaurants and some private locations. The city still refuses to give enough diffusion and support to these events, regardless of the amount of people that comes from other places.

Overall, I think it's a nice city to hang around, and it seems to be evolving slowly, but until this evolution doesn't happen, it doesn't seem to be an option for a young person for living, but for some months is more than enough.