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MASTER DISSERTATION

Theme

Prevalence and antibiotic susceptibility of Salmonella strains isolated from milk and dairy products

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List of abreviation

List of abbreviations

ATM : aztreonam

AMP : ampicilline

AMC :*amoxicilline*

CIP : Ciprofloxacine

CEZ:cefazoline

CTX :ceftazidim

ERT : ertapenem

FOX :cefoxitine

GEN : Gentamicine

GMT : gelose touati modified

H2S :hydrogene sulfure

ISO : International organization for standarization

LEM : laboratory of ecologymicrobial

MDR : Multiple Drug Resistance

MIC : Minimum inhibitory Concentration

NTS : non-typhoidal Salmonella

RV: Rappaport Vassiliadis

TDA: Tryptophan desaminase

TSI: Triple sugar Iron Agar

WHO: World Health Organization

XLD: Gelose xylose lysine desoxycholate

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INTRODUCTION

Salmonella is a Gram-negative zoonotic pathogen (Yang and al., 2018)it is one of the major causes of foodborne infectious diseases, posing a serious threat to public health. It can enter the food supply chain at different stages of production, processing, distribution, and marketing (Shen and al., 2021). Gastroenteritis is the most common manifestation of *Salmonella* worldwide, followed by bacteremia and enteric fever (Heredia and García, 2018) *Salmonella* is mainly found in poultry, eggs and dairy products Fresh fruits and vegetables are other food sources implicated in the transmission of *Salmonella* (Eng and al., 2015)

In general, food animals such as pigs, poultry and cattle are the main sources of *Salmonella* infection. The main routes of dissemination of pathogens are trade-in animals and uncooked animal food products. The slaughter process of food animals in abattoirs is considered to be one of the main sources of contamination of organs and carcasses with *Salmonella*. The emergence of antibiotic-resistant foodborne pathogens has raised public concern as these pathogens are more virulent and lead to the increased mortality rate of infected patients (Eng *and al.*, 2015)

Although different serotypes have been associated with salmonellosis, a limited number of them are responsible for most human infections; S. enterica Enteritidis being the most common in the EU (39.5% in 2013) and the USA (14.5% in 2012), followed by *S. enterica Typhimurium* (including its monophasic variant) (28, 8% in EU, 2013; 11.6% in the USA) *Salmonella* Enteritidis is commonly associated with poultry and its products, while *S. Typhimurium* has a wider range of species, including pigs and poultry. Cattle as well as poultry. Therefore, foods of animal origin, especially contaminated poultry products (eggs and poultry meat), have been considered the main vectors of *Salmonella* infection and associated with the global outbreak of S. Enteritidis In Furthermore, various epidemiological studies have confirmed the great contribution of poultry-based foods to the burden of salmonellosis (Antunes *and al.*, 2016)Increasing human population and urbanization, per capita income, globalization, and changes in consumption trends (more protein in the diet) have increased the consumption of animal products. The consumption of these products is estimated to reach 376 million tonnes in 2030 (Dhama and al. 2013).

This high demand for animal products results in intensive animal production and processing of products, with an increased circulation of food on a global level. This situation could lead to faulty processing practices and an increased risk of contamination with foodborne pathogens at any point in the farm-to-table chain. Animal origin is a serious concern because it is difficult to control. Many factors can cause contamination, including those from the environment (associated wildlife, water from different sources, disposal of animal waste, etc.) and human handling of animals (slaughtering and processing practices, storage procedures, etc. Microbial pathogens can cause disease through the consumption of animal products contaminated with microorganisms or their toxins.(Heredia and García, 2018)

These microorganisms can proliferate via the food chain and a pool of resistance genes can be transmitted to human pathogens, reducing the availability of effective drugs to treat infectious diseases caused by these microorganisms The increasing isolation of *Salmonella* antibiotic-resistant in humans and other animals is a public health concern *Salmonella* that is resistant to antibiotics can be found in multiple surveys using pulsed gel electrophoresis. Strains of *Salmonella* are reported to possess the blacmy gene, which results in plasmid-level resistance to ceftiofur and ceftriaxone, and are multidrug-resistant (Medeiros *and al.*, 2011) Thus, the main objectives of our survey were to report the prevalence of *Salmonella* present in the carcass and intestine of chicken and chicken offal and dairy products (cheese, cream, milk) in Bejaia. Furthermore, the antibiotic resistance profiles of these isolates.

Bibliographic synthesis

Taxonomy and nomenclature

Salmonella is an enterobacteria of the Enterobacteriaceae family. The genus Salmonella has 2 species: Salmonella enterica, and Salmonella bongori (Judicial Commission Of The International Committee On Systematics Of Prokaryotes 2005). Salmonella received its name from Daniel E. Salmon, the veterinarian who was the first to isolate (what was then called) "Bacillus choleraesuis» from pig intestines in 1884. This name was changed in 1900 to "Salmonella choleraesuis" by Lignieres.

Today, the genus *Salmonella* is divided into only two species: *Salmonella* enterica and *Salmonella bongori*, with *S. enterica* being divided into 6 additional subspecies. In the past, the subspecies of *S. enterica* were considered as subgenera and the serovars/serotypes of *Salmonella* as distinct species, which, according to what is done today, would give more than 2,600 species of *Salmonella*. The terms "serovars" and "serotypes" are generally considered synonymous. The World Health Organization (WHO)/Institut Pasteur use the term "serovar", while the Centers for Disease Control (CDC) and the American Society for Microbiology (ASM) originally used the term "serotype", but gradually replaced it with "sérovar" to maintain international consistency (Oludairo *and al.*, 2022).

The nomenclature of *Salmonella* is complex, and scientists use different systems to refer to and communicate about this genus. However, consistency in *Salmonella* is necessary for communication between scientists, health officials, and the public. Unfortunately, current usage often combines multiple nomenclature systems that inconsistently divide the genus into species, subspecies, subgenera, groups, subgroups, and serovars (serovars), which is confusing(Brenner and al. 2000). The nomenclature of the genus *Salmonella* has evolved from the initial concept of a serotype and a species proposed by Kauffmann based on the serological identification of O (somatic) and H (flagellar) antigens. Each serotype was considered a separate species (eg, *S. Paratyphi* A, *S.* Newport, and *S. Enteritidis*); this concept,if used today, would result in 2,463 species of *Salmonella*. Other taxonomic proposals have been based on the clinical role of a strain, on the biochemical characteristics that divide serotypes into subgenera, and finally, on genomic relatedness. Proposals for nomenclature changes within the genus have been summarized previously (Brenner *and al.*, 2000).

Salmonella enterica is divided into six subspecies, enterica (I), salamae (II), arizonae (IIIa), diarizonae (IIIb), indica (IV), houtenae (VI) (Wang et al., 2020) More 60% of all

Salmonella and 99% of serovars causing disease in warm-blooded animals are members of subspecies I. Other *Salmonella*, particularly subspecies IIIa (Arizona) and S. bongori, are associated with disease in cold-blooded organisms with Arizona and are occasionally responsible for systemic disease in humans. What is particularly intriguing about subspecies I serovars is that their ability to cause disease in animals encompasses a spectrum of host specificity and disease severity (Chan *and al.*, 2003).

Bacteriology

Salmonella is a genus belonging to the Enterobacteriaceae family. It is a Gramnegative, facultatively anaerobic, oxidase-negative, catalase-positive, non-spore-forming bacillus. The size varies between 2.0 and 5.0 μ m in length by 0.7 to 1.5 μ m in width. Most Salmonella species are motile by peritrichous flagella, except for S. Gallinarum, S. Pullorum and some mutants. The optimum growth temperature for Salmonella is 37°C. However, minimal growth was recorded between 2 and 4°C and at a maximum temperature of 54°C (Boubendir ;2019).

Transmission

Transmission of nontyphoidal *Salmonella* to humans can occur through ingestion of contaminated food or water, consumption of infected animals, or direct or indirect contact with *Salmonella*-infected animals in homes, veterinary clinics, zoos, or other public or private places (Eng *and al.*, 2015). The consumption of raw or undercooked meat remains a risk factor for salmonellosis.

Kitchen practices such as frequency of cleaning surfaces or not using a cutting board for raw meat are specific risk factors for *Salmonella* infection (Mughini-Gras and al., 2017). Contact with animals is responsible for several cases of human salmonellosis each year, and the risk to public health varies between animal species, age group, husbandry practice and state of health. Certain human subpopulations are considered more at risk due to biological or behavioral risk factors. Many human infections caused by direct contact with animals such as cattle, horses, cats, dogs etc. are attributed to occupational causes, the agricultural environment and contamination at the family level (Hoelzer and al., 2011).

Reservoir and Host

The host specificity of certain *Salmonella* depends on the ability of the serovar to adapt to the environment of its hosts. This specific ability to adapt to the host environment is regulated by several microbial characteristics, which are responsible for the expression of clinical manifestations in specific hosts. Other important determinants are the infectious dose of a particular serovar, the animal species infected, the age of the host, and the immune response. It has been shown that a particular mechanism making a serovar virulent for a particular animal species could make the same serovar less or even avirulent for another animal species. This phenomenon is called "serovar host specificity" or "serovar host adaptation" (Jajere; 2019)

Salmonella is an intestinal pathogen (D'Aoust; 1994) present in the intestines of humans and animals -their main reservoir- they can, following fecal contamination, survive in the environment (water and soil) for several months (Korsak; 2004) Their ubiquity results in a large spectrum of reservoirs: humans and animals, mammals, birds. Their ability to survive also allows them to persist in secondary reservoirs such as foods of animal origin, fruits and vegetables (Todd, Greig and al. 2008).

Antibiotic resistance

In developed countries, antimicrobial drug resistance in non-typhoidal *Salmonella* organisms is an almost inevitable consequence of the use of antimicrobial drugs in food-producing animals. Such drugs may be used either therapeutically or prophylactically, or for growth promotion (feed additives). Despite legislation targeted at controlling the overall usage of antimicrobials in food-producing animals, in recent years there have been significant increases in developed countries in the occurrence of resistance in non-typhoidal *Salmonella* spp. Such increases have been observed in many countries, not only in Europe but also in North America. Of particular concern in such organisms is the development of resistance to key antimicrobials such as fluoroquinolones and more recently extended-spectrum β -lactamases.

In developed countries, it is increasingly accepted that most of these strains are of zoonotic origin and acquire their resistance in the animal host before being transmitted to humans through the food chain (Threlfall ;2002)

In the early 1960s, the first incidence of resistance of *Salmonella* to a single antibiotic, namely chloramphenicol, was reported. Since then, the frequency of isolation of *Salmonella* strains with resistance to one or more antimicrobial agents has increased in many countries, including the United States, United Kingdom and Saudi Arabia. Antimicrobial agents such as ampicillin, chloramphenicol, trimethoprim and sulfamethoxazole are used as traditional first-line treatments for *Salmonella* infections. *Salmonella* spp. resistant to these agents are called multidrug-resistant (MDR) (Eng and al; 2015)

Of particular importance in the 1990s was a multidrug-resistant strain of *Salmonella* typhimurium of definitive phage type (DT) 104, showing resistance to six commonly used antimicrobials, with approximately 15% of isolates also showing reduced susceptibility to ciprofloxacin. Mutations in the *gyrA* gene of these isolates were characterized by a LightCycler PCR-based *gyrA* mutation assay, and at least four different mutations were identified. Multiple resistances (to four or more antimicrobials) are also common in poultry-associated pathogens (Eng and al; 2015).

Discussion

Raw milk and milk products are basic components of a healthy human diet, have a high nutritional content and provide a good environment for the growth and multiplication of microorganisms, including *Salmonella*. Milk and milk products can be contaminated directly or indirectly from different sources. Pathogens in milk are a major cause of foodborne infections, which is a collective public health problem for researchers and consumers. Salmonellosis remains the leading foodborne human illness transmitted through the consumption of contaminated milk and milk products.(Garbaj and al ;2022)

This study aimed to identify *Salmonella* pp. in raw milk and dairy products such as cheeseand ice cream from different localities of Bejaia using conventional techniques and to test the isolated strains against a variety of antibiotics to determine their succeptibility.

The present study on Salmonella spp. evaluated a total of 165 (102 Milk; 12 Cheese; 51 Ice) samples of milk and other dairy products collected at different locations in Bejaia, all samples were transported in a cooler to the LEM.

All samples collected in the study were first added to buffered peptone water which is a noninhibiting nutrient medium. Then, a selective enrichment broth which is Vassiliadis ratio was used to allow continuous growth of Salmonella and for isolation selective differentiation media such as XLD, mackonkey and GMT are used. Finally, the presumptive colonies obtained on the plating media were subjected to a series of biochemical tests for strain identification (TSI, urea, sucrose and mannitol test).

The study showed a prevalence of 1.25% of *Salmonella* spp (n=2/165) in the samples studied. Cheese (1 of 12), ice cream (1 of 52) had a prevalence of 8.33% and 1.96%, respectively. In Libya; Aboubaker M. Garbaj isolated 131 samples from dairy products (16%). *Salmonella* was identified in 4/18 (22.2%) of Maasora cheese and 6 out of 13 (46.1%) of Ricotta cheese. Aboubaker explained that Ricotta cheese has a higher water content than Maasora, which could be more favorable to bacterial growth.(Garbaj and al ;2022)

Another study conducted by Torres-Vitela in Mexico, which examined 200 samples of two types of fresh cheese (Panela and Adobera) for *Salmonella* spp. and found an incidence of 56% in Panela and 20% in Adobera samples (Torres-Vitela *and al.*, 2012). The results of Aboubaker seem to be consistent with those of Torres-Vitela and al. while our results present a low prevalence compared to the rate of *Salmonella* found in the two previous studies, The differences observed in prevalence could be explained by contamination during preparation and processing or by contamination of equipment and utensils. Epidemics due to cheese consumption have most often been linked to the use of raw milk or a mixture of raw and pasteurized milk in the manufacturing process. Alternatively, the milk in the bulk tank

may be contaminated in the udder or by feces. *Salmonella* spp. are one of the leading causes of foodborne illness and milk consumption has been implicated in salmonellosis outbreaks.

The incidence of *Salmonella* in raw cow's milk was 16% the pathogen was recovered in 9 of 56 samples in libya (Garbaj *et al.*, 2022). in Lebanon a total of 195 raw milk samples were collected during two consecutive years from 48 sources The incidence of *Salmonella* was35% (Joubrane *and al.*, 2022). Finally in our results, no *salmonella* was isolated in the 102 milk samples collected so the prevalence is 0%. However the consumption of raw milk is recognized as a common means of transmission of *salmonella*.

Previous studies have shown a higher rate of isolation compared to the results of this study which could be attributed to the difference in the number of samples collected in the studies or the differences in prevalence (16%, 20. 5%, 35% and 0%) may be due to several factors, including inadequate management and hygienic conditions of the herd, milking parlour, mammary gland, milkers, possible contamination of drinking water or food with the pathogen, The presence of asymptomatic shedders, among others, can also play an important role in the presence and spread of the microorganism within the herds and also the contamination of milk storage tanks.

Consumer awareness of the hazards associated with the consumption of unpasteurized milk is necessary and Since various studies have identified Salmonella spp. as one of the major pathogens associated with foodborne illness in humans with the risk of consumption of raw milk or milk products made from raw milk it is necessary to put in place measures to prevent contamination and minimize the prevalence of *salmonella*.

Ice cream is a frozen dairy dessert made from a variety of ingredients. *Salmonella* does not survive typical minimal pasteurization processes. The presence of such microorganisms indicates that this process was improper or that contamination occurred after pasteurization. Therefore, sanitation is essential to ensure that dairy products are not decontaminated (Garbaj *et al*; 2022). In a study by Aboubaker none of the 13 ice cream samples showed growth of *Salmonella* spp 0%. In our study 52 samples were examined and low incidence levels for *Salmonella* 1.92% were found. Our results appear to be consistent with Aboubaker. Giventhat different studies have identified *Salmonella* spp. as one of the main pathogens associated with foodborne illness in humans with the risk of consumption of raw milk or raw milk products, the need to establish hygiene standards throughout the production chain is important. (Garbaj *et al.*, 2022)

The increased emergence of MDR bacteria is of concern and has become a critical issue worldwide Coordination across sectors under the One Health concept is needed to understand

the development of resistant pathogens in humans, animals and environment. the main source of antibiotics in animal products is due to overuse or misuse of antimicrobial agents in animal food ,These practices have increased antimicrobial resistance among bacterial strains, especially in developing countries (Garbaj *et al.*, 2022). in our study the isolated *salmonella* were tested for their susceptibility to antibiotics by disc diffusion on Mueller-Hinton agar plates. The isolates showed variable zones of inhibition around the 4 antibiotic discs. All isolates were susceptible to ampicillin, gentamicin, cefozolin and ciprofloxacin. while in Libya, Twenty-four antibiotics were used for antimicrobial resistance testing of *Salmonella* spp. with the agar disk diffusion method (Kirby-Bauer technique). Aboubaker found the isolates to behighly resistant to awide range of antibiotics commonly used .All isolates were resistant to amoxicillin, bacitracin, penicillin G, lincomycin, vancomycin, clindamycin and cloxacillin with an ARI of 0.042. In contrast, all strains tested were sensitive to levofloxacine ,doxycycline and ciprofloxacine . In addition , all tested isolates (100%) were resistant to more than one antibiotics. (Garbaj *et al.*, 2022)

A global action plan to combat antimicrobial resistance is needed to preserve our ability to prevent and treat disease and also reduce multiresistant strains.

Conclusion

Milk and milk products are basic components of a healthy human diet many African countries especially in Algeria, the consumption of milk has increased and the latter continues to constitute the first source of animal protein (after mutton) because these products have a high nutritional value and several necessary elements which make them a complete food. Therefore, dairy products are susceptible to microbial contamination and constitute а favorable environment for the growth of various microorganisms, including pathogenic bacteria such as salmonella. During this study, the prevalence of Salmonella in different municipalities of Béjaïa as well as their antimicrobial resistance profile were determined. The results obtained in this study showed that 2 of 165 (1.25%) samples had Salmonella spp ,based on conventional culture methods in RV broth, XLD agar and biochemical identification. the existence of a low rate of Contamination by Salmonella in the milk and its products sold and obtained directly from the udder in different farms in Béjaïa (1.21%) demonstrates that the good hygienic conditions throughout the production process have move from breeder to market, but this is not enough, because this study should be deepened by targeting larger samples and more farms to more accurately determine the real prevalence and also have more time to carry out this survey and sampling . although the need to put in place measures to prevent contamination and reduce the problem remains a priority, which will result in milk and dairy products with high standards of safety and quality and reduce the risk of diseases of origin eating . isolated salmonella were found to be sensitive to all antibiotics tested. The increased emergence of MDR bacteria is of concern and has become a critical issue worldwide due to the misuse (overuse or abuse) of antibiotics. another therapeutic strategy, other than antibiotics, is recommended to prevent outbreaks of multidrug-resistant Salmonella, and also Antibiotic use monitoring programs and strict biosecurity measures should be implemented in order to avoid contamination crossing foods of animal origin. **Recommendation**:

- Further studies are needed to investigate the relationship between food production and outbreaks of human salmonellosis.
- The application of rapid diagnostic procedures (eg PCR) is necessary to trace the sources of infection and aid in rapid diagnosis.

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Résumé

La salmonellose est une maladie d'origine alimentaire (FDI) qui affecte la santé publique et peut entraîner la mort de personnes. De nombreuses épidémies de salmonellose ont été signalées en raison de la contamination du lait cru et des produits laitiers par l'agent pathogène de salmonelle. Cette présente étude visait à isoler, identifier et déterminer la prévalence des souches de Salmonella isolées à partir de produits laitiers non pasteurisés tels que le lait cru provenant de différentes fermes, le fromage et la crème glacée provenant de différentes machines et à tester la sensibilité des souches à certains antibiotiques. .Au total, 165 échantillons (102 laits ; 12 fromages, 51 glaces) ont été collectés dans différentes communes de Béjaïa. Après isolement, les isolats suspects ont été identifiés à l'aide de tests biochimiques. Après cela, les souches ont été soumises à des tests de sensibilité antimicrobienne avec 4 antibiotiques en utilisant la méthode CIM (Détection de concentration minimale inhibitrice). Un total de 2 échantillons positifs ont été rapportés donnant une prévalence globale de 1,21 % (1 de fromage 8,33 % ; 1 de crème glacée 1,96%). Les isolats de Salmonella se sont révélés sensibles à tous les antibiotiques : céfazoline, ampicilline, gentamicine, ciprofloxacine ; et ont montré divers degrés de sensibilité.La prévalence de Salmonella spp. dans le lait n'a pas encore fait l'objet d'études approfondies à Béjaïa. Cette étude souligne la nécessité de mettre en œuvre des mesures pour prévenir la contamination et réduire les problèmes résultant du lait et des produits laitiers avec des normes élevées de sécurité et de qualité, réduisant ainsi le risque de maladies d'origine alimentaire.

Mots clé : prévalence, salmonella , sensibilité antimicrobiens, lait , fromage, créme glacée .

Abstrat

Salmonellosis is a foodborne illness (FDI) that affects public health and can lead to the death of people. Many salmonellosis outbreaks have been reported due to contamination of raw milk and dairy products with the salmonella pathogen. This present study aimed to isolate, identify and determine the prevalence of strains of Salmonella isolated from unpasteurized dairy products such as raw milk taken from different farms, cheese, and ice cream taken from different machines and to test the sensitivity of the strains to certain antibiotics. A total of 165 samples (102 milk; 12 cheeses, 51 ice cream) were collected in different municipalities of Bejaïa. After isolation, the suspected isolates were identified using biochemical tests. After that, the strains were subjected to antimicrobial susceptibility testing with 4 antibiotics using the following CIM (Minimum inhibitory concentration detection).A total of 2 positive samples were reported giving an overall prevalence of 1.21% (1 from cheese 8.33%; 1 from ice cream 1.96%). Salmonella isolates have been shown to be sensitive to all antibiotics: Cefazolin, Ampicillin, Gentamicin, ciprofloxacin; and showed varying degrees of sensitivity. The prevalence of Salmonella spp. in milk has not yet been the subject of in-depth studies in Béjaïa. This study highlights the need to implement measures to prevent contamination and reduce problems resulting from milk and milk products with high standards of safety and quality, thereby reducing the risk of foodborne illnesses.

Keywords: prevalence, salmonella, antimicrobial susceptibility, milk, cheese, ice cream