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Mohammed El Bachir El Ibrahimi University of B.B.A
كلية علوم الطبيعة والحياة وعلوم الأرض والكون
Faculty of Nature and Life Sciences and of Earth and Universe Sciences
قسم العلوم البيولوجية
Department of Biological Sciences

DISSERTATION

Submitted in Partial Fulfillment of the Requirement for the Master's Degree

Domain: Nature and Life Sciences
Field: Biological Sciences
Specialty: Applied Microbiology

Theme:

Prevalence & antimicrobial resistance pattern of *Staphylococcus aureus* from animal-based food in Bordj Bou Arreridj, Algeria.

Presented by

SEKKAL Maroua & ZERROUGUI Imane

Publically defended On 12 / 06 / 2024, In Front of Jury Members:

	Full name	Rank	Affiliation / institution
President:	Ms. BOUGUERRA Asma	Class A Lecturer	University of Bordj Bou Arreridj
Examiner:	Mr. MERIBAI Abdelmalek	Class B Lecturer	University of Bordj Bou Arreridj
Supervisor:	Ms CHENOUF Nadia Safia	Class B Lecturer	University of Bordj Bou Arreridj

Academic year 2023/2024

Acknowledgments

Dedication

To my dearest family members, to my parents SEHILI Leila and ZERROUGUI Youcef, your love, guidance, and sacrifices have shaped me into the person I am today. Thank you for always believing in me and supporting me unconditionally.

To my brothers KARIM, AZIZ, FATEH, SAMY, and my sister MERIEM, your strength, humor, and companionship have been a constant source of inspiration. Thank you for being there through thick and thin.

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To my partner SEKKAL Maroua your hard work and collaboration have been invaluable. Together, we've tackled challenges and achieved milestones.

Thank you for your commitment to our project.

I am grateful for the bond we share and the memories we've created together.

Last but not least, I want to thank ME! For never quitting.

IMANE

Dedication

I devoutly dedicate this work to the souls of my loving parents, SEKKAL Abdallah and BENMAHAMED Dalila. "My lord, have mercy upon them as they brought me up small" [17:24].

To my precious sister MOUNIRA, and my amazing brothers ABDESLAM, TAMIME, SAMIR, NACER, and his wife CHAHINEZ: I am forever indebted to you for your sacrifices, efforts, inspiration, and unconditional love. Thank you for supporting me through my hardships and encouraging me to reach my potential. You are truly God's blessing to me.

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Last but not least, I want to thank ME! For never quitting.

MAROUA

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In the name of Allah, the Most Gracious, the Most Merciful. All praise is due to Allah, Lord of the worlds, and peace and blessings be upon the Prophet Muhammad, his family, and his companions.

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List of Abbreviations

ATCC: The American Type Culture Collection.

BBA: Bordj Bou Arreridj.

BHIB: Brain Heart Infusion Broth.

C: Chloramphenicol.

CIP: Ciprofloxacin.

CNS: Coagulase negative staphylococci.

COT : Cotrimoxazole.

CPS : Coagulase positive staphylococci.

DA: Clindamycin.

ERY: Erythromycin.

FC: Fusidic acid.

FOX: Cefoxitin.

GEN: Gentamycin.

I: Intermediate.

LEV: Levofloxacin.

MDR: Multi Drug Resistance.

MH: Muller Hinton.

MRSA: Methicillin-resistant *Staphylococcus aureus.*

MSA: Mannitol Salt agar.

MSSA : Methicillin sensitive *staphylococcus aureus.*

NA: Nutrient agar.

PBP2a: Penicillin Binding Protein 2a.

R: Resistant.

S: Susceptible.

STR: Streptomycin.

SA : *Staphylococcus aureus.*

SCCmec: Staphylococcal Cassette Chromosome mec.

SE: Staphylococcal enterotoxins.

SFP: Staphylococcal Food Poisoning.

WHO: World Health Organization.

Introduction

1. Introduction

Animal-based foods are considered high-quality products and excellent sources of nutrients, thereby playing a crucial role in constructing healthy human diets and nutrition by providing essential proteins, vitamins, and minerals **(Murphy et Allen, 2003).** Milk in general and raw milk in particular is a primary source of calcium and other nutrients, consumed either fresh or as an ingredient in numerous dairy products. Meat products such as sausages and shawarma are both appreciated for their rich flavors and protein content while gaining popularity as fast-food dishes in many countries, including Algeria.

However, foodborne diseases caused by microbial contamination are an important health risk associated with the processing and handling of these animal-based foods **(WHO, 2022).** Amongst these microbial agents, *Staphylococcus aureus* (SA), a Gram-positive coccal foodborne pathogen from the *Micrococcaceae* family is responsible for a wide range of diseases in humans ranging from skin and soft tissue infections to life-threatening septicemia. Some toxigenic SA strains can also cause what we call the staphylococcal food poisoning (SFP) and in some cases SFP outbreaks. It is an acute intoxication that results from the consumption of foods contaminated with preformed staphylococcal enterotoxins **(Grundmann *et al.*, 2006)**. SA produces over 25 staphylococcal enterotoxins, heat-stable structures that can endure food processing and handling, leading to foodborne intoxications characterized by symptoms such as nausea, vomiting, diarrhea, and abdominal pain, 2 to 8h after the ingestion of the contaminated foods **(Titouche *et al.*, 2020).** Likewise, in animals, SA is regarded as a common cause of bovine mastitis in dairy cattle, which may result in huge economic losses **(Chenouf *et al.*, 2021).**

On the other hand, antimicrobial resistance is, today, one of the biggest threats to global health, food security and development. It is rising worldwide, and new resistance mechanisms are emerging and spreading **(Michael *et al.*, 2014)**. The misuse and the overuse of antimicrobials in both human and veterinary medicines have enormously contributed to the emergence of resistant bacteria such as methicillin-resistant SA (MRSA) **(Borg *et al.*, 2007)**. In April 2014, the World Health Organization (WHO) published its first antimicrobial resistance global report, in which resistant bacteria were categorized into three classes according to their priority, and MRSA was classified as "of high priority" **(WHO, 2014)**.

Methicillin resistance arises through acquisition of the *mecA* gene, which encodes an alternative penicillin-binding protein, PBP2a, with a low affinity for most β-lactam antibiotics, therefore, MRSA is considered resistant to almost all β-lactam antimicrobials. MRSA is known to be located on a mobile genetic element termed "staphylococcal cassette chromosome mec (*SCC*mec)" **(Borg *et al.*, 2007)**. Nowadays, MRSA is considered a major *nosocomial* pathogen worldwide, and countless studies reported their high prevalence in health-care settings. Similarly, the presence of MRSA in foods and food-producing animals such as milk and meat products has been commonly reported worldwide, raising public health concerns, as these multi-drug resistant bacteria can be transmitted to human through the food chain.

The WHO pointed out the need to deal with the crisis of antimicrobial resistance under a “One Health” approach, which focuses on the interactions between humans, animals and their environments. It’s in this light that our study was designed. It aimed to assess the prevalence of SA in three different varieties of animal-based foods commercialized in Bordj Bou Arreridj (BBA) city: raw milk and meat products samples (sausage and shawarma), and to determine the antimicrobial resistance patterns of SA isolates. The prevalence of MRSA among the isolates was also investigated.

Materials & Methods

2. *Materials and Methods*

2.1. Sample collection

During the period from February 4th to May 15th, 40 samples of animal-based foods (20 raw milk samples, 10 shawarma samples and 10 sausage samples) were collected from distinct regions in the city of Bordj Bou Arreridj North-Est of Algeria (figure 1). Raw milk samples were obtained from traditional milk stores (also termed "Lebben" as a vernacular name), while meat samples were collected from various butcher shops and fast-food restaurants.

During sampling, a survey was conducted in order to collect the following data: milk source, transportation conditions, general hygiene conditions and storage (annex 1). All samples were collected in small sterile containers or bags and immediately transported in a 4°C cooler to the laboratory of the department within 1h to 2h (one sample per store).

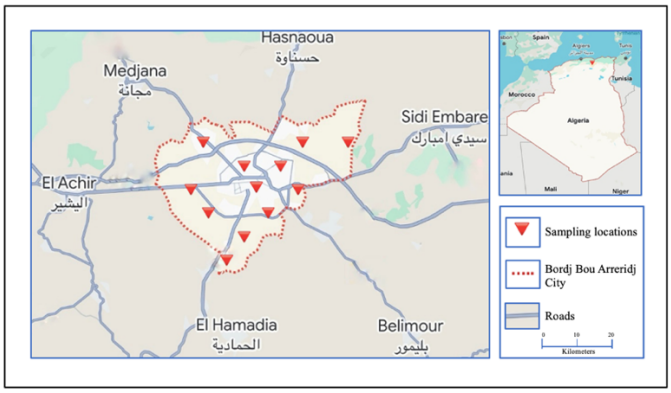

Figure 1: Bordj Bou Arreridj city map illustration of the regions selected for samples

2.2. Sample processing

Sample processing was carried out according to ISO 6888-3 **(ISO, 2003)**. Unlike milk samples which did not require processing (figure 2), 10g of meat samples were precisely weighed and minced into thin pieces using sterile scissors and knives then placed in sterile bags to be homogenized in 90 ml of a 0.9% sodium chloride solution.

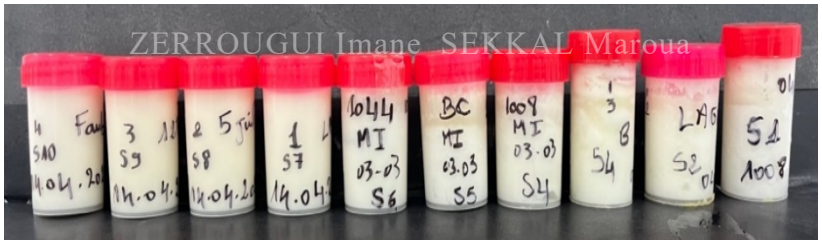

Figure 2: Raw milk samples

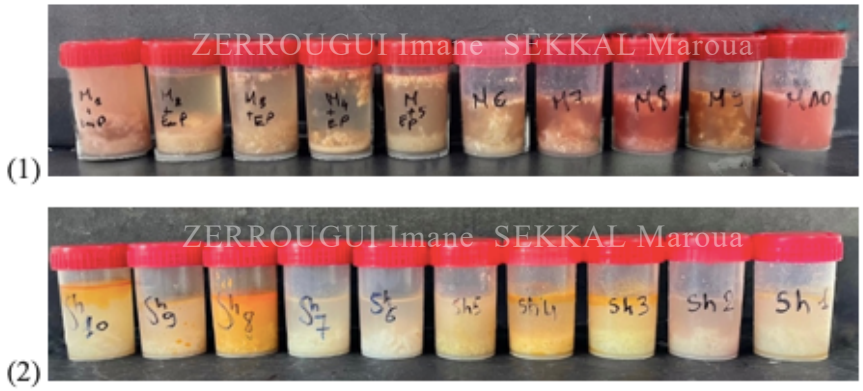

Figure 3: Sample processing of meat samples (1) sausage (2) shawarma

2.3. Enrichment

A micropipette was used to transfer 1ml of milk/meat solution into 9ml of Giolitti-Cantoni broth (Microbiotech, Sétif, Algeria) supplemented with 1% potassium tellurite. The tubes were incubated at 37°C for 24 h**. (Wu *et al.*, 2018)**

Figure 4: Enrichment in Giolitti-Cantoni broth

Positive-enriched cultures were identified through the appearance of a black color due to the reduction of tellurite to metallic tellurium. **(Chenouf *et al.*, 2021)**

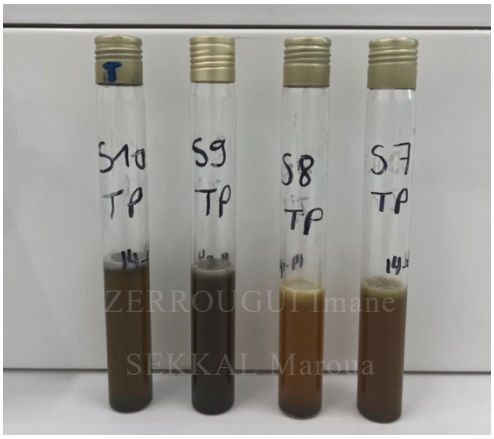

Figure 5: Positive enriched cultures

2.4. Isolation

As illustrated in the figure below, a loopful of each positive enriched broth was streaked onto Mannitol Salt Agar (MSA) plates (Microbiotech, Sétif, Algeria), following the T-streak method. These plates were then incubated at 37°C for 24 h, and further observed for bacterial growth. In the case of SA, the acidity of the media will cause the pH indicator, phenol red, to turn yellow.

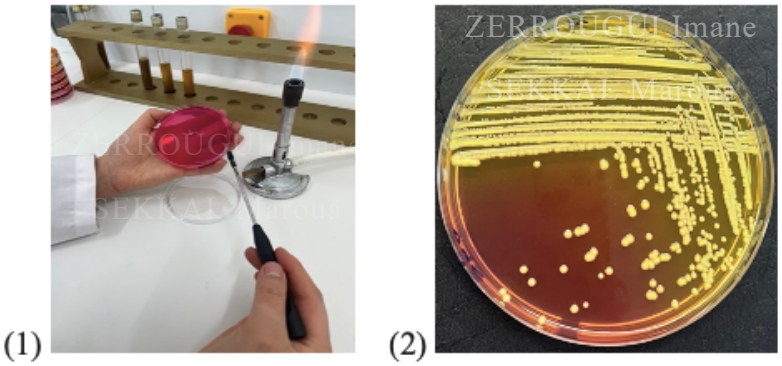

Figure 6: (1) Isolation on MSA (2) Macroscopic aspect of SA colonies

2.5. Purification

Only colonies with presumptive SA morphology were selected for purification on Nutrient Agar (NA) plates (Pasteur Institute of Algeria) using a sterile inoculating loop. Afterwards, all plates were incubated at 37°C for 24 h.

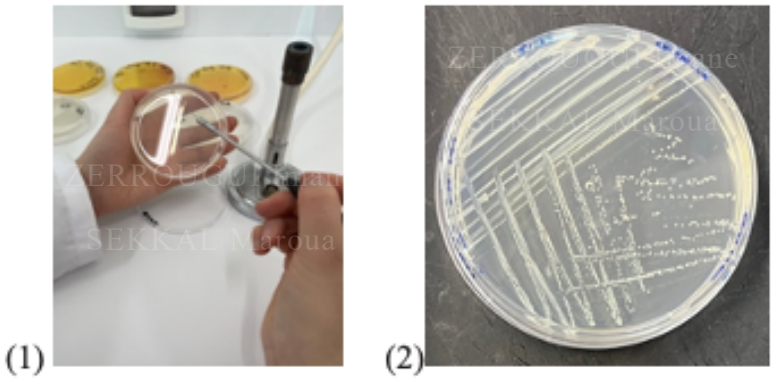

Figure 7: (1) Purification on NA (2) Macroscopic aspect of SA colonies

2.6. Identification

2.6.1. Gram staining

First introduced in 1882, Gram-staining remains one of the most crucial staining techniques in microbiology. The first step is the use of crystal violet dye for the slide's initial staining. The next step, also known as fixing the dye, involves using iodine to prevent easy removal of the dye. Subsequently, a decolorizer, often ethanol, is used to remove the dye. The basic principle of Gram-staining involves the ability of the bacterial cell wall to retain the crystal violet dye during solvent treatment. SA are Gram- positive bacteria that are small round in shape (cocci) and occur as clusters appearing like a bunch of grapes (figure 8).

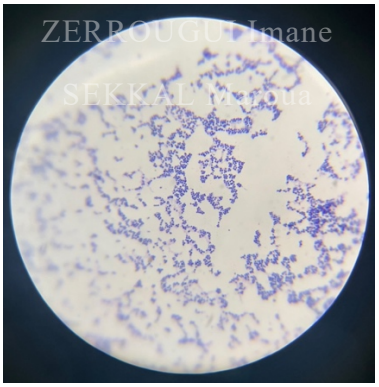

Figure 8: Observation of SA under optical microscope (x100)

2.6.2. Biochemical identification

Three biochemical tests were conducted to confirm the identification of SA **(Thaker *et al.*, 2013)**:

2.6.2.1. Catalase test:

A single drop of 3% H_2O_2 was placed onto a sterile microscope slide. Then, a small amount of the bacteria was collected from a well-isolated 18 to 24-hour colony streaked on NA and placed on the slide, using a sterile inoculating loop. Positive reactions were evident by immediate effervescence (bubble formation) (figure 9).

Figure 9: A positive Catalase test

2.6.2.2. Coagulase test: Two methods were used:

1/ Tube coagulase: A swab of young bacterial colonies growing on nutrient agar plates was directly mixed in a larger volume of human plasma in a small test tube to be examined 24h later. **(Katz, 2010)**

2/ Enriched tube coagulase: Strains were incubated overnight in Brain Heart Infusion Broth (BHIB) at 37°C.

the test was performed by adding 1ml from the broth culture to 2ml of human plasma. The tubes were incubated and examined after 2 and 4 hours (figure 10) **(Kateete *et al.*, 2010).**

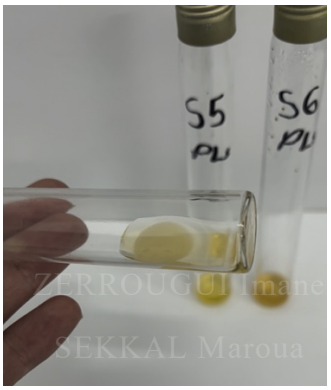

Figure 10: A positive coagulase test

2.6.2.3. DNase test:

The test strain is inoculated on DNA Agar, a medium which contains DNA, using sterile swabs. After overnight incubation, the colonies are tested for DNAse production by flooding the plate with a hydrochloric acid solution. The acid precipitates unhydrolyzed DNA. Finally, DNAse-producing strains are, therefore, found to be surrounded by clear areas indicating DNA hydrolysis (figure 11). **(Kateete *et al.*, 2010)**.

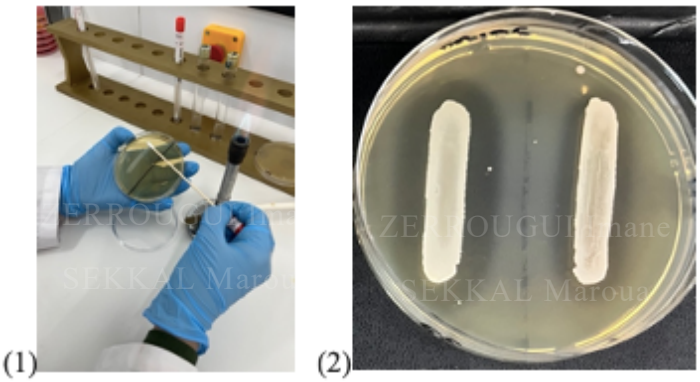

Figure 11: (1) DNase test inoculation (2) A positive DNase test

It is worth mentioning that in all identification tests, a reference strain *S. aureus* ATCC 25923 was used as a positive control.

2.7. Antimicrobial susceptibility testing

The susceptibility of the confirmed SA strains was tested according to the Kirby-Bauer disk diffusion method on Muller Hinton (MH) Agar. **(Bauer *et al.*, 1966)**

2.7.1. Inoculum preparation

Well-isolated young colonies growing on NA were carefully selected and suspended in 5ml of a 0.9% sodium chloride solution to prepare a bacterial suspension with a turbidity value of the standard of 0,5 McFarland Units. The suspension is then vortexed before being used within the 15 minutes of preparation.

2.7.2. Inoculation of MH plates

A sterile swab was dipped into the inoculum tube and then carefully rotated against the side of the tube to remove excess fluid. The surface of an MH plate was inoculated through streaking the swab three times over the entire MH surface, with a rotation of approximately 60° (figure 12).

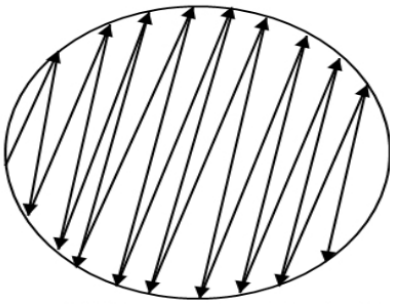

Figure 12: The inoculation technique used in antibiogram

2.7.3. Placement of the antibiotic disks

As mentioned in the tables I and II below, ten antibiotic-impregnated disks belonging to 8 different antibiotic classes were carefully placed on the MH plate surface. Once placed, a disk should never be moved (figure 13). Regarding plates incubation, a temperature range of 35°C ± 2°C is required.

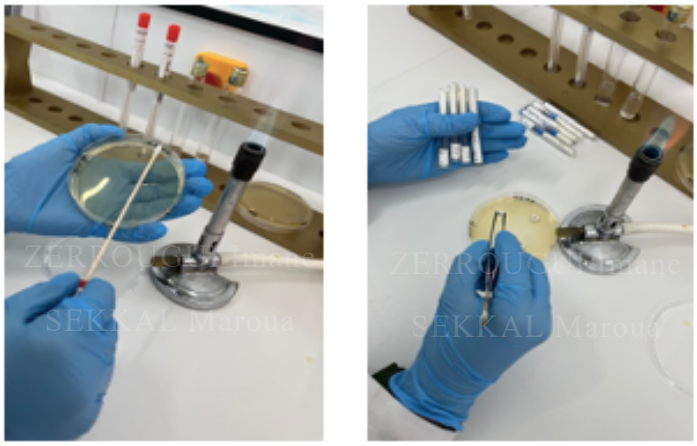

Figure 13: Placement of antibiotic disks

Table I: List of antibiotics tested

Family	**Class**	**Charge (µg)**	**Abbreviation**	**Brand, country**
β-Lactams	Cefoxitin	30	FOX	Liofilchem, Italy
Macrolides	Erythromycin	15	E	Himedia, India
Aminoglycosides	Gentamycin	10	GEN	Himedia, India
	Streptomycin	10	STR	Liofilchem, Italy
Pheynicol	Chloramphenicol	30	C	Liofilchem, Italy
Fusidin	Fusidic acid	10	FC	Himedia, India
Lincomycins	Clindamycin	2	DA	Liofilchem, Italy
Fluoroquinolones	Ciprofloxacin	5	CIP	Himedia, India
	Levofloxacin	5	LEV	Himedia, India
Diaminopyrimidines	Cotrimoxazole	25	COT	Himedia, India

Table II: Antibiotics tested per Petri plate

Plate	Antibiotic discs used				
1	E	DA	COT	CIP	C
2	FC	LEV	FOX	GEN	S

2.7.4. Inhibition zone Measurement

Following incubation, the inhibition zone was measured to the nearest millimeter using a ruler. The diameter of the disk in the was included in measurement (figure 14).

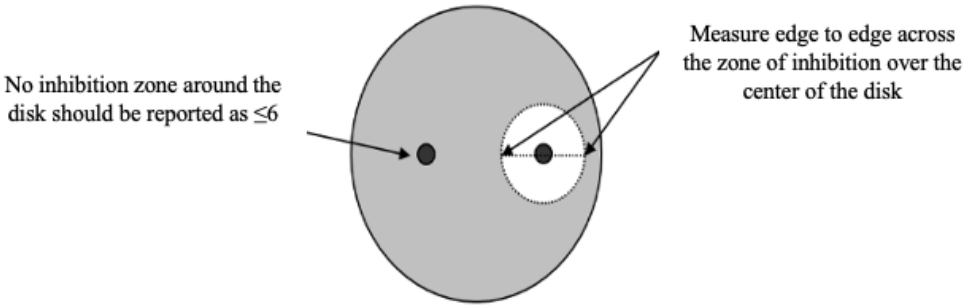

Figure 14: Inhibition zones Measurement

2.7.5. Interpretation of the results

As outlined in annex 2, SA strains were categorized as resistant (R), intermediate (I) or susceptible (S), according to the published CLSI guidelines **(CLSI, 2020)**.

2.8. Complementary tests

2.8.1. Detection of Methicillin-resistant *Staphylococcus aureus* (MRSA)

MRSA isolates were detected using a 30µg cefoxitin disk diffusion test in the standard conditions of antibiogram. MRSA isolates showed cefoxitin inhibition zone diameters of <21mm **(CLSI, 2020).**

2.8.2. Detection of inducible Clindamycin resistance (D-test)

As for the detection of inducible clindamycin resistance, a D-test, involving the placement of an erythromycin disk in proximity to a clindamycin disk, was applied to detect eventual inducible clindamycin resistance (figure 15) **(Chenouf *et al.*, 2021).** The test is considered positive if a D image appears.

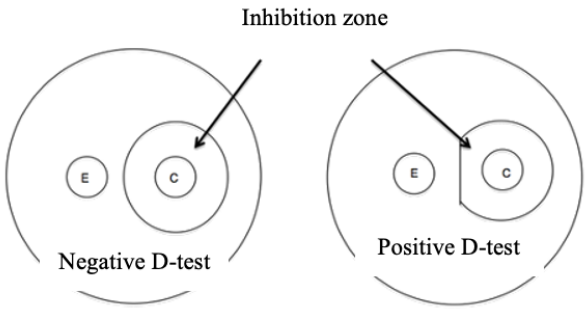

Figure 15: The D-test

Results & Discussion

3. *Results and Discussion*

3.1 Results

3.1.1. Contamination and isolation frequency per food type

Of the 40 milk and meat samples, 32 were contaminated with *Staphylococci* species (80% of total samples). As shown in table III and figure 16, milk samples showed the highest isolation frequency estimated at 62%, indicating a higher contamination rate as all milk samples were contaminated, followed by 25% and 13% for shawarma and sausage samples, respectively.

Table III: Contamination of animal-based food samples by Staphylococci species

Sample type	Number of contaminated samples	Percentage
Milk	**20/20**	**20/32 (62%)**
Sausage	**4/10**	**4/32 (13%)**
Shawarma	**8/10**	**8/32 (25%)**
Total number of *Staphylococci* species	**32/40**	**32/32 (100%)**

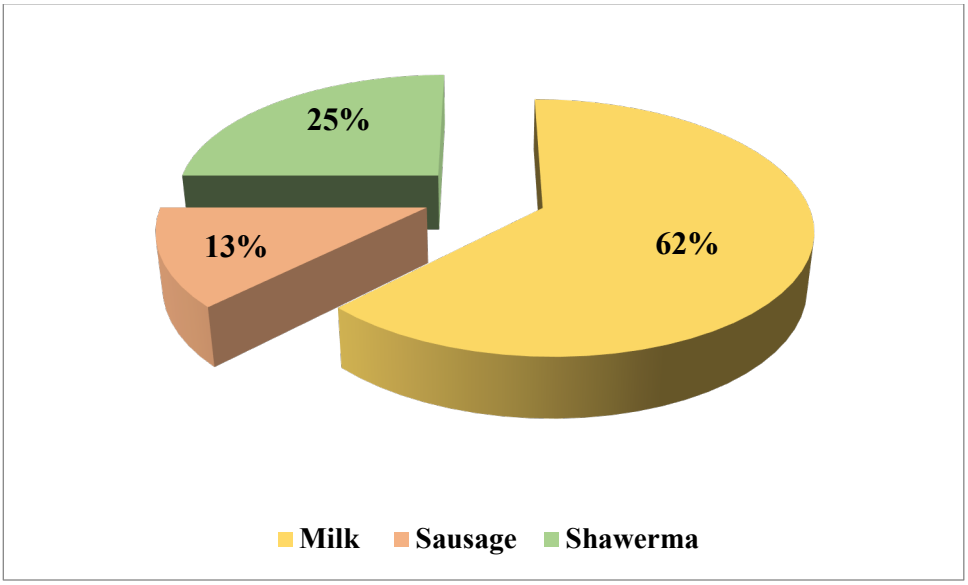

Figure 16: Isolation frequency per food type

3.1.2. Prevalence of coagulase-positive (CPS) and -negative (CNS) Staphylococci

Among the 32 pure *Staphylococci* isolates, 18 were determined as CPS, all isolated from milk, while the remaining 14 isolates were assigned as CNS. The distribution of *Staphylococci* species per food type is given in table IV and figure 17.

Table IV: Distribution of Staphylococci species per food type

Samples	CPS	CNS
Milk	18	2
Sausage	0	4
Shawarma	0	8
Total	18	14

CPS: Coagulase-positive *Staphylococci*; **CNS:** Coagulase-negative *Staphylococci*

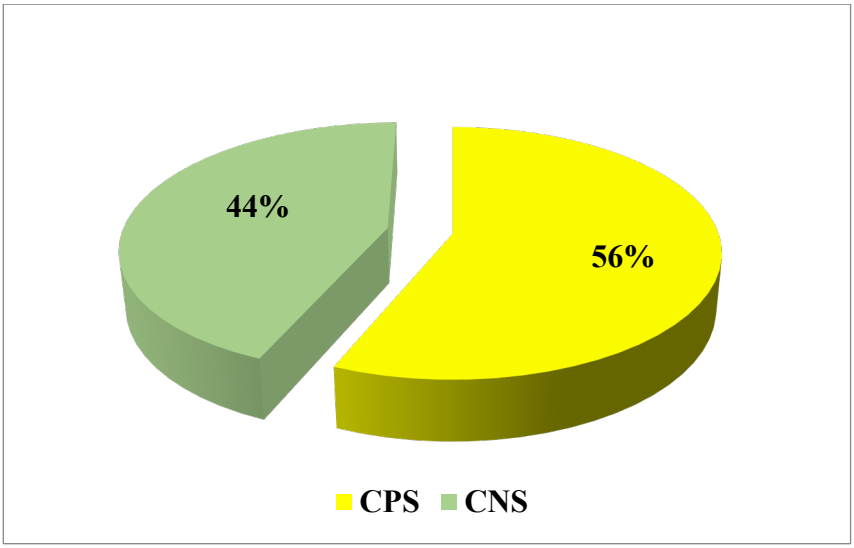

Figure 17: Prevalence of CPS and CNS isolates

3.1.3. Prevalence of *Staphylococcus aureus* (SA)

Based on the macroscopic aspect on MSA, Gram-staining, catalase, coagulase, and DNase tests, all 18 CPS originating from milk samples were identified as SA (figure 18 and 19). In contrast, no SA strain was revealed in sausage and shawarma samples.

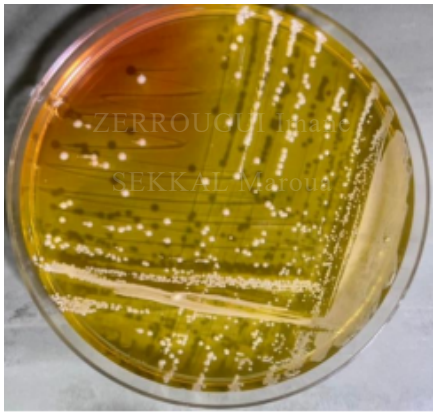

Figure 18: Macroscopic aspect of SA on MSA

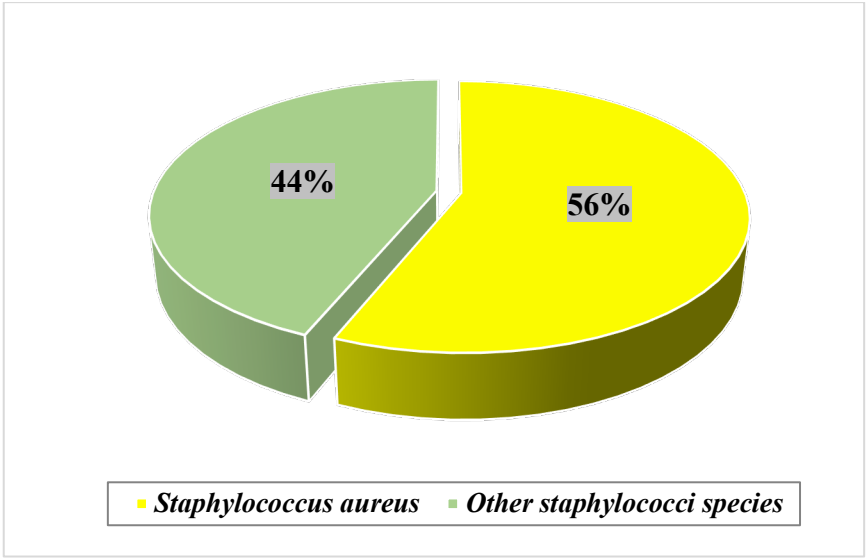

Figure 19: Prevalence of *Staphylococcus aureus*

3.1.4. Antimicrobial resistance patterns of *Staphylococcus aureus* strains

The antimicrobial susceptibility testing performed using the disk diffusion method according to the guidelines of CLSI (2020) showed remarkable differences among the 18 SA strains. As shown in table V, 8 SA strains, designated S2, S3, S7, S8, S9, S13, S14 S19 were susceptible to all antibiotics. Six SA strains were resistant to Erythromycin and Clindamycin. Interestingly, one SA strain showed inducible Clindamycin resistance (5% of total SA strains) (figure 22). Moreover, 2 SA strains resisted to Gentamycin, and 2 to Cefoxitin. Only one SA strain was resistant to Ciprofloxacin. In contrast, all SA strains displayed susceptibility towards Levofloxacin, Fusidic acid, Cotrimoxazole, Chloramphenicol, and Streptomycin (figures 20 and 21).

Table V: Antimicrobial resistance patterns of SA strains

SA Strain number	SA strain code	Antimicrobial resistance phenotype
1	S1	FOX
2	S2	Susceptible to all antibiotics
3	S3	Susceptible to all antibiotics
4	S4	ERY-DA
5	S6	ERY-DA*-FOX
6	S7	Susceptible to all antibiotics
7	S8	Susceptible to all antibiotics
8	S9	Susceptible to all antibiotics
9	S11	ERY-DA
10	S12	CIP
11	S13	Susceptible to all antibiotics
12	S14	Susceptible to all antibiotics
13	S15	GEN
14	S16	ERY-DA
15	S17	ERY-DA
16	S18	GEN
17	S19	Susceptible to all antibiotics
18	S20	ERY-DA

FOX: Cefoxitin; **ERY**: Erythromycin; **CLI**: Clindamycin; **CIP**: Ciprofloxacin; **GEN**: Gentamycin; *: Positive D-test (inducible Clindamycin resistance).

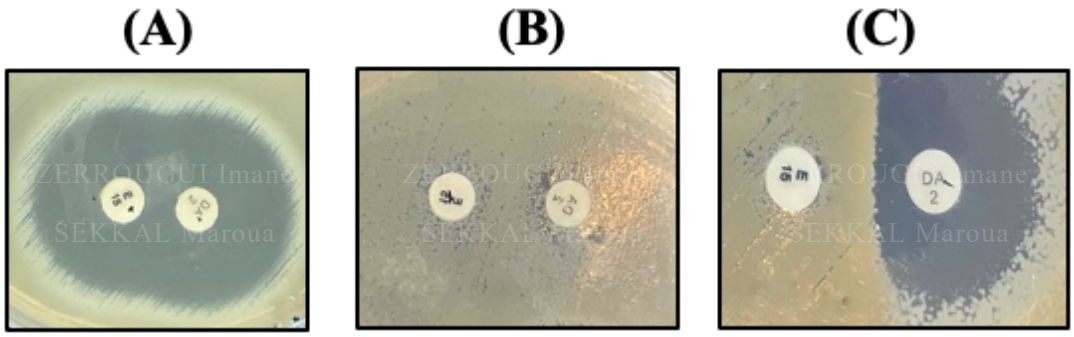

Figure 20: D-test of SA strains

(A) Erythromycin- and Clindamycin-sensitive SA strain, **(B)** Erythromycin- and Clindamycin-resistant SA strain, **(C)** inducible Clindamycin resistant SA strain

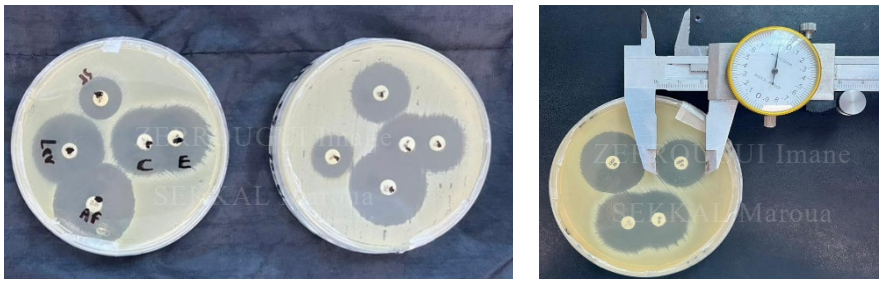

Figure 21: Susceptible SA strains (8, 11 and 12)

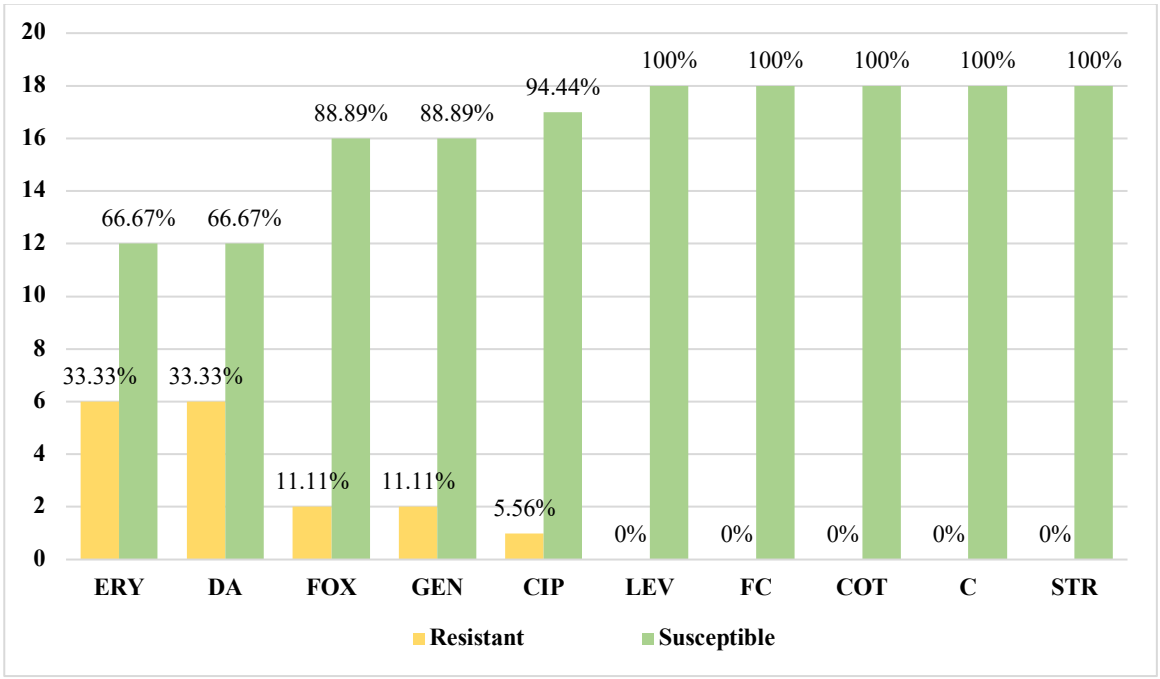

Figure 22: Antimicrobial resistance patterns of SA strains

(ERY) Erythromycin, **(DA)** Clindamycin, **(FOX)** Cefoxitin, **(GEN)** Gentamycin, **(CIP)** Ciprofloxacin, **(LEV)** Levofloxacin, **(FC)** Fusidic acid, **(COT)** Cotrimoxazole, **(C)** Chloramphenicol, **(STR)** Streptomycin.

3.1.5. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA)

As shown in the figures below, of the 18 SA strains, two were resistant to Cefoxitin (S1: zone diameter= 20; S6: zone diameter= 18). Therefore, they were categorized as MRSA (11% SA strains, and 5% of total samples), while the remaining 16 SA strains were recognized as methicillin-susceptible SA (MSSA). Interestingly, a multidrug-resistant phenotype, defined as resistance to ≥3 drug classes, was revealed in one MRSA strain (S6).

Figure 23: Two MRSA strains (Cefoxitin inhibition zone ≥ 22mm)

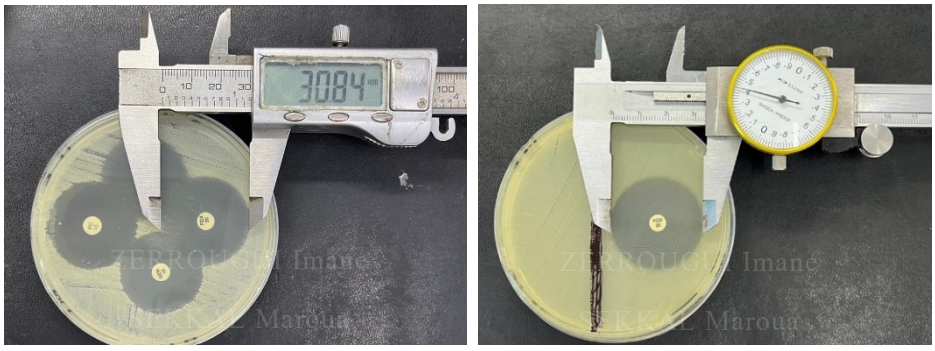

Figure 24: A MSSA strain (Cefoxitin inhibition zone ≤ 21mm)

3.2 Discussion

Animal-based foods are fundamental elements in human nutrition and diet. However, they may constitute a potential health hazard if they harbor pathogenic and/or toxigenic pathogens. Countless studies aimed at investigating the microbiological safety of these products specifically shedding light on SA presence and the prevalence of MRSA.

As a matter of fact, the prevalence of *SA* strains from animal-derived food is well documented in several countries, including Algeria. In our study, we isolated 32 *Staphylococci* species from a diverse range of animal-based food samples. The distribution of *Staphylococci* species based on coagulase type varied according to the food class. All 18 CPS were isolated from raw milk while most CNS 56% were found in meat products (sausage and shawarma samples). This outcome can be attributed to the fact that CNS are common inhabitants of human and animal skin/mucous membranes and therefore can be transferred to meat products during slaughter processing, mincing or preparation. Additionally, **Osman *et al.* (2017)** & **Gundog *et al.* (2024)** outlined that the biofilm formation ability of CNS protects them from antimicrobial agents allowing their persistence in the food processing environment.

The percentage of food specimens contaminated with SA was 44% (18/40), all derived from the 20 milk samples. These results were higher than those found by **Tamendjari *et al.* (2021),** who isolated 29 strains out of 87 cow milk samples (33.33%) collected from Tiaret and Souk-Ahras, whereas **Titouche *et al.* (2020)** published a prevalence of 37.04% in raw milk collected in Tizi Ouzou area. This difference in prevalences could be explained by the limited number of our samples as well as the isolation methods used.

In contrary, shawarma and sausage samples were contaminated neither by CPS nor SA, unlike the results shown by **Chaalal *et al.* (2018).** This result looks satisfying compared to other previous studies, where SA was commonly detected with relatively high rates. In her other study, **Chaalal. (2013)** confirmed that 20% of the *Staphylococci* strains isolated from sausage drawn from butcher shops of Tiaret region were identified as SA. Furthermore, the study conducted by **Cohen *et al.* (2006)** in Morocco, SA was detected in 16% of the samples of fresh sausages obtained from different places of

preparation or sale. Attribute to the sample sizes, sample types, and geographic locations of investigation, it is maybe the reason for these differences.

In general, meat products are known to be often contaminated by SA results from poor hygiene practices meat processing, preparation and/or storage stages. If one of the meat handlers has a staphylococcal infection (impetigo, folliculitis, abscess and boils), he can easily transmit it through food. However, the strains that produce staphylococcal enterotoxins (SE) and mainly the staphylococcal enterotoxin A (SEA), encoded by the *sea* gene, cause food poisoning outbreaks.

In the light of the results obtained, raw milk is more heavily contaminated by SA than the other animal-based foods. The high isolation rate in milk samples is mainly due to the absence of a pasteurization process. Moreover, Contamination of raw milk with staphylococcal species may have several origins, but is mostly related to poor hygiene practices, during the different processes of milking, handling, and transportation. Nasal carriage by cows and nasal or hand carriage by farm workers are also important sources of SA and CNS. Additionally, the presence of *Staphylococci* species in raw milk can be a sign of intramammary infection (mastitis) in dairy cows **(Titouche *et al*., 2020)**.

The *in vitro* antibiotic susceptibility test revealed total susceptibility of SA strains to Levofloxacin, Fusidic acid, Cotrimoxazole, Chloramphenicol and Streptomycin, which allow them to be efficient therapeutic alternatives, notably in the case of MDR or MRSA SA strains. On the other hand, 33% of the strains were resistant to Clindamycin and Erythromycin. This rate seems interestingly much higher than the rate of 9.1% reported in Medea and Ain Defla **Achek *et al.* (2018)**. Overall, SA strains resist to macrolide and lincosamide antibiotics in 3 ways: (1) through target-site modification by methylation or mutation that prevents the binding of the antibiotic to its ribosomal target, (2) through efflux of the antibiotic, and (3) through drug inactivation **(Miklasińska-Majdanik, 2021)**.

In our study, only one SA strain showed inducible Clindamycin resistance phenotype (5%). **Garg *et al.* (2022)** reported that the total percentage of inducible Clindamycin resistance was 21.9%. It is worth mentioning that in vitro routine tests for Clindamycin susceptibility may fail to detect inducible Clindamycin resistance resulting in treatment failure, thus the need to detect such resistance using a simple D test is crucial.

In our study, two and one SA strains were resistant to Gentamycin and Ciprofloxacin, with rates of 11% and 5%, respectively. **Ren *et al.* (2020)** stated rates 12.3% and Ciprofloxacin 4.6% for Gentamycin and Ciprofloxacin, respectively in southern Xinjiang, China. Furthermore **Zhang *et al.* (2022)** his meta-analysis demonstrates that the prevalence of antibiotic resistance to Gentamicin increased over time. Aminoglycosides are one of the classes of antibiotics that play an important role in the treatment of staphylococcal infections. The main mechanism of resistance to aminoglycosides is the inactivation of antibiotics by aminoglycoside-modifying enzymes (AMEs**).** Regarding quinolones, the most important resistance mechanism is mutation in the target **(Rahimi, 2016).**

Notably, we identified 2 MRSA strains (11%) representing a high prevalence among collected samples. **Titouche *et al.* (2019)** identified 9 MRSA strains sourced out from raw milk (4,1%), and **Chaalal. (2013)** reported a prevalence of 3.5% in raw milk and meat. In their seconf publication, **Chaalal *et al.* (2018)** revealed an MRSA prevalence of 21.5% in various food products, including raw milk and meat. Yet, most MRSA represented moderate multidrug resistance rates in Algeria according to **(Achek *et al.*, 2018)**. MRSA's ability to resist antibiotics is attributed to a unique structure called staphylococcal cassette chromosome *mec* (SCCmec). This structure conceals the *mec*A gene, which produces penicillin-binding protein 2a (PBP2a). With a reduced affinity for antibiotics such as methicillin and the broader β- lactam family, PBP2a enables MRSA to withstand its effects. The resistance of the isolated SA strains to cefoxitin suggests the presence of *mec*A gene and therefore the possibility of withstanding other β-lactam antibiotics. In addition, one MRSA strain was revealed to be MDR. These results indicate a serious public health issue, considering that these bacteria may be transferred to humans via milk consumption, as well as the danger of therapeutic failures. The presence of MRSA among our SA strains could be explained by the fact that he β-lactam class is extensively used in Algerian dairy cattle against for preventive and therapeutic purposes, notably the staphylococcal mastitis **(Chenouf *et al.*, 2021)**.

Finally, complementary studies including molecular characterization of the MRSA strains are required to better investigate the dissemination and epidemiology of MRSA in animal-based food.

Conclusion

3. Conclusion

The prevalence of SA and MRSA in particular in animal-based food represents a major public health concern due to the antimicrobial resistance phenomenon and the treatment challenges it poses. In this study, a total of 40 samples of animal-based foods (20 raw milk samples, 10 shawarma samples and 10 sausage samples) were drawn from different retail markets of Bordj Bou Arreridj city, and were assessed for the prevalence of SA strains, and their antimicrobial resistance patterns.

Amidst the 40 samples, 32 were contaminated with *Staphylococci* species (80% of total samples), and milk samples showed the highest contamination rate estimated (62%). Of the 32 *Staphylococci* isolates, 44% were CNS while 18 were determined as CPS, all isolated from raw milk samples (56%).

Regarding the antimicrobial resistance patterns, 8 SA strains exhibited susceptibility to all antibiotics tested, and six were resistant to both Erythromycin and Clindamycin. Interestingly, one SA strain showed inducible Clindamycin resistance (5% of total SA strains). Two SA strains were resistant to Cefoxitin, therefore, categorized as MRSA (11%), one of which was MDR. Moreover, 2 SA strains resisted to Gentamycin and only one showed resistance to Ciprofloxacin. In contrast, all SA strains displayed susceptibility towards Levofloxacin, Fusidic acid, Cotrimoxazole, Chloramphenicol, and Streptomycin.

The detection of MRSA and other SA resistant strains in animal-based foods commercialized in Bordj Bou Arreridj city is considered an alarming finding. Potential risks could be generated from transferring these strains to humans either directly by consuming raw milk and contaminated animal-based products or indirectly through improper handling.

The antimicrobial resistance levels observed in our study are relatively low but still punctuate the need for strict and supervised antibiotic use policies in barns and dairy farms, as well as raising awareness among animal husbandry to limitate and control of the spread of resistant bacteria. Moreover, new safe biological antimicrobial alternatives are urgently needed.

Conclusion

The overall results of our work call for increased surveillance and vigilance of food safety measures in order to prevent contamination and ensure the safety of both animals and animal-based food products through extending to mandatory regulations to guarantee suitable hygiene practices (uniforms, sanitizing, and regular medical examinations), proper cooking, storage, monitoring and reporting any signs of food contamination or illness to health authorities.

Scientific production

Unraveling the resistance mechanisms of methicillin-resistant *Staphylococcus aureus* (MRSA)

Maroua SEKKAL[1], Imane ZERROUGUI[1], Nadia Safia CHENOUF[1,2,3], Chafik Redha MESSAI[1,4]

[1] Faculty of Nature and Life Sciences and of Earth and Universe Sciences, University Mohamed El-Bachir El-Ibrahimi of Bordj Bou Arréridj
[2]Laboratory of Exploration and Valorization of Steppe Ecosystems (EVES), Department of Biology, Faculty of Nature and Life Sciences, University of Djelfa, Algeria.
[3]Laboratoire de Biologie des Systèmes Microbiens (LBSM), BP92, 16050, Ecole Normale Supérieure de Kouba, Algiers, Algeria.
[4]Laboratoire de Santé et Productions Animales, Rue Issad Abbes, Oued Smar 16000, Ecole Nationale Supérieure Vétérinaire, Algiers, Algeria.

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) emerged in 1961 as a significant challenge in treating infections caused by penicillin-resistant strains. Its prevalence and extensive antimicrobial resistance emphasize critical risks in food safety and public health sectors. Addressing these risks needs a profound understanding of MRSA resistance mechanisms, genetic determinants, and related factors. The objective of this study is to unravel the resistance mechanisms of MRSA, and provide comprehensive insights into the intricate biology of MRSA. In this study, 21 published review and research articles sourced from PubMed, and ScienceDirect databases have been examined and included. MRSA ability to resist antibiotics is attributed to its possession of a unique structure called staphylococcal cassette chromosome *mec* (SCCmec). This structure harbors the *mec*A gene, responsible for producing penicillin-binding protein 2a (PBP2a). With reduced affinity for antibiotics such as methicillin and the broader B Lactam family, PBP2a enables MRSA to withstand their effects. The survival of MRSA relies on the integrity of the cell wall. Penicillin-binding proteins (PBPs), including the distinctive PBP2a encoded by the *mec*A gene within SCCmec, play a key role in cell-wall synthesis. MRSA resistance mechanism involves ß-lactamase production and the acquisition of *mec*A, allowing for the synthesis of PBP2a with reduced sensitivity to ß-lactam antibiotics. Additionally, the efflux-mediated resistance, a less-explored mechanism, has gained attention as bacterial pumps can extrude various antimicrobial compounds, contributing to multidrug resistance. These pumps, classified into families like MFS, SMR, MATE, RND, and ABC, play a crucial role in expelling antibiotics from the cell. MRSA possesses over ten multidrug efflux pumps encoded in the chromosome or plasmids. Investigating the resistance mechanisms employed by MRSA offers invaluable insights into its antibiotic evasion and resistance development. This knowledge is a prominent contribution in identifying prospective and alternative therapeutic interventions for effectively combating MRSA infections.

Keywords: MRSA, antimicrobial resistance, efflux pumps, *mec*A, SCCmec

SNVBNR 2024: 1 Séminaire Valorisation de la Biomasse Végétale Naturelle et Résiduelle

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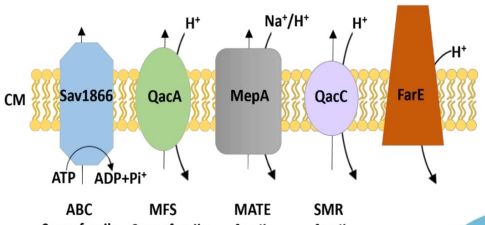
Objective

The objective of this study is to unravel the resistance mechanisms of MRSA, and provide comprehensive insights into the intricate biology of MRSA.

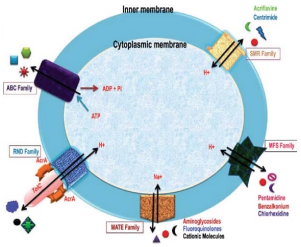
Material and Methods

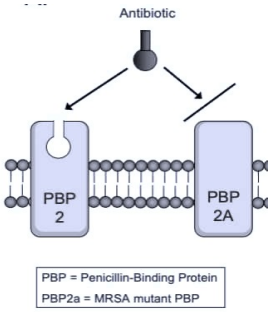
21 published review and research articles sourced from PubMed, and ScienceDirect databases have been examined and included **in this bibliogrqphic research**

Results and discussion



SCCmec





SCCmec

Efflux mediated resistance (efflux pumps)

Conclusion

Investigating the resistance mechanisms employed by MRSA offers invaluable insights into its antibiotic evasion and resistance development. This knowledge is a prominent contribution in identifying prospective and alternative therapeutic interventions for effectively combating MRSA infections.

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Co-authors: ZERROUGUI Imane; CHENOUF Nadia Safia; MESSAI Chafik Redha.

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شكارة بوزياني محمد

Head Of The Laboratory

أ.د/موسي عبد الحميد

Charting the future of MRSA treatment: old-revived and nnovel antibiotics

Imane Zerrougui*[1], Maroua Sekkal[1], Nadia Safia Chenouf[2,3], and Messai Chafik Redha[4]

[1]Faculty of Nature and Life Sciences, University of Bordj Bou Arréridj (SNV-STU) – Algeria
[2]Exploration and Valorization of Steppe Ecosystems, University of Djelfa (EVES) – Algeria
[3]Laboratoire de Biologie des Systèmes Microbiens, Ecole Normale Supérieure de Kouba (LBSM) – Algeria
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Abstract

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Keywords: Staphylococcus aureus, MRSA, antimicrobial resistance, novel antibiotics.

*Speaker

ID: 543788

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I. Introduction

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II. Objective

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III. Materials and Methods

A comprehensive literature review, including 11 recently published peer-reviewed articles from PubMed database, has been conducted. Recently published researches have summarized new licensed anti-staphylococcal drugs.

IV. Results

Figure 1: Shematic representation of timeline of antimicrobial drug developement and subequent emergence of resistance.

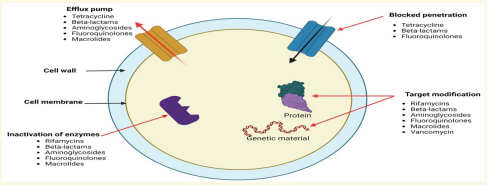

Figure 2: Antimicrobial drug resistance mechanisms in microorganisms.

V. Discussion

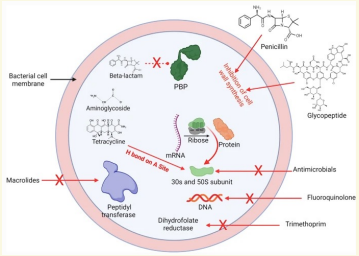

Figure 3: Therapeutic medications and their modes of action for general microbial infections

VI. Conclusion

The older antibiotics laid the groundwork for bacterial treatment, while the exploration of new antibiotic classes has yielded promising leads for future MRSA treatment strategies, combat resistance and broaden the spectrum of efficiency.

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Author email: imane.zerrougui@univ-bba.dz

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Faculty of Sciences and Technology

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ZERROUGUI Imane

Presented an E- Poster communication entitled

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At the *1st Pan Arab Symposium on Parasitology and Infectious Diseases (PASPID24)* on *Mai 18th & 19th, 2024*

Co-authors*: Maroua Sekkal, Nadia Safia Chenouf, Messai Chafik Redha*

President of *PASPID24*
Dr. AROUSSI Abdelkrim

Dean of Sciences and Technology Faculty
Dr. BAGHDADI Djilali

Inspecting staphylococcal enterotoxins (SEs): food safety implications

Maroua Sekkal*[1], Imane Zerrougui[1], Nadia Safia Chenouf[2,3], and Chafik Redha Messai[4]

[1]Faculty of Nature and Life, Earth and Universe, University of Bordj Bou Arréridj (SNV-STU) – Algeria

[2]Laboratory of Exploration and Valorization of Steppe Ecosystems, University of Djelfa (EVES) – Algeria

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[4]Laboratoire de Santé et Productions Animales, Ecole Nationale Supérieure Vétérinaire (ENSV) – Algeria

Abstract

Staphylococcal enterotoxins (SEs) are predominant virulence factors produced by *Staphylococcus aureus*, a foodborne pathogen and leading cause of food poisoning outbreaks (FPO) worldwide. This study aims to provide concise insights into these toxins' classification, function, and contribution to the persistence of foodborne diseases and outbreaks. A comprehensive literature review, including 14 articles from PubMed database, has been examined. To date, over 24 toxins have been identified, extending from SEA to SElV2, with Staphylococcal Enterotoxin A being the most prominent toxin in staphylococcus-related food poisoning. These single-chain molecules with a molecular weight ranging from 22 to 30kDa exhibit remarkable stability under acidic, and heat conditions. They share structural similarities and are classified into four phylogenetic groups based on their amino acid sequences. After their ingestion, SEs display two major toxic activities. An emetic activity sets off the vagus nerve and the emetic center of the brain, triggering symptoms such as nausea, violent vomiting, abdominal cramping, and diarrhea, with variations observed among different SE types. Some enterotoxins lacking emetic activity are referred to as SE-like toxins (SEls). In addition, a super antigenic activity accountable for the non-specific stimulation of massive populations of T lymphocytes causing acute inflammatory responses translated to strong fever and potentially leading to toxic shock syndrome. As to the SE production and regulation, SEs genes are encoded on mobile genetic elements such as plasmids, bacteriophages, or pathogenicity islands, facilitating their transfer between different *S. aureus* strains. A profound understanding of *Staphylococcus aureus* enterotoxin's function and correlation to FPO decipher the hazard these toxins bear upon human health and food safety, emphasizing the need for momentous preventive measures, and therapeutic approaches to tackle staphylococcal food poisoning.

Keywords: Staphylococcus aureus, food poisoning, staphylococcal enterotoxins, emetic activity

*Speaker

ID: 543748

Inspecting staphylococcal enterotoxins (SEs): food safety implications.

SEKKAL Maroua[1], ZERROUGUI Imane[1], CHENOUF Nadia Safia [1,2,3], MESSAI Chafik Redha[1,4]

[1]Faculty of Nature and Life Sciences and of Earth and Universe Sciences, University Mohamed El-Bachir El-Ibrahimi of Bordj Bou Arréridj

[2]Laboratory of Exploration and Valorization of Steppe Ecosystems (EVES), Department of Biology, Faculty of Nature and Life Sciences, Unv of Djelfa, Algeria.

[3]Laboratoire de Biologie des Systèmes Microbiens (LBSM), BP92, 16050, Ecole Normale Supérieure de Kouba, Algiers, Algeria.

[4]Laboratoire de Santé et Productions Animales, Rue Issad Abbes, Oued Smar 16000, Ecole Nationale Supérieure Vétérinaire, Algiers, Algeria.

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Keywords

Staphylococcus aureus, food poisoning, staphylococcal enterotoxins (SEs), emetic activity, superantigens.

I. Introduction

Staphylococcal enterotoxins (SEs) are predominant virulence factors produced by *Staphylococcus aureus*, a foodborne pathogen and leading cause of food poisoning outbreaks (FPO) worldwide.

II. Objective

This study aims to provide concise insights into these toxins' classification, function, and contribution to the persistence of foodborne diseases and outbreaks.

III. Materials and Methods

A comprehensive literature review, including 14 articles from PubMed database, has been examined.

IV. Results

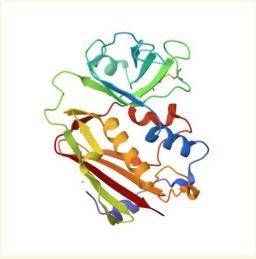

Figure 1: 3D structure of staphylococcal enterotoxin A

V. Discussion

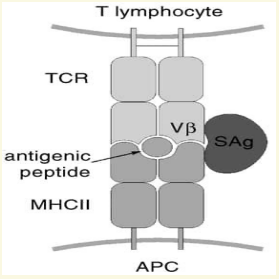

Figure 2: Superantigen function

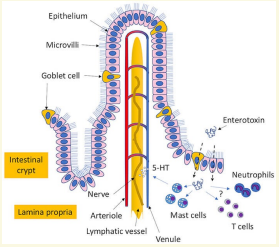

Figure 3: Mechanism of the emetic activity of staphylococcal enterotoxins

VI. Conclusion

A profound understanding of *Staphylococcus aureus* enterotoxin's function and correlation to FPO decipher the hazard these toxins bear upon human health and food safety, emphasizing the need for momentous preventive measures, and therapeutic approaches to tackle staphylococcal food poisoning.

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Author email: maroua.sekkal@univ-bba.dz

People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research
University of Relizane
Faculty of Sciences and Technology

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RELIZANE UNIVERSITY

Certificate

This to certify that

SEKKAL Maroua

Presented an E- Poster communication entitled

Inspecting *staphylococcal* enterotoxins (SEs): food safety implications

At the *1st Pan Arab Symposium on Parasitology and Infectious Diseases (PASPID24)* on *Mai 18th & 19th, 2024*

Co-authors*: Imane Zerrougui, Nadia Safia Chenouf, Chafik Redha Messai*

President of *PASPID24*
Dr. AROUSSI Abdelkrim

Dean of Sciences and Technology Faculty
Dr. BAGHDADI Djilali
د . جيلالي بغدادي
عميد كلية العلوم والتكنولوجيا

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Annexes

Annex 1: Samples data

Food type	Sample code	Sampling date	Market location	General hygiene	Number of Supply farms	Supply farms location	Delivery date	Transport process
Milk	S1	04/02/2024	1008 (BBA)	+ +	**1**	Laitrie centrale (BBA)	03/02/2024	Closed milk cans
	S2	04/02/2024	Lagraf (BBA)	+ +	**1**	Yachir (BBA)	04/02/2024	Closed milk cans
	S3	04/02/2024	Blad (BBA)	+ + +	**2**	Medjana (BBA) Zemoura (BBA)	04/02/2024	Closed milk cans
	S4	03/03/2024	1008 (BBA)	+ +	**1**	Laitrie centrale (BBA)	03/03/2024	Closed milk cans
	S5	03/03/2024	BC (BBA)	+ +	**1**	Laitrie centrale (BBA)	02/03/2024	Closed milk cans
	S6	03/03/2024	1044 (BBA)	+ +	**1**	Laitrie centrale (BBA)	02/03/2024	Closed milk cans
	S7	14/04/2024	Lagar (BBA)	+ +	**1**	Laitrie centrale (BBA)	14/04/2024	Closed milk cans
	S8	14/04/2024	5 juillet (BBA)	+	**2**	Zemoura (BBA) Ras Al Ouad (BBA)	13/04/2024	Closed milk cans
	S9	14/04/2024	12hectare (BBA)	+ + +	**1**	Laitrie centrale (BBA)	14/04/2024	Closed milk cans
	S10	14/04/2024	Faubourd (BBA)	+ +	**2**	Lachbor (BBA) Ras Al Ouad (BBA)	14/04/2024	Closed milk cans

	S11	21/04/2024	Blad (BBA)	+ +	**1**	Medjana (BBA)	21/04/2024	Closed milk cans
	S12	21/04/2024	5 juillet (BBA)	+	**1**	Laitrie centrale (BBA)	20/04/2024	Closed milk cans
	S13	21/04/2024	BC (BBA)	+ +	**1**	Ras Al Ouad (BBA)	21/04/2024	Closed milk cans
	S14	21/04/2024	Faubourd (BBA)	+ +	**1**	Yachir (BBA)	21/04/2024	Closed milk cans
	S15	21/04/2024	Faubourd (BBA)	+ + +	**1**	Medjana (BBA)	21/04/2024	Closed milk cans
	S16	04/05/2024	Blad (BBA)	+ +	**1**	Laitrie centrale (BBA)	04/05/2024	Closed milk cans
	S17	04/05/2024	1008 (BBA)	+ +	**2**	Yachir (BBA) Lachbor (BBA)	04/05/2024	Closed milk cans
	S18	04/05/2024	1044 (BBA)	+ +	**1**	Laitrie centrale (BBA)	03/05/2024	Closed milk cans
	S19	04/05/2024	BC (BBA)	+ +	**1**	Yachir (BBA)	04/05/2024	Closed milk cans
	S20	04/05/2024	5 juillet (BBA)	+	**2**	Zemoura (BBA) Medjana (BBA)	03/05/2024	Closed milk cans

Food type	Sample code	Sampling date	Market location	General hygiene	Preparation date
Merguez	M1	15/04/2024	Lagar (BBA)	+ + +	15/04/2024
	M2	15/04/2024	20 Aout (BBA)	+ +	15/04/2024
	M3	15/04/2024	1008 (BBA)	+ + +	15/04/2024
	M4	15/04/2024	Les 500 (BBA)	+ + +	15/04/2024
	M5	15/04/2024	Les 400 (BBA)	+ + +	15/04/2024
	M6	28/04/2024	12hectare (BBA)	+ +	28/04/2024
	M7	28/04/2024	Lagar (BBA)	+ + +	28/04/2024
	M8	28/04/2024	Leblad (BBA)	+ +	28/04/2024
	M9	28/04/2024	Mounia (BBA)	+ + +	28/04/2024
	M10	28/04/2024	1008 (BBA)	+ + +	27/04/2024
Shawarma	SH1	28/04/2024	12hectare (BBA)	+ + +	28/04/2024
	SH2	28/04/2024	Faubourd (BBA)	+ +	28/04/2024
	SH3	28/04/2024	Faubourd (BBA)	+ + +	28/04/2024
	SH4	28/04/2024	Mounia (BBA)	+ + +	28/04/2024
	SH5	28/04/2024	1008 (BBA)	+ +	28/04/2024
	SH6	06/05/2024	Souk Alfelah (BBA)	+ +	06/05/2024
	SH7	06/05/2024	Souk Alfelah (BBA)	+	06/05/2024
	SH8	06/05/2024	Lblad (BBA)	+ +	06/05/2024
	SH9	06/05/2024	Les 350 (BBA)	+ + +	06/05/2024
	SH10	06/05/2024	Belle vue (BBA)	+ + +	06/05/2024

Annex 2: Recommended antimicrobial critical values

	Antibiotics	Critical values (mm)			Source
		R	I	S	
1	Cefoxitin (30µg)	≥21	-	≤22	CLSI (2020)
2	Erythromycin (15µg)	≥13	14-22	≤23	CLSI (2020)
3	Gentamycin (10µg)	≥12	13-14	≤15	CLSI (2020)
4	Streptomycin (10µg)	≥13	-	≤15	CASFM (2017)
5	Chloramphenicol (30µg)	≥12	13-17	≤18	CLSI (2020)
6	Fusidic acid (10µg)	≥24	-	≤24	CASFM (2023)
7	Clindamycin (2µg)	≥14	15-20	≤21	CLSI (2020)
8	Ciprofloxacin (5µg)	≥15	16-20	≤21	CLSI (2020)
9	Levofloxacin (5µg)	≥15	16-18	≤19	CLSI (2020)
10	Cotrimoxazole (25µg)	≥16	11-15	≤10	CLSI (2020)

R, Resistant **S,** Susceptible **I,** Intermediate.

Annex 3:

Computer tools:

- Word Microsoft Office 2020
- Excel Microsoft Office 2020
- Endnote 20

Laboratory equipment:

- Petri plates;
- Sterile containers;
- Sterile swab sticks;
- Antibiotic disks;
- Physiological serum 0,9%;
- Distilled water;
- Test tubes;

- Pasteur Pipette;
- Erlenmeyer;
- 180ml sterile flasks;
- 70°C alcohol;
- Bleach;
- Hydrochloric Acid.

Culture media and reagents:

- Giolitti & Contoni;
- Mannitol salt agar;
- Brain heart infusion broth ;
- DNA agar ;
- Mueller Hinton agar (MH);
- Nutrient agar (NA) ;
- Potassium tellurite;
- Stains : Fuschine, Lugol, crystal violet ;
- Immersion oil.

Laboratory instruments:

- Autoclave 121°C;
- Incubator 37°C;
- Water bath;
- Bunsen burner
- Refrigerator;
- Magnetic stirrer +hot plate;
- BecBunsen, racks;
- Pince, Scissors;
- Inoculation loop;
- Calibre;
- Ruler;
- Micropipette, tips;
- Balance;
- Syringes.

Abstract

Abstract

Staphylococcus aureus (SA), a major contributor to foodborne illnesses in Algeria and worldwide, is recurrently isolated from meat and dairy products with some strains exhibiting resistance against a variety of antibiotics. The aim of our study was to compute the prevalence of SA in 40 animal-based food samples collected in Bordj Bou Arreridj city (raw milk, shawarma and sausage), and decipher its antimicrobial resistance patterns. Overall, 32 samples (80%) were contaminated and 18 SA were isolated from milk. Eight SA strains were susceptible to all antibiotics while low resistance rates were recorded to Clindamycin and Erythromycin (33%), Gentamycin (11%) and Ciprofloxacin (5%)**.** One SA strain showed inducible Clindamycin resistance (5%), and 2 strains were MRSA (11%), one of which was MDR.

Keywords: *Staphylococcus aureus*, animal-based food, MRSA, antibiotic resistance, Bordj Bou Arreridj.

Résumé

Staphylococcus aureus (SA)*,* un contributeur majeur aux maladies d'origine alimentaire en Algérie et dans le monde, est fréquemment isolé dans la viande et les produits laitiers, certaines souches étant résistantes à divers antibiotiques. Notre étude visait à déterminer la prévalence de SA dans 40 échantillons d'aliments d'origine animale prélevés à Bordj Bou Arreridj (20 laits crus, 10 shawarma et 10 saucisses), et à déterminer leurs phénotypes de résistance aux antibiotiques. Au total, 32 échantillons (80 %) ont été contaminés et 18 SA ont été isolées du lait. Huit souches de SA étaient sensibles à tous les antibiotiques tandis que de faibles taux de résistance ont été enregistrés à l'encontre de la clindamycine et à l'érythromycine (33 %), la gentamycine (11 %) et la ciprofloxacine (5%). Une souche de SA présentait une résistance inductible à la clindamycine (5 %) et 2 souches SARM (11 %) ont été détectées, dont une était MDR.

Mots clés : *Staphylococcus aureus*, aliments d'origine animale, SARM, résistance aux antibiotiques, Bordj Bou Arreridj.

ملخّص

تعد المكورات العنقودية مساهما رئيسيا في الأمراض المنقولة عن طريق الطعام في الجزائر وجميع أنحاء العالم، وقد تكرر عزلها من منتجات اللحوم والألبان مع بعض السلالات التي اظهرت مقاومة ضد مجموعة متنوعة من المضادات الحيوية. تهدف دراستنا الى حساب مدى انتشار بكتيريا المكورات العنقودية في 40 عينة من أغذية ذات أصل حيواني تم جمعها في مدينة برج بوعريريج (20عينة من الحليب الخام و10 منا لشاورما و10 من النقانق)، وأيضا تحديد أنماط مقاومتها لمجموعة من المضادات الحيوية. بصفة عامة، كانت 32 عينة (80٪) ملوثة وتم عزل 18 عينة المكورات العنقودية من الحليب. كانت 8 سلالات SA حساسة لجميع المضادات الحيوية بينما تم تسجيل معدلات مقاومة منخفضة ضد الكليندامايسين والإريثرومايسين (33٪)، الجنتامايسين (11٪) والسيبروفلوكساسين (5٪) كما أظهرت إحدى السلالات مقاومة محفزة للكليندامايسين (5٪)، وكانت سلالتان مقاومة للميثيسيلين (11٪)، إحداهما اتصفت بالمقاومة المتعددة للمضادات الحيوية.

الكلمات المفتاحية: المكورات العنقودية، الأغذية ذات الأصل الحيواني، المكورات العنقودية المقاومة للميتيسيلين، مقاومة المضادات حيوية، برج بوعريريج.