



## Article

# Antibiotic Susceptibility Profiles of Bacterial Isolates Recovered from Abscesses in Cattle and Sheep at a Slaughterhouse in Algeria

Chahrazed Yousfi <sup>1,2,3</sup> , Saoussen Oueslati <sup>3,4</sup> , Dina Daaboul <sup>3</sup> , Delphine Girlich <sup>3</sup> , Alexis Proust <sup>5</sup>,  
Chafia Bentchouala <sup>1,6</sup> and Thierry Naas <sup>3,4,7,\*</sup>

- <sup>1</sup> Centre Hospitalo-Universitaire Ben Badis, Service de Microbiologie, Constantine 25000, Algeria; chahrazedyousfi06@gmail.com (C.Y.); c.bentchouala@yahoo.fr (C.B.)
  - <sup>2</sup> Institut des Sciences Vétérinaires, Université des Frères Mentouri Constantine 1, Constantine 25000, Algeria
  - <sup>3</sup> Team ReSIST, UMR1184, INSERM, School of Medicine, OI Healthi, Université Paris-Saclay, CEA, 94270 Le Kremlin-Bicêtre, France; oueslati.saoussen@gmail.com (S.O.); dinadaaboul58@gmail.com (D.D.); delphine.girlich@universite-paris-saclay.fr (D.G.)
  - <sup>4</sup> Bacteriology-Hygiene Unit, Bicêtre Hospital, APHP Paris-Saclay, 94270 Le Kremlin-Bicêtre, France
  - <sup>5</sup> Department of Hormonal Biochemistry, Hôpital de Bicêtre, Assistance Publique-Hôpitaux de Paris, 75610 Le Kremlin-Bicêtre, France; alexis.proust@aphp.fr
  - <sup>6</sup> Faculté de Médecine, Université Salah Boubnider Constantine 3, Constantine 25000, Algeria
  - <sup>7</sup> French National Reference Center for Antibiotic Resistance: Carbapenemase-Producing Enterobacterales, 94270 Le Kremlin-Bicêtre, France
- \* Correspondence: thierry.naas@aphp.fr; Tel.: +33-1-45212986

**Abstract:** Abscesses represent the most prominent emerging problem in the red meat industry, leading to great economic constraints and public health hazards. Data on etiological agents present in these purulent lesions in Algeria are very scarce. The aim of this study was to identify the bacteria responsible for these abscesses and to determine their antibiotic susceptibility profiles. A total of 123 samples of abscesses from 100 slaughtered sheep and 23 slaughtered cattle were cultured in several media. A total of 114 bacterial isolates were cultured from 103 abscesses. Bacteria were identified using MALDI-TOF, and antibiotic susceptibility was determined by the disk diffusion method on Mueller–Hinton agar. A total of 73.6% (n = 84) corresponded to Enterobacterales, of which four were multidrug-resistant (MDR). These isolates, together with *Staphylococcus aureus*, coagulase negative *Staphylococci*, and seven randomly chosen susceptible *Escherichia coli* isolates, were further characterized using WGS. Resistome analysis of the four MDR Enterobacterales isolates revealed the presence of OXA-48 carbapenemase in two *Klebsiella pneumoniae* ST985 and one *E. coli* ST10 isolates and a CTX-M-15 ESBL in one *E. coli* isolate ST1706. Two coagulase-negative *Staphylococci* isolates were found to carry the *mecA* gene. WGS showed the presence of different resistance genes and virulence genes. Our study revealed 5% of MDR Enterobacterales (including ESBLs and carbapenemases) identified from abscesses, thus urging the need for abscess monitoring in slaughterhouses.

**Keywords:** abscesses; bacteria; antimicrobial resistance; cattle; sheep; slaughterhouse



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## 1. Introduction

An abscess can be defined as an accumulation of pus surrounded by fibrous tissue. They can occur anywhere in the body where pyogenic bacteria can establish and multiply. Among the common causes of abscesses in cattle and sheep are the following:

Injuries to the feet, such as puncture wounds, bruising, and abscesses, which can result from excessive wear of moist feet on rough and abrasive flooring and poor needle practice, leading to abscesses at injection sites. Caseous lymphadenitis (CL) in sheep and goats is caused by *Corynebacterium pseudotuberculosis* and results in abscesses of peripheral and internal lymph nodes. In cattle, skin abscesses may occur at vaccination sites when

vaccination is performed under suboptimal conditions, while liver abscesses are often associated with acidosis in fattening animals [1].

Abscesses are responsible for tremendous economic losses at the farm with a decline in the market value of animals, through a decrease in animal reproductive and productive efficiency, and variable mortality rates. Thus, during the inspection process of production in slaughterhouses, if the carcass presents a problem that could compromise food safety, a total or partial condemnation may occur. This condemnation represents financial losses for the production lines [2–4]. In addition, subcutaneous abscesses may be ruptured during the skinning process, and the bacteria responsible for the infection (zoonotic bacteria) may contaminate the surface of the carcass [5–7], which represents a public health hazard to slaughterhouse workers and meat inspectors as well as consumers [8].

Many species of zoonotic bacteria may be involved in the etiology of abscesses [6], including *Staphylococcus* spp., *Streptococcus* spp., *Corynebacterium* spp., *Pasteurella* spp., *Pseudomonas aeruginosa*, *Escherichia coli*, and other Gram-negative rods [9,10]. In many instances, samples showed more than one species of bacteria isolated from a single abscess [11].

Local antibiotics (including infusion into the abscess) tend to be more effective than systemic antibiotics. Many antibiotics cannot easily penetrate the capsule of an abscess and/or may not be effective in the abscess environment due to changes in pH and other factors in the pus [12]. In addition, antibiotic choices are limited in food animals, and strict adherence to withdrawal times is required to protect food safety. Most wound infections contain multiple bacterial species and should be cultured to determine optimal antibiotic therapy if needed. Therefore, identification of the specific agents involved in the abscess is necessary to adequately implement effective prophylactic or treatment strategies that could lead to the reduction of microbiological pressure at the farm level and eliminate risk factors [13,14].

In Algeria, a few studies were carried out on the epidemiological aspect of the abscesses, but none of these studies addressed the bacteriological aspects and the implication of micro-organisms in relation to their zoonotic potential as well as their antibiotic-resistance profiles and their molecular mechanisms of multidrug resistance [13,15]. The present work was designed to analyze the characteristics of bacteria growing from abscesses in slaughtered cattle and sheep at abattoirs in the Constantine region, northeast of Algeria.

## 2. Materials and Methods

### 2.1. Origin of Isolates

The study was conducted during the period from February 2019 to March 2020 in the largest slaughterhouse in the Constantine region, northeast of Algeria. During the study period, 677 cattle and 978 sheep (N = 1 655) were slaughtered and routinely inspected. The population of both cattle and sheep was predominantly male (561 vs. 116 in cattle and 975 vs. 3 in sheep, for males and females, respectively). A total of 123 abscess lesions were recorded (n = 23, 1.39%) and (n = 100, 6.04%) in cattle and sheep, respectively.

An information sheet was systematically established for each slaughtered animal, including sex, age, and weight status. The localization of examined abscesses in sheep were crural region, liver, lung, lymph node, udder, neckline, peritoneum, precrural region, prescapular region, sternum, cutaneous, and testicles, while in cattle: lung, liver, peritoneum, lymph node, and pericardium.

Intact abscesses from slaughtered cattle and sheep were excised individually, placed in sterile plastic bags, labeled, placed in ice-filled coolers, and transported to the microbiology laboratory of the University hospital Ibn Badis of Constantine, Algeria, for further characterization.

### 2.2. Microbiological Methods

The abscesses were opened by grasping the surface of the abscesses with a hot spatula and incising the capsule with a sterile scalpel. Observations were made of the abscess size and the consistency, color, and odor of the exudate. A loopful of the material contained

in the abscess was streaked directly onto different culture medias including Trypticase Soy Agar, Columbia agar supplemented with 5% of sheep blood, Mac Conkey agar, and mannitol salt agar (BioMérieux, Marcy L'Etoile, France). The incubation was made in aerobic conditions at 37 °C for 24 h. Growing bacterial colonies were subcultured separately on the appropriate media to obtain pure cultures (after another incubation at 37 °C for 24 h).

Bacterial identification was carried out through biochemical galleries (API, bioMérieux), at the Microbiology laboratory of the University hospital Ibn Badis, and subsequently confirmed using MALDI-TOF: matrix-assisted laser desorption ionization–time of flight (Biotyper, Bruker, Hannover, Germany) at the University hospital Bicêtre, Le Kremlin-Bicêtre, France.

### 2.3. Antimicrobial Susceptibility Testing and MIC Determination

All isolates were submitted to susceptibility testing against antimicrobial agents using the Kirby–Bauer disc diffusion assay on Muller–Hinton agar, and the results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing guidelines CA-SFM (Comité de l'antibiogramme de la Société Française de Microbiologie) as updated in 2022 (<http://www.eucast.org> (accessed on 24 January 2024)).

The antibiotic disks (bioMérieux) for bacteria belonging to Enterobacterales order were as follows: amoxicillin-clavulanic acid (AMC, 20/10 µg), ampicillin (AMP, 10 µg), amoxicillin (AMX, 25 µg), piperacillin (PIP, 100 µg), ticarcillin (TIC, 75 µg), Temocillin (TEM, 30 µg), cefazolin (CZ, 30 µg), ceftazidime (CAZ, 30 µg), ceftazidime (CAZ, 30 µg), aztreonam (ATM, 30 µg), imipenem (IPM, 10 µg), ertapenem (ETP, 30 µg), ciprofloxacin (CIP, 5 µg), nalidixic acid (NA, 30 µg), gentamicin (GM, 10 µg), kanamycin (K, 30 µg), amikacin (AN, 30 µg), tetracycline (TET, 30 µg), chloramphenicol (C, 30 µg), fosfomicin (FOS200, 200 µg), and trimethoprim/sulfamethoxazole (SXT, 1.25 + 23.7 µg).

*Aeromonas* spp. isolates were tested towards ceftazidime (CAZ, 30 µg), cefepime (FEP, 30 µg), aztreonam (ATM, 30 µg), ciprofloxacin (CIP, 5 µg), levofloxacin (LEV, 5 µg), and trimethoprim/sulfamethoxazole (SXT, 1.25 + 23.7 µg).

Gram-positive cocci were tested against penicillin (P, 10 Units), oxacillin (OX, 1 µg), ceftazidime (CAZ, 30 µg), gentamicin (GM, 10 µg), kanamycin (K, 30), Tobramycin (TOB, 10 µg), erythromycin (ERY, 15 µg), clindamycin (CM, 2 µg), chloramphenicol (C, 30 µg), tetracycline (TE, 30 µg), Tigecycline (TGC 15 µg), ciprofloxacin (CIP, 5 µg), ofloxacin (OFX, 5 µg), Levofloxacin (LEV, 5 µg), Quinupristin/dalfopristin (QD, 15 µg), Linezolid (LNZ, 30 µg), trimethoprim/sulfamethoxazole (SXT, 1.25 + 23.7 µg), Rifampin (RA, 5 µg), fusidic Acid (FA, 10 µg), and Nitrofurantoin (F, 300 µg).

Finally, rod-shaped Gram-positive isolates were tested against meropenem (MER, 10 µg), imipenem (IPM, 10 µg), ciprofloxacin (CIP, 5 µg), erythromycin (ERY, 15 µg), clindamycin (CM, 2 µg), vancomycin (VAN, 30 µg), and Linezolid (LNZ, 30 µg).

Gram-negative isolates resistant to expanded spectrum cephalosporins and/or to carbapenems were further characterized by broth microdilution method using customized Sensititre plates (Thermo Fisher Scientific, Les Ulis, France). The minimum inhibitory concentrations (MICs) of beta-Lactams, ciprofloxacin, levofloxacin, and tobramycin were determined and interpreted using the EUCAST guidelines. The detection of a carbapenem-hydrolysis was carried out using the homemade Carba NP, and the presence of one of the 5 main carbapenemases was confirmed by the NG-Test CARBA 5 Lateral Flow ImmunoAssay (NG Biotech, Guipry, France) as previously described [16].

### 2.4. Whole-Genome Sequencing and Bioinformatic Analysis

Total DNA of twenty bacterial isolates were extracted using the PureLink™ Genomic DNA Mini-Kit (ThermoFisher Scientific, Les Ulis, France) following the manufacturer's instructions and stored at −20 °C. DNA libraries were prepared using the NEBNext Ultra II FS DNA Library Prep Kit for Illumina (New England Biolabs, Evry, France) according to the manufacturer's instructions, and run on a NextSeq 500 sequencer (Illumina, Évry-

Courcouronnes, France) to generate paired-end 150-bp reads, as previously described [16]. Raw WGS data were assembled de novo using the CLC genomics 10.2 program (Qiagen, Les Ulis, France), and the genomes were analyzed online using software available at the center for genomic epidemiology-CGE (<https://cge.food.dtu.dk/> (accessed on 24 January 2024)). The latter included MLST 2.0 software to determine the sequence types (ST), ResFinder 4.1 to determine the acquired resistome, PlasmidFinder 2.1, to identify known plasmid replicon types and VirulenceFinder 2.0. for the presence of potential virulence genes [16]. Additionally, the virulence factor database (VFDB) (<http://www.mgc.ac.cn/VFs/main.htm> (accessed on 20 December 2023)) was also used to search for virulence factors. Reference plasmid sequences were retrieved from the NCBI database (<https://www.ncbi.nlm.nih.gov> (accessed on 24 January 2024)). Contigs carrying carbapenemase genes were mapped to reference plasmids, using CLC genomics 10.2 program (Qiagen). Mutations in the quinolone-resistance-determining region (QRDR) of *gyrA* and *parC* were also analyzed.

### 2.5. Statistical Analysis

Collected data were statistically treated using Microsoft Excel 2019 and STATA (version 11.1). Pearson's chi-square test and Fisher's exact test, as appropriate, were applied to analyze the categorical variables. A two-tailed *p*-value < 0.05 was considered statistically significant.

### 2.6. Nucleotide Sequence Accession Number

The whole-genome sequences generated in the study have been submitted to the Genbank nucleotide sequence database under Bioproject PRJNA948715.

## 3. Results

### 3.1. Abscess Characteristics

During the study period, 677 cattle and 978 sheep (N = 1655) were slaughtered and routinely inspected. The population of both cattle and sheep was predominantly male (561 vs. 116 in cattle and 975 vs. 3 in sheep, for males and females, respectively). A total of 123 abscess lesions were recorded (n = 23, 1.39%) and (n = 100, 6.04%) in cattle and sheep, respectively. A total of 123 abscess lesions were recorded from the 1655 slaughtered animals, giving an overall prevalence of 7.43%. This prevalence was significantly lower in cattle (n = 23, 1.39%) than in sheep (n = 100, 6.04%) (*p* < 0.001).

In sheep, 97 (97%) and 3 (3%) of the abscesses were recorded from males and females, respectively, while, in cattle, abscesses recorded from males and females were 19 (82.61%) and 4 (17.39%), respectively. The abscesses were mostly recorded from young animals ((N = 115, 93.5%); sheep (n = 97, 78.87%), and cattle (n = 18, 14.63%)) (Table 1).

**Table 1.** Frequency of abscesses in relation to age and sex in sheep and cattle.

Age	Sheep		Cattle		
	≤12 Months	>12 Months	≤ 2 Years	2–5 Years	>5 Years
Male	97	/	18	/	1
Female	/	3	/	3	1

Abscesses in sheep were more frequently located in lymph nodes (n = 27, 31.8%), preapular region (n = 17, 19.8%), and lung (n = 28, 21.2%) (Figure 1A). In cattle, abscesses were frequently located in the lungs (n = 8, 44.4%) and liver (n = 6, 33.3%) (Figure 1B).

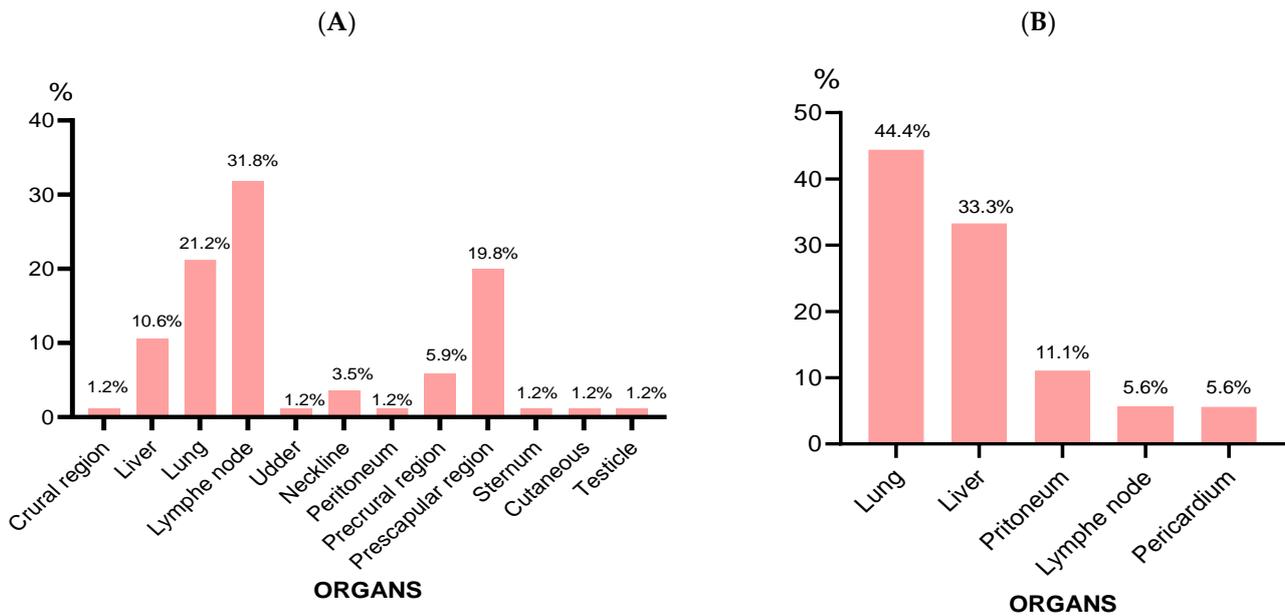


Figure 1. Abscess seats location in sheep (A) and cattle (B).

Finally, the characteristics of abscesses vary according to the bacterial species involved, as illustrated in Table 2.

Table 2. Clinical presentations of abscesses according to the bacterial species.

Pus Characteristics / Bacteria	<i>Staphylococcus aureus</i>	<i>Staphylococcus Coagulase negative</i>	<i>Aeromonas spp.</i>	<i>Bacillus spp.</i>	<i>Enterobacteriales</i>
Consistency	Grumbling viscous Homogeneous viscous	Homogeneous viscous Homogeneous fluid	Homogeneous viscous Thick homogeneous	Homogeneous viscous Thick homogeneous	Grumbling fluid Grumbling viscous Homogeneous fluid Homogeneous viscous Thick grumbling Thick homogeneous Viscous grumbling hemorrhagic
Color	White Light yellow	Light yellow Yellow white Green	Green White Yellow	Green Yellow	Green White Yellow
Odor	Fade	Fade	Fade	Fade	Nauseating Fade

### 3.2. Bacterial Isolates

Of the 123 abscesses analyzed, 103 gave a positive culture (18 in cattle and 85 in sheep), of which 114 bacterial isolates were identified (91 from sheep and 23 from cattle) and further characterized. Bacterial identification revealed Enterobacteriales (*Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Morganella morganii*, *Citrobacter brackii*, *Citrobacter freundii*, *Enterobacter spp.*, *Serratia marcescens*, *Proteus vulgaris*, *Lelliottia spp.*) (n = 84, 73.6%), *Staphylococcus spp.* (n = 15, 13.1%), *Aeromonas spp.* (n = 13, 11.4%), and *Bacillus spp.* (n = 2, 1.9%) (Table 3). *Escherichia coli* represented 61.3% of the isolated bacteria, and 83% of the Enterobacteriales.

**Table 3.** Bacterial species isolated from abscesses in cattle and sheep.

Bacterial Species	Animal Species		Total	Percentage (%)
	Sheep	Cattle		
<i>Escherichia coli</i>	56	14	70	61.3%
<i>Staphylococcus aureus</i>	5	2	7	6.1%
<i>Aeromonas veronii</i>	3	3	6	5.2%
<i>Aeromonas hydrophila</i>	1	3	4	3.5%
<i>Klebsiella pneumoniae</i>	3		3	2.6%
<i>Morganella morganii</i>	2	1	3	2.6%
<i>Staphylococcus epidermidis</i>	3		3	2.6%
<i>Klebsiella oxytoca</i>	2		2	1.7%
<i>Citrobacter brackii</i>	1		1	0.9%
<i>Citrobacter freundii</i>	1		1	0.9%
<i>Enterobacter</i> spp.	1		1	0.9%
<i>Serratia marcescens</i>	1		1	0.9%
<i>Proteus vulgaris</i>	1		1	0.9%
<i>Lelliottia</i> spp.	1		1	0.9%
<i>Staphylococcus lentus</i>	1		1	0.9%
<i>Staphylococcus cohnii</i>	1		1	0.9%
<i>Staphylococcus simulans</i>	1		1	0.9%
<i>Staphylococcus pasteurii</i>	1		1	0.9%
<i>Staphylococcus vitulinus</i>	1		1	0.9%
<i>Aeromonas bestiarum</i>	1		1	0.9%
<i>Aeromonas salmonicida</i>	1		1	0.9%
<i>Aeromonas eucrenophila</i>	1		1	0.9%
<i>Bacillus cereus</i>	1		1	0.9%
<i>Bacillus mojavenensis</i>	1		1	0.9%
<b>Total</b>	<b>91</b>	<b>23</b>	<b>114</b>	<b>100%</b>

In the 11 abscesses in which 2 species had been recovered (6 in sheep and 5 in cattle), the different combinations were as follows: *E. coli*/*Aeromonas* spp. (n = 5), *E. coli*/*S. aureus* (n = 5), and *E. coli*/*Morganella morganii* (n = 1).

### 3.3. Antibiotic Susceptibility Testing

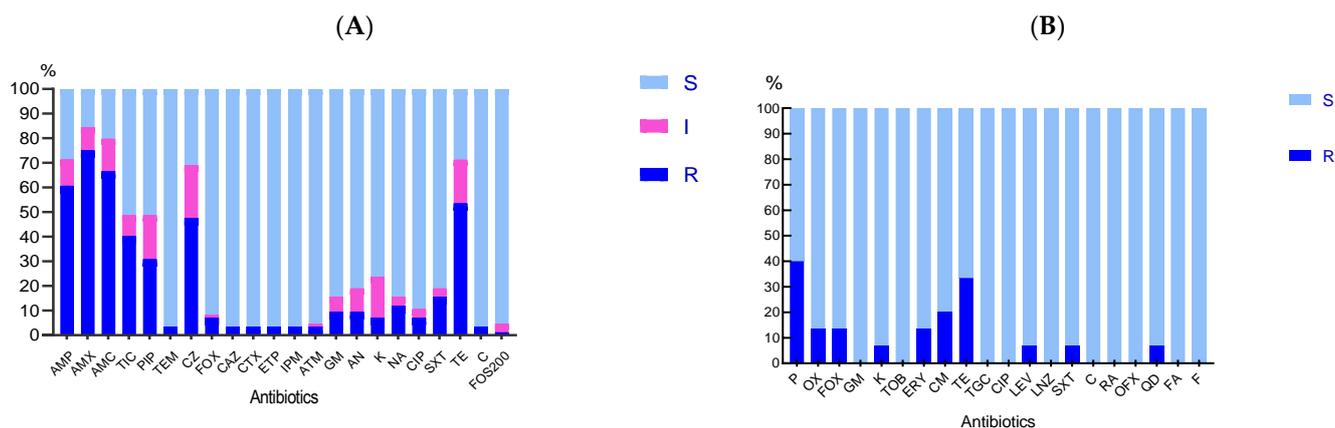
Different rates of resistance were observed for the Enterobacterales isolates, with resistance to amoxicillin, ticarcillin, ampicillin, amoxicillin-clavulanic acid, and tetracycline being the highest rates recorded (Figure 2).

Three isolates with reduced susceptibility to carbapenems were identified. Two *K. pneumoniae* isolates (O103B2 and O103B1) had MICs of 2 µg/mL, 1 µg/mL, and 0.5 µg/mL for ertapenem, imipenem, and meropenem, respectively, and one *E. coli* isolate (O103A10) displayed MICs of 0.5 µg/mL for both ertapenem and imipenem, and 0.12 µg/mL for meropenem (Table 3). These isolates presented additionally high MICs for temocillin 512 µg/mL and 256 µg/mL, for *E. coli* O103A10 and *K. pneumoniae* O103B2 and O103B1, respectively (Table 4). These isolates gave positive results using the Carba NP, suggesting the likely presence of a carbapenem-hydrolyzing enzyme. The NG-Test CARBA 5 confirmed the presence of an OXA-48-like carbapenemase.

*E. coli* O103A9 displayed resistance to expanded-spectrum cephalosporins (ESCs) and exhibited a synergy image in the DD-test, indicating the presence of extended-spectrum beta-lactamases (ESBLs).

The *Aeromonas* spp. were susceptible to all the antibiotics tested except for two that were resistant to cotrimoxazole.

Two coagulase-negative isolates (*S. epidermidis* and *S. pasteurii*) were considered methicillin-resistant as they were resistant to oxacillin and ceftiofur. High resistance rates to tetracycline were observed (Figure 2). The two isolates of *Bacillus* spp. were pan-susceptible to all the antibiotics tested.



**Figure 2.** Susceptibility results of bacteria isolated from the abscesses: (A) Enterobacterales; (B) Staphylococci. Results were interpreted according to Eucast breakpoints 2022. (S: sensitive, I: intermediate, R: resistance). Amikacin (AN), amoxicillin (AMX), amoxicillin-clavulanic acid (AMC), ampicillin (AMP), aztreonam (ATM), cefazolin (CZ), cefotaxime (CTX), ceftazidime (CAZ), chloramphenicol (C), ciprofloxacin (CIP), clindamycin (CM), ertapenem (ETP), erythromycin (ERY), fosfomycin (FOS200), fusidic Acid (FA), gentamicin (GM), imipenem (IPM), kanamycin (K), Levofloxacin (LEV), Linezolid (LNZ), nalidixic acid (NA), Nitrofurantoin (F), ofloxacin (OFX), Oxacillin (OX), penicillin (P), piperacillin (PIP), Quinupristin/dalfopristin (QD), Rifampin (RA), Temocillin (TEM), tetracycline (TE), ticarcillin (TIC), Tigecycline (TGC), Tobramycin (TOB), and trimethoprim/sulfamethoxazole (SXT). Disk loads are indicated in Section 2.

**Table 4.** MICs of Enterobacterales harboring bla<sub>OXA-48</sub> gene.

Antimicrobial (s)	<i>K. pneumoniae</i> O103B2 *	<i>K. pneumoniae</i> O103B1 *	<i>E. coli</i> O103A10 *
Amoxicillin	>32 (R)	>32 (R)	>32 (R)
Amoxicillin + CLA	>128 (R)	>128 (R)	>128 (R)
Ticarcillin	>32 (R)	>32 (R)	<4 (S)
Piperacillin	>32 (R)	>32 (R)	<4 (S)
Piperacillin-tazobactam	>32 (R)	>32 (R)	<4 (S)
Temocillin	256(R)	256 (R)	512 (R)
Tigecycline	1 (R)	1 (R)	1 (R)
Ceftazidime	16 (R)	16 (R)	0.25 (S)
Ceftazidime/Avibactam	0.25 (S)	0.25 (S)	0.25 (S)
Cefotaxime	8 (R)	8 (R)	0.5 (S)
Ceftolozane/Tazobactam	16 (R)	16 (R)	0.5 (S)
Cefepime	8 (I)	8 (I)	0.5 (S)
Cefiderocol	1 (S)	1 (S)	0.12 (S)
Aztreonam	16 (R)	16 (R)	0.12 (S)
Imipenem	1 (S)	1 (S)	0.5 (S)
Imipenem/Relebactam	0.5 (S)	0.5 (S)	0.25 (S)
Meropenem	0.5 (S)	0.5 (S)	0.12 (S)
Meropenem/Vaborbactam	0.5 (S)	0.5 (S)	0.12 (S)
Ertapenem	2 (R)	2 (R)	0.5 (S)
Ciprofloxacin	2 (R)	2 (R)	0.12 (S)
Tobramycin	8 (R)	8 (R)	1 (S)
Levofloxacin	0.5 (S)	1 (I)	0.25 (S)
Colistin	0.5 (S)	0.5 (S)	0.5 (S)

\* Values are in micrograms per milliliter. S and R stand for susceptible and resistant.

### 3.4. Resistome, MLST, Plasmidome, and O-Serogroups

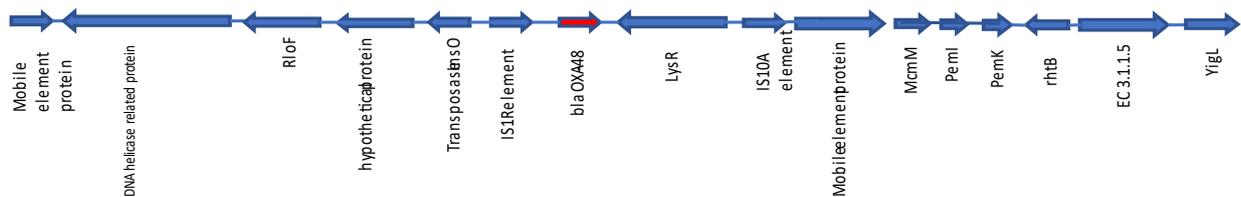
Twenty strains were further investigated by WGS. Gram-positive isolates were chosen based on their resistance profile such as *mecA*-positive *Staphylococci* or their likelihood to carry virulence factors such as *S. aureus* isolates. Enterobacterales were chosen based

on their resistance profile, such as expanded spectrum cephalosporin- or carbapenem-resistance on randomly chosen multisusceptible *Escherichia coli* isolates to obtain an overview of their STs or virulence factors likely involved in abscess formation.

The two *K. pneumoniae* isolates O103B1 and O103B2 harbored, in addition to *bla*<sub>OXA-48</sub>, several  $\beta$ -lactam resistance genes, including the ESBL *bla*<sub>CTX-M-15</sub>, *bla*<sub>OXA-1</sub>, *bla*<sub>SHV-187</sub>, and *bla*<sub>TEM-1B</sub> genes. Additionally, these isolates carried several other resistance genes to different antibiotic classes (Table 5).

The *E. coli* isolate O103A10 carries the carbapenemase *bla*<sub>OXA-48</sub> gene, as well as *tet*(B) and *mph*(B) genes that are responsible for tetracycline and macrolide resistance, respectively (Table 5).

Furthermore, the remaining *E. coli* strain O103A9 harbored the ESBL *bla*<sub>CTX-M-15</sub> and *bla*<sub>TEM-1B</sub> genes, along with aminoglycoside resistance genes *aph*(6)-I<sub>d</sub> and *aph*(3'')-I<sub>b</sub>, *qnrS1* for quinolones, and *sul2* for sulphonamides (Table 5). In silico MLST typing assigned the two *E. coli* O103A9 isolate to ST1706 and ST10 for O103A10, while the two *K. pneumoniae* isolates belonged to the same ST985. Several replicon-types were identified in the four MDR Enterobacterales (Table 5). The CTX-M-15-producing *E. coli* O103A9 harbored an IncY plasmid, while *E. coli* O103A10 carried several plasmids (Table 5), including Col156, IncFIA, IncFIB, and IncFII, but no IncL plasmid known to carry *bla*<sub>OXA-48</sub> gene. A careful analysis of the contig carrying the *bla*<sub>OXA-48</sub> gene suggested a chromosomal location, as the *E. coli* chromosomal genes are present on both sides of *bla*<sub>OXA-48</sub> gene (Figure 3). In addition, electroporation experiments with plasmids extracted from *E. coli* O103A10 failed to transfer any plasmid carrying *bla*<sub>OXA-48</sub> gene to *E. coli* Top10.



**Figure 3.** Genetic environment of *bla*<sub>OXA-48</sub> gene located on the chromosome in *E. coli* O103A10. *Bla*<sub>OXA-48</sub> gene is indicated in red.

The two carbapenemase-producing *K. pneumoniae* isolates O103B1 and O103B2 also carried several plasmids, including an IncL plasmid, likely carrying *bla*<sub>OXA-48</sub> gene (Table 5). Mapping the reads against the prototypical OXA-48 plasmid revealed 100% sequence coverage, suggesting the presence of the entire plasmid in these isolates [17,18].

The resistome of seven randomly chosen *E. coli* isolates revealed few resistance genes. Five isolates produced *tetA* and *tetB* genes, together with aminoglycoside resistance genes (*aadA1*, *aadA2b*, *aph*(3')-I<sub>a</sub>, *aph*(3'')-I<sub>b</sub>, *aph*(6)-I<sub>d</sub>) in two of the seven *E. coli* isolates. Furthermore, two *E. coli* isolates expressed a sulfonamide resistance gene (*sul2* and/or *sul3*). These *E. coli* isolates belonged to seven different STs: ST88, ST101, ST224, ST155, ST223, ST206. Only three plasmid replicon types were detected in the investigated *E. coli* isolates; these are IncFII, IncFIA, and IncFIB. Additionally, O-serogroups that could potentially pose public health concerns were also identified, including O8, O23, O37, O42, O116, O123, O144, and O153 (Table 5).

The *S. epidermidis* strain harbored several antibiotic resistance genes, including *mecA* (which encodes PLP2A conferring resistance to methicillin), *blaZ* (which encodes a narrow spectrum penicillinase), *ant*(6) and *aph*(3') (which encode resistance to aminoglycosides), and *fosB*, *fusB*, and *tet*(K) (which encode resistance to fosfomycin, fusidic acid, and tetracycline, respectively). The *S. pasteurii* strain harbored only *mecA* and *blaZ* genes. Four isolates of *S. aureus* (O104F5, O104F7, O104F9, and O104F10) carried the *blaZ* gene, while the *tet*(K) and *erm*(T) genes were detected only in isolates O104F4 and O104F10, respectively (Table 6).



**Table 6.** Resistance genes, MLST, and virulence factors detected in *Staphylococci* isolates, as revealed with ResFinder-4.1, MLST 2.0, PlasmidFinder 2.0, and VirulenceFinder-2.0 softwares available at CGE the center for genomic epidemiology. (<https://cge.food.dtu.dk/> (accessed on 24 January 2024)).

Isolates <sup>1</sup>	Clinical Features			MLST	Antibiotic Resistance Genes										Virulence Factors																					
					Beta-lactams		Aminoglycosides		Fosfomycin	Fusidic acid	Tetracycline	Erythromycin	Adherence		Enzyme					Immune Evasion			Toxin													
	Animal <sup>2</sup>	Site <sup>3</sup>	Date of Isolation <sup>4</sup>		<i>bla<sub>z</sub></i>	<i>mecA</i>	<i>ant(6)</i>	<i>aph(3')</i>	<i>fosB</i>	<i>fusB</i>	<i>tet(K)</i>	<i>erm(T)</i>	<i>ebp</i>	<i>sdnC</i>	<i>sdnG</i>	<i>icaA,B,C</i>	<i>hlg</i>	<i>hukD,E</i>	<i>sak</i>	<i>spl</i>	<i>sspA</i>	<i>sspB</i>	<i>sspC</i>	<i>geh</i>	<i>lip</i>	<i>aur</i>	<i>nuc</i>	<i>capB</i>	<i>scn</i>	<i>hly</i>	<i>sec</i>	<i>sea</i>	<i>sel</i>	<i>cytR2</i>		
<i>Sepi</i> O104F3	S	PR	23/04	ST61	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
<i>Spas</i> O104G1	S	NK	21/05		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
<i>Sa</i> O104F4	S	LN	18/06	ST522	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
<i>Sa</i> O104F5	C	Li	21/04	Unk	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
<i>Sa</i> O104F6	S	NK	24/02	ST522	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Sa</i> O104F7	S	LN	06/03	ST700	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
<i>Sa</i> O104F8	C	L	29/05	ST97	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
<i>Sa</i> O104F9	S	LN	05/03	ST700	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
<i>Sa</i> O104F10	S	LN	05/05	ST398	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	

(1) Sa: *S. aureus*; Sepi: *S. epidermidis*; Spas: *S. pasteurii* (2) S: sheep; C: cattle; (3) PR: prescapular region; LN: lymph node; Li: liver; NK: neckline; L: lung; (4) day/month/2019. Filled boxes indicated presence of a given allele.

*S. epidermidis* strain belonged to ST61, and the six *S. aureus* isolates belonged to five different STs: ST700 for two isolates, ST522 for two isolates and ST398 from sheep, and ST97 and another novel allele from cattle.

### 3.5. Virulome

As these bacterial isolates were responsible for purulent infections, the search for virulence genes was of the utmost importance (Tables 6 and 7). The ESBL-producing *E. coli* strain O103A9 was found to harbor only three known virulence genes: *ompT*, *terC*, and *yehD*, which encode an outer membrane protease, a tellurite resistance protein C, and a fimbrial protein, respectively. The *E. coli* strain-producing OXA-48 carried three genes as well, including *terC*, *csgA*, and *traT*, which encode a tellurite resistance protein C, a curli fimbriae subunit A, and a serum-resistance-associated protein, respectively.

Among the two *K. pneumoniae* isolates, O103B2 harbored 82 virulence genes, while O103B1 had 79 genes. Both strains shared common virulence genes such as type 1 fimbriae (*fimABCDEFGHIK*) and type 3 fimbriae operon (*mrkABCFHIJ*), enterobactin gene clusters (*entABCDEFGS* and *fepABCDG*), Type IV pili (*pilW*), the T6SS-II (*impAFGHJ*) operon, the *stbABCD* operon, and the siderophore *iroAN* cluster. Additionally, both strains were found to contain the integrative and conjugative element (*ICEKp*) containing the *yersiniabactin* gene cluster (*ybtAEPQSTUX*), *irp1*, *irp2*, and *fyuA* genes. In addition to these genes, other genes were found (Table 7).

The virulome of the seven susceptible *E. coli* isolates was also investigated, and the most common factors found were *terC*, *fimH*, *nlpI*, *lpfA*, *fyuA*, *hlyF*, *iutA*, and *cvaC* (Table 7).

The WGS analysis of *S. aureus* isolates revealed that all but one (O104F6) carried the hemolysin-encoding genes *hlgA*, *hlgB*, and *hlgC*. Leucocidin genes *lukD* and *lukE* were identified in five strains. The serine protease-encoding genes *splA* and *splB* were detected in all six strains, while *splE* was detected in only four strains. Enterotoxin genes *sec* and *sel* were detected in strains O104F7 and O104F9, and the immune evasion gene *sak* was detected in three isolates (O104F4, O104F6, and O104F8), while the *scn* gene was detected in two isolates (O104F8 and O104F10) (Table 6).



#### 4. Discussion

This study revealed a concerning rate of multidrug resistance in bacteria isolated from abscesses in one slaughterhouse in Algeria. In low- to middle-income countries, the intensification of farming is on the rise, primarily driven by a scarcity of available land and the continuous growth of human populations. This intensified farming demands greater use of antibiotics to combat infectious diseases, subsequently fostering the transfer of antibiotic resistance genes (ARGs) between microbes, resulting in multidrug-resistant bacteria in livestock but also in domestic animals and quick spread of resistant microorganisms, which have serious consequences on animal health, productivity, and food production that pose both economic and human health problems [19,20]. Livestock animals are at risk of infectious diseases, especially with pyogenic organisms that cause abscesses at various sites of the body, and consequently have an impact on productivity, fertility, and in general to livestock health [2,21,22]. Infected carcasses at slaughterhouses can be partially or completely discarded [23].

In this study, we reported 123 abscesses, including 100 from sheep and 23 from cattle. Abscesses occur commonly in sheep as ovines are more susceptible to developing them than other ruminants. It cannot be determined solely based on clinical examination and generally relies on postmortem inspection of carcasses in slaughterhouses [24,25]. The analysis of bacterial isolates from pus showed the predominance of Enterobacterales isolates, particularly *E. coli*, followed by *Staphylococcus* spp. Enterobacterales, and *E. coli* especially have been found to be major players in the formation of abscesses in animals [5,26,27], although several studies have isolated *S. aureus* from abscesses as the quintessential suppurative pathogen in large proportions [13,14,28,29]. These bacterial species isolated are zoonotic pathogens, which places public health at risk.

The presence of ESBLs, and in particular CTX-M-15, in animals has previously been linked to the human sector before it was also detected in animals and the environment [30]. Additionally, ceftiofur, a third-generation cephalosporin, is the main cephalosporin used in veterinary medicine due to its effectiveness in treating bacterial infections in food-producing animals; for this reason, these antibiotics could provide selection pressure that favors co-selection of plasmids carrying mobile genes (transposon, integron, cassette gene) that result in carbapenem-resistant (CR) strains [31,32]. Here, we identified a CTX-M-15 producing *E. coli* ST1706, an ST that has previously been described in Japan from different pig farms [33].

The presence of OXA-48-carbapenemase producers is very worrying, as carbapenems remain the last-resort therapy for treating human infections caused by MDR Gram-negative and Gram-positive bacteria [31]. However, the clinical use of these antibiotics is presently at risk due to the global proliferation of  $\beta$ -lactamases (BLs) with the ability to degrade them, and the increase in the worldwide emergence of carbapenem-resistant organisms (CROs), which constitute a critical growing public health threat [34]. In livestock or veterinary fields, carbapenems are not licensed and have no legal indication, so their use is prohibited [32,35,36]. Nevertheless, many studies conducted in Algeria have reported CROs in livestock, companion animals, and birds [37–39].

In our study, we isolated, for the first time, OXA-48 carbapenemase-producing Enterobacterales from abscesses of farm animals. OXA-48 is the most common carbapenemase in Enterobacterales and one of the most frequently isolated around the Mediterranean rim [40]. It is most frequently detected in *K. pneumoniae* and *E. coli* but can also occur in other Enterobacterales species [40]. Both *K. pneumoniae* isolates belong to ST985, an ST type that has been isolated from various sources and geographic locations. MDR *K. pneumoniae* ST985 isolates carrying up to 16 different resistance genes, including *bla*<sub>CTX-M-55</sub> gene, were isolated from rectal swab samples of dairy cows from Quetta in Pakistan [41]. In Austria, *bla*<sub>CTX-M-15</sub>-producing *K. pneumoniae* ST985 were isolated from river water samples that were identical to clinical isolates from Austrian hospitals [42]. Finally, *bla*<sub>CTX-M-15</sub>-producing *K. pneumoniae* ST985 have been involved in an outbreak in Israel in a neonatal intensive care unit [43]. The simultaneous presence of *bla*<sub>OXA-48</sub> and *bla*<sub>CTX-M-15</sub> genes in

*K. pneumoniae* strains ST985 is of particular concern, as this ST seem to be responsible for human and animal infections, and as the combination of these two  $\beta$ -lactamases lead to the resistance to almost all  $\beta$ -lactams. Extended-spectrum  $\beta$ -lactamase (ESBL) and carbapenemase genes are often associated with an MDR phenotype [44], as illustrated in our study by the presence of different resistance to different classes of antibiotics.

The *Bla*<sub>OXA-48</sub> gene is usually located on transferable Inc L plasmids [45]; however, there have also been reports of chromosomally located *bla*<sub>OXA-48</sub> genes in some STs, such as ST38 CPE [46–48]. Here, we report a chromosomal localization of the *bla*<sub>OXA-48</sub> gene in *E. coli* ST 10. Chromosomal insertion of resistance genes is believed to favor the stability of resistance genes in the absence of selective pressure [49,50].

Methicillin-resistant *staphylococci* are among the emerging pathogens that now constitute a threat to human and animal health. Due to its rapid development of antibiotic resistance in clinical settings, methicillin-resistant *S. aureus* (MRSA) is regarded as one of major life-threatening pathogens. The recent isolation of MRSA strains in several animals is thought to be one of the main factors in the spread of infection and disease in both humans and animals [51,52]. In our study, we identified a methicillin-susceptible *S. aureus* ST398, an ST spreading worldwide in animals and humans and often referred to as livestock-associated MRSA (LS-MRSA) [53,54].

This resistance, as our study shows, concerned coagulase-negative Staphylococci (CoNS: *S. epidermidis* and *S. pasteuri*) isolated from abscesses and harbored the *mecA* gene, indicating methicillin resistance. Methicillin-resistant coagulase-negative *staphylococci* have been less studied, but their importance as pathogens is increasing. For a long time, *S. aureus* was thought to be the predominant pathogenic *Staphylococci* species. However, recent investigations have shown the increasing role of CoNS in causing antibiotic-resistant infections [55,56]. In a previous study conducted in Algeria, which focused on unpasteurized cow's milk, three isolated CoNS were resistant to methicillin, and all were *mecA*-positive [57]. A few studies carried out on CoNS in livestock have revealed that food-producing animals constitute a large reservoir of multiresistant CoNS [58].

Virulence factors of Enterobacterales or *Staphylococci* are based on their ability to adhere to host cells, produce toxins, and resist host immune defenses [59]. The identification of specific virulence genes is crucial for understanding the pathogenic potential and for the development of preventive strategies, effective vaccines, and novel therapeutics [60]. For Enterobacterales, important virulence factors, including adhesins, fimbriae, intimin, capsules, iron metabolism, siderophores, heme/hemoglobin transport proteins, and cell invasion, were identified. Furthermore, a study conducted in Western Algeria in 2017 to determine the prevalence of carbapenemase-producing Enterobacteriaceae (CPE) in chicken meat revealed that carbapenemase-producing *K. pneumoniae* isolates harbored several virulence factors, such as *fimH* type 1 fimbriae virulence gene, *ureA*, involved in the hydrolysis of urea to ammonia, *mrkD*, encoding a type 3 fimbriae that promotes biofilm development, *uge*, that codes for a UDP galacturonate 4-epimerase, and *wabG*, encoding the biosynthesis of the core lipopolysaccharide [61]. The relationship between antimicrobial resistance and virulence factors in bacteria is complex. There are common characteristics shared between virulence and resistance, such as the involvement of efflux pumps, porins, and cell wall alterations. Additionally, some studies have found a significant association between certain virulence genes and antimicrobial resistance, suggesting that acquiring resistance to some antibiotics may impact the expression of virulence factors [62–64]. In contrast to other studies, KPC-producing Enterobacterales, such as *K. pneumoniae*, typically exhibit lower levels of virulence compared to non-carbapenemase-producing strains. Overall, the presence of carbapenemase genes does not necessarily imply a reduction in virulence, although there might be trade-offs between virulence and resistance capabilities in certain strains [65].

## 5. Conclusions

The presence of *bla*<sub>ESBL</sub>, *bla*<sub>OXA-48</sub>, and *mecA* genes in animal abscesses highlights the importance of monitoring the use of antimicrobial in animals. Preventing the spread of multidrug-resistant bacteria in livestock animals should, therefore, be a priority for public health, which can be achieved through the reduction in and proper use of antimicrobial agents in animal husbandry and in humans, and also acting at the farm level, improving hygiene and biosecurity measures, based primarily on the elimination of risk factors and vaccination of small ruminants.

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**Data Availability Statement:** Raw data are available upon request, and WGS sequences have been deposited at NCBI.

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## References

1. Misk, T.N.; El-Sherry, T.; Misk, N.A. Retrospective study on body surface abscesses in farm animals. *Assiut Vet. Med. J.* **2020**, *66*, 47–61. [[CrossRef](#)]
2. Mariappan, S.; Mydeen, S.N.M.; Samuel, S.J.R.; Jegathambigai, J.; Rajan, K.E.; Sudhakar, S. *Bacillus* sp. Causing Abscessation in Sheep and Goat Population. *Curr. Sci.* **2012**, *103*, 921–925.
3. Awad, A.; Awadin, W.F. Pathological and bacteriological studies on some recorded internal abscesses in slaughtered cattle. *Assiut Vet. Med. J.* **2016**, *63*, 52–61.
4. Nagati, S.F.; El Shafii, S.S.A.; Abd El Mawgoud, S.R.A. Effectiveness of Disinfectants on Environmental Multidrug Resistance Contaminants Causing Skin Abscess in Farm Animals. *Am. J. Anim. Vet. Sci.* **2021**, *16*, 128–138. [[CrossRef](#)]
5. Tavassoli, M.; Imani, A.; Yousefnia, M.; Tukmechi, A.; Tajik, H. Bacteria associated with subcutaneous abscesses of cattle caused by *Hypoderma* spp. larvae in north of Iran. *Vet. Res. Forum* **2010**, *1*, 123–127.
6. AL-Tufflyli, Y.K.; Shekhan, M.I. Clinical and Bacteriological Study of Subcutaneous Abscesses Caused by Gram Positive Bacteria in Cow and Sheep in Al-Qadisiyah Province. *Al-Qadisiyah J. Vet. Med. Sci.* **2012**, *11*, 17–24.
7. Ninios, T.; Lundén, J.; Korkeala, H.; Fredriksson-Ahomaa, M. *Meat Inspection and Control in the Slaughter House*, 1st ed.; John Wiley & Sons: Chichester, UK, 2014; pp. 154–157.
8. Aljameel, M.A.; Halima, M.O.; ElTigani-Asil, E.A.; El-Eragi, A.M. Bacteriological and histopathological studies on pulmonary abscesses in camels (*Camelus dromedarius*) slaughtered at Nyala Slaughterhouse, South Darfur State, Sudan. *J. Vet. Med. Anim. Prod.* **2013**, *4*, 26–38.
9. Herenda, D.; Chambers, P.G.; Ettriqui, A.; Seneviratna, P.; Da Silva, T.J.P. *Manuel on Meat Inspection for Developing Countries*; FAO: Rome, Italy, 1994; pp. 58–60.
10. Buba, D.M.; Gurumyen, G.Y.; Oragwa, O.A.; Oziegbe, S.D.; Patrobas, M.N.; Dunka, H.I. Retrospective Analysis of Cutaneous Abscess in Cattle, Goats and Pigs Slaughtered at the Jos Abattoir, Nigeria. *Sokoto J. Vet. Sci.* **2020**, *17*, 44. [[CrossRef](#)]
11. Al-nakeeb, N.K.; HameedAl-Fetly, D.R. Anaerobic Bacterial Isolation with Histopathological Exam of Liver Abscesses in Cattle, Sheep, and Camels in Al-Qadisiyah Province. *Al-Qadisiyah J. Vet. Med. Sci.* **2016**, *15*, 40–46.
12. Wagner, C.; Sauermann, R.; Joukhadar, C. Principles of Antibiotic Penetration into Abscess Fluid. *Pharmacology* **2006**, *78*, 1–10. [[CrossRef](#)]
13. Alloui, M.N.; Kaba, J.; Alloui, N. Prevalence and risk factors of caseous lymphadenitis in sheep and goats of Batna area (Algeria). *Res. Opin. Anim. Vet. Sci.* **2011**, *1*, 162–164.

14. Ferreira, A.; Monteiro, J.M.; Vieira-Pinto, M. The Importance of Subcutaneous Abscess Infection by *Pasteurella* spp. and *Staphylococcus aureus* as a Cause of Meat Condemnation in Slaughtered Commercial Rabbits. *World Rabbit. Sci.* **2014**, *22*, 311–317. [[CrossRef](#)]
15. Saadi, C. Les Motifs de Saisie des Viandes Rouges et Abats les Plus Fréquents au Niveau de L'abattoir de Frère Ben Aissa Biskra. Master's Thesis, University Mohamed Khider Biskra, Biskra, Algeria, 2018.
16. Perez-Palacios, P.; Girlich, D.; Soraa, N.; Lamrani, A.; Maoulainine, F.M.R.; Bennaoui, F.; Amri, H.; El Idrissi, N.S.S.; Bouskraoui, M.; Birer, A.; et al. Multidrug-Resistant Enterobacterales Responsible for Septicaemia in a Neonatal Intensive Care Unit in Morocco. *J. Glob. Antimicrob. Resist.* **2023**, *33*, 208–217. [[CrossRef](#)] [[PubMed](#)]
17. Aubert, D.; Naas, T.; Héritier, C.; Poirel, L.; Nordmann, P. Functional Characterization of IS1999, an IS4 Family Element Involved in Mobilization and Expression of  $\beta$ -Lactam Resistance Genes. *J. Bacteriol.* **2006**, *188*, 6506–6514. [[CrossRef](#)] [[PubMed](#)]
18. Bonnin, R.A.; Nordmann, P.; Carattoli, A.; Poirel, L. Comparative Genomics of IncL/M-Type Plasmids: Evolution by Acquisition of Resistance Genes and Insertion Sequences. *Antimicrob. Agents Chemother.* **2013**, *57*, 674–676. [[CrossRef](#)]
19. Ferriol-González, C.; Domingo-Calap, P. Phage Therapy in Livestock and Companion Animals. *Antibiotics* **2021**, *10*, 559. [[CrossRef](#)] [[PubMed](#)]
20. Wang, H.; Qi, J.F.; Qin, R.; Ding, K.; Graham, D.W.; Zhu, Y.G. Intensified Livestock Farming Increases Antibiotic Resistance Genotypes and Phenotypes in Animal Feces. *Commun. Earth Environ.* **2023**, *4*, 123. [[CrossRef](#)]
21. Hatem, M.E.; Arab, R.H.; Ata, S. Nagwa Bacterial Abscessation in Sheep and Goat in Giza Governorate with Full Antibiogram Screening. *Glob. Vet.* **2013**, *10*, 372–381.
22. Tamai, I.A.; Mohammadzadeh, A.; Mahmoodi, P.; Pakbin, B.; Salehi, T.Z. Antimicrobial Susceptibility, Virulence Genes and Genomic Characterization of Trueperella Pyogenes Isolated from Abscesses in Dairy Cattle. *Res. Vet. Sci.* **2023**, *154*, 29–36. [[CrossRef](#)]
23. Rodrigues, R.M.; Martins, T.O.; Procópio, D.P. Economic Loss from the Main Causes of Whole Bovine Carcass Condemnation in Slaughterhouses Supervised by the Federal Inspection Service in São Paulo State from 2010 to 2019. *Acta Sci.* **2022**, *44*, e55220. [[CrossRef](#)]
24. Al-Harbi, K.B. Prevalence and Etiology of Abscess Disease of Sheep and Goats at Qassim Region, Saudi Arabia. *Vet. World* **2011**, *4*, 495–499. [[CrossRef](#)]
25. Omar Ali, A.S.; Mohammed, Z.M.A. Gross and Histopathological Findings on Lung Abscesses in Slaughtered Sheep in Libya. *Eur. J. Vet. Med.* **2023**, *3*, 1–4. [[CrossRef](#)]
26. Mcvey, D.S.; Kennedy, M.; Chengappa, M.M. *Veterinary Microbiology*, 3rd ed.; Wiley-Blackwell: Ames, IA, USA, 2013; pp. 184–194.
27. Madhav Mugale, N.; Balachandran, C.; Dillibabu, V.; Kirubharan, J.; Dhinakar Raj, G.; Sridhar, R.; Selvasubramaniam, S. Hepatic Abscess in Sheep and Goat Caused by O26 Escherichia Coli Serotype: An Emerging Pathogen. *Indian Vet. J.* **2015**, *92*, 76–79.
28. Al-Anbagi, N.A. Isolation and Identification some bacterial causes of lung abscesses sheep by chromogenic media. *Basrah J. Vet. Res.* **2016**, *15*, 360–370. [[CrossRef](#)]
29. Didkowska, A.; Żmuda, P.; Kwiecień, E.; Rzewuska, M.; Klich, D.; Wędzina, M.K.; Witkowski, L.; Żychska, M.; Kaczmarkowska, A.; Orłowska, B.; et al. Microbiological Assessment of Sheep Lymph Nodes with Lymphadenitis Found during Post—Mortem Examination of Slaughtered Sheep: Implications for Veterinary—Sanitary Meat Control. *Acta Vet. Scand.* **2020**, *62*, 48. [[CrossRef](#)] [[PubMed](#)]
30. Haenni, M.; Lupo, A.; Madec, J.-Y. Résistance Aux Carbapénèmes Chez Les Animaux En l'absence d'usage. *Bull. Acad. Vet. Fr.* **2018**, *171*, 4–8. [[CrossRef](#)]
31. Das, S. The Crisis of Carbapenemase-Mediated Carbapenem Resistance across the Human–Animal–Environmental Interface in India. *Infect. Dis. Now* **2023**, *53*, 104628. [[CrossRef](#)]
32. Ramírez-Castillo, F.Y.; Guerrero-Barrera, A.L.; Avelar-González, F.J. An Overview of Carbapenem-Resistant Organisms from Food-Producing Animals, Seafood, Aquaculture, Companion Animals, and Wildlife. *Front. Vet. Sci.* **2023**, *10*, 1158588. [[CrossRef](#)]
33. Norizuki, C.; Kawamura, K.; Wachino, J.I.; Suzuki, M.; Nagano, N.; Kondo, T.; Arakawa, Y. Detection of Escherichia coli Producing CTX-M-1-Group Extended-Spectrum  $\beta$ -Lactamases from Pigs in Aichi Prefecture, Japan, between 2015 and 2016. *Jpn. J. Infect. Dis.* **2018**, *71*, 33–38. [[CrossRef](#)]
34. Naas, T.; Dabos, L.; Bonnin, R.A.  $\beta$ -Lactamase Genes without Limits. *Microorganisms* **2023**, *11*, 1200. [[CrossRef](#)]
35. Madec, J.Y.; Haenni, M.; Nordmann, P.; Poirel, L. Extended-Spectrum  $\beta$ -Lactamase/AmpC- and Carbapenemase-Producing Enterobacteriaceae in Animals: A Threat for Humans? *Clin. Microbiol. Infect.* **2017**, *23*, 826–833. [[CrossRef](#)] [[PubMed](#)]
36. Uyanik, T.; Çadirci, Ö.; Gücükoğlu, A.; Can, C. Investigation of Major Carbapenemase Genes in ESBL-Producing Escherichia Coli and Klebsiella Pneumoniae Strains Isolated from Raw Milk in Black Sea Region of Turkey. *Int. Dairy J.* **2022**, *128*, 105315. [[CrossRef](#)]
37. Yaici, L.; Haenni, M.; Saras, E.; Boudehouche, W.; Touati, A.; Madec, J.Y. bla<sub>NDM-5</sub>-Carrying IncX3 Plasmid in Escherichia Coli ST1284 Isolated from Raw Milk Collected in a Dairy Farm in Algeria. *J. Antimicrob. Chemother.* **2016**, *71*, 2671–2672. [[CrossRef](#)] [[PubMed](#)]
38. Loucif, L.; Chelaghma, W.; Bendjama, E.; Cherak, Z.; Khellaf, M.; Khemri, A.; Rolain, J.M. Detection of bla<sub>OXA-48</sub> and mcr-1 Genes in Escherichia Coli Isolates from Pigeon (*Columba livia*) in Algeria. *Microorganisms* **2022**, *10*, 975. [[CrossRef](#)] [[PubMed](#)]

39. Loucif, L.; Chelaghma, W.; Cherak, Z.; Bendjama, E.; Beroual, F.; Rolain, J.M. Detection of NDM-5 and MCR-1 Antibiotic Resistance Encoding Genes in Enterobacterales in Long-Distance Migratory Bird Species *Ciconia ciconia*, Algeria. *Sci. Total Environ.* **2022**, *814*, 152861. [[CrossRef](#)] [[PubMed](#)]
40. Hamprecht, A.; Sommer, J.; Willmann, M.; Brender, C.; Stelzer, Y.; Krause, F.F.; Tsvetkov, T.; Wild, F.; Riedel-Christ, S.; Kutschenreuter, J.; et al. Pathogenicity of Clinical OXA-48 Isolates and Impact of the OXA-48 IncL Plasmid on Virulence and Bacterial Fitness. *Front. Microbiol.* **2019**, *10*, 2509. [[CrossRef](#)]
41. Habib, S.; Gibbon, M.J.; Couto, N.; Kakar, K.; Habib, S.; Samad, A.; Munir, A.; Fatima, F.; Mohsin, M.; Feil, E.J. The Diversity, Resistance Profiles and Plasmid Content of *Klebsiella* spp. Recovered from Dairy Farms Located around Three Cities in Pakistan. *Antibiotics* **2023**, *12*, 539. [[CrossRef](#)]
42. Frenk, S.; Rakovitsky, N.; Temkin, E.; Schechner, V.; Cohen, R.; Kloyzner, B.S.; Schwaber, M.J.; Solter, E.; Cohen, S.; Stepansky, S.; et al. Investigation of Outbreaks of Extended-Spectrum Beta-Lactamase-Producing *Klebsiella pneumoniae* in Three Neonatal Intensive Care Units Using Whole Genome Sequencing. *Antibiotics* **2020**, *9*, 705. [[CrossRef](#)]
43. Lepuschitz, S.; Schill, S.; Stoeger, A.; Pekard-Amenitsch, S.; Huhulescu, S.; Inreiter, N.; Hartl, R.; Kerschner, H.; Sorschag, S.; Springer, B.; et al. Whole genome sequencing reveals resemblance between ESBL-producing and carbapenem resistant *Klebsiella pneumoniae* isolates from Austrian rivers and clinical isolates from hospitals. *Sci. Total Environ.* **2019**, *662*, 227–235. [[CrossRef](#)]
44. Nordmann, P.; Poirel, L. Strategies for Identification of Carbapenemase-Producing Enterobacteriaceae. *J. Antimicrob. Chemother.* **2013**, *68*, 487–489. [[CrossRef](#)]
45. Poirel, L.; Bonnin, R.A.; Nordmann, P. Genetic Features of the Widespread Plasmid Coding for the Carbapenemase OXA-48. *Antimicrob. Agents Chemother.* **2012**, *56*, 559–562. [[CrossRef](#)]
46. Hendrickx, A.P.A.; Landman, F.; de Haan, A.; Witteveen, S.; van Santen-Verheuevel, M.G.; Schouls, L.M. *bla*<sub>OXA-48</sub>-like Genome Architecture among Carbapenemase-Producing *Escherichia coli* and *Klebsiella pneumoniae* in The Netherlands. *Microb. Genom.* **2021**, *7*, 000512.
47. Lee, C.R.; Lee, J.H.; Park, K.S.; Kim, Y.B.; Jeong, B.C.; Lee, S.H. Global Dissemination of Carbapenemase-Producing *Klebsiella pneumoniae*: Epidemiology, Genetic Context, Treatment Options, and Detection Methods. *Front. Microbiol.* **2016**, *7*, 895. [[CrossRef](#)]
48. Mairi, A.; Pantel, A.; Sotto, A.; Lavigne, J.P.; Touati, A. OXA-48-like Carbapenemases Producing Enterobacteriaceae in Different Niches. *Eur. J. Clin. Microbiol. Infect. Dis.* **2018**, *37*, 587–604. [[CrossRef](#)]
49. Bonnet, R.; Marchandin, H.; Chanal, C.; Sirot, D.; Labia, R.; De Champs, C.; Jumas-Bilak, E.; Sirot, J. Chromosome-Encoded Class D  $\beta$ -Lactamase OXA-23 in *Proteus mirabilis*. *Antimicrob. Agents Chemother.* **2002**, *46*, 2004–2006. [[CrossRef](#)]
50. Beyrouthy, R.; Robin, F.; Delmas, J.; Gibold, L.; Dalmasso, G.; Dabboussi, F.; Hamzé, M.; Bonnet, R. IS1R-Mediated Plasticity of IncL/M Plasmids Leads to the Insertion of *Bla*<sub>OXA-48</sub> into the *Escherichia coli* Chromosome. *Antimicrob. Agents Chemother.* **2014**, *58*, 3785–3790. [[CrossRef](#)] [[PubMed](#)]
51. Venugopal, N.; Mitra, S.; Tewari, R.; Ganaie, F.; Shome, R.; Rahman, H.; Shome, B.R. Molecular Detection and Typing of Methicillin-Resistant *Staphylococcus aureus* and Methicillin-Resistant Coagulase-Negative *Staphylococci* Isolated from Cattle, Animal Handlers, and Their Environment from Karnataka, Southern Province of India. *Vet. World* **2019**, *12*, 1760–1768. [[CrossRef](#)]
52. Zaher, H.A.; El Baz, S.; Alothaim, A.S.; Alsalamah, S.A.; Alghonaim, M.I.; Alawam, A.S.; Eraqi, M.M. Molecular Basis of Methicillin and Vancomycin Resistance in *Staphylococcus aureus* from Cattle, Sheep Carcasses and Slaughterhouse Workers. *Antibiotics* **2023**, *12*, 205. [[CrossRef](#)] [[PubMed](#)]
53. Silva, V.; Araújo, S.; Monteiro, A.; Eira, J.; Pereira, J.E.; Maltez, L.; Igrejas, G.; Lemsaddek, T.S.; Poeta, P. *Staphylococcus aureus* and MRSA in Livestock: Antimicrobial Resistance and Genetic Lineages. *Microorganisms* **2023**, *11*, 124. [[CrossRef](#)]
54. Bouiller, K.; Bertrand, X.; Hocquet, D.; Chirouze, C. Human Infection of Methicillin-Susceptible *Staphylococcus aureus* CC398: A Review. *Microorganisms* **2020**, *8*, 1737. [[CrossRef](#)] [[PubMed](#)]
55. Bonvegna, M.; Grego, E.; Sona, B.; Stella, M.C.; Nebbia, P.; Mannelli, A.; Tomassone, L. Occurrence of Methicillin-Resistant Coagulase-Negative *Staphylococci* (Mrcons) and Methicillin-Resistant *Staphylococcus aureus* (MRSA) from Pigs and Farm Environment in Northwestern Italy. *Antibiotics* **2021**, *10*, 676. [[CrossRef](#)] [[PubMed](#)]
56. Chajęcka-Wierzchowska, W.; Gajewska, J.; Zadernowska, A.; Randazzo, C.L.; Caggia, C. A Comprehensive Study on Antibiotic Resistance among Coagulase-Negative *Staphylococci* (CoNS) Strains Isolated from Ready-to-Eat Food Served in Bars and Restaurants. *Foods* **2023**, *12*, 514. [[CrossRef](#)] [[PubMed](#)]
57. Chenouf, N.S.; Mama, O.M.; Messaï, C.R.; Ruiz-Ripa, L.; Fernández-Fernández, R.; Carvalho, I.; Zitouni, A.; Hakem, A.; Torres, C. Detection of Methicillin-Resistant Coagulase-Negative *Staphylococci* and PVL/MecA Genes in Cefoxitin-Susceptible *Staphylococcus aureus* (T044/ST80) from Unpasteurized Milk Sold in Stores in Djelfa, Algeria. *J. Dairy Sci.* **2021**, *104*, 2684–2692. [[CrossRef](#)]
58. Bhargava, K.; Zhang, Y. Multidrug-Resistant Coagulase-Negative *Staphylococci* in Food Animals. *J. Appl. Microbiol.* **2012**, *113*, 1027–1036. [[CrossRef](#)] [[PubMed](#)]
59. Bujňáková, D.; Puvača, N.; Čirković, I. Virulence factors and antibiotic resistance of enterobacterales. *Microorganisms* **2022**, *10*, 1588. [[CrossRef](#)] [[PubMed](#)]
60. Pakbin, B.; Brück, W.M.; Rossen, J.W. Virulence factors of enteric pathogenic *Escherichia coli*: A review. *Int. J. Mol. Sci.* **2021**, *22*, 9922. [[CrossRef](#)] [[PubMed](#)]
61. Chaalal, N.; Touati, A.; Bakour, S.; Aissa, M.A.; Sotto, A.; Lavigne, J.-P.; Pantel, A. Spread of OXA-48 and NDM-1-Producing *Klebsiella pneumoniae* ST48 and ST101 in Chicken Meat in Western Algeria. *Microb. Drug Resist.* **2021**, *27*, 492–500. [[CrossRef](#)]

62. Schroeder, M.; Brooks, B.D.; Brooks, A.E. The complex relationship between virulence and antibiotic resistance. *Genes* **2017**, *8*, 39. [[CrossRef](#)]
63. Beceiro, A.; Tomás, M.; Bou, G. Antimicrobial resistance and virulence: A successful or deleterious association in the bacterial world? *Clin. Microbiol. Rev.* **2013**, *26*, 185–230. [[CrossRef](#)]
64. Miranda-Estrada, L.I.; Ruíz-Rosas, M.; Molina-López, J.; Parra-Rojas, I.; González-Villalobos, E.; Castro-Alarcón, N. Relationship between virulence factors, resistance to antibiotics and phylogenetic groups of uropathogenic *Escherichia coli* in two locations in Mexico. *Enfermedades Infecc. Microbiol. Clin. (Engl. Ed.)* **2017**, *35*, 426–433. [[CrossRef](#)]
65. Mendes, G.; Santos, M.L.; Ramalho, J.F.; Duarte, A.; Caneiras, C. Virulence factors in carbapenem-resistant hypervirulent *Klebsiella pneumoniae*. *Front. Microbiol.* **2023**, *14*, 1325077. [[CrossRef](#)]

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