Universidade de Aveiro
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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Microbiologia, realizada sob a orientação científica da Doutora Ana Cláudia Pereira, do Centro para Saúde Translacional e Investigação em Biotecnologia Médica da Escola Superior de Saúde do Instituto Politécnico do Porto, e coorientação científica da Doutora Sónia Mendo, Professora Auxiliar com Agregação do Departamento de Biologia da Universidade de Aveiro

Dedico aos meus pais.

o júri

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palavras-chave

Obesidade; Inflamação Crónica; Resistência Antimicrobiana; Secretoma; Macrófagos

resumo

A obesidade é caraterizada pelo excesso de gordura no tecido adiposo, continuando a ser descrita como um problema à saúde pública. Além disso, está associada ao desenvolvimento e agravamento de comorbidades como diabetes tipo II, doenças cardiovasculares, hipertensão e síndrome metabólica. Outro problema à saúde pública incide nas resistências antimicrobianas (AMR). Ao longo do tempo, têm vindo a ganhar proporções alarmantes à escala Mundial, devido à capacidade de adquirir novos mecanismos de resistência, ameaçando o tratamento de infeções adquiridas na comunidade e nos hospitais, do qual resulta numa doença prolongada, incapacidade e morte. O estado de obesidade acarreta um desequilíbrio na produção de fatores pró-inflamatórios e anti-inflamatórios que contribuem para a suscetibilidade das infeções. Essa suscetibilidade traduz-se em comprometimento respiratório e da barreira cutânea, comorbidades relacionadas à obesidade, cicatrização mais lenta e terapia antimicrobiana ineficiente. A capacidade alarmante das bactérias em adquirir resistência tem levantado obstáculos à terapia antimicrobiana em pacientes com inflamação crônica, como indivíduos obesos. Este estudo teve como objetivo compreender a influência de um ambiente inflamatório que mimetiza a obesidade, no crescimento de *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Klebsiella pneumoniae*, *Escherichia coli* e *Pseudomonas aeruginosa* com diferentes resistências a antibióticos. Para tal, as linhagens celulares Raw 264.7 (macrófagos) e 3T3-L1 (adipócitos) foram escolhidas para servir como meio condicionado para o crescimento bacteriano. As estirpes bacterianas foram expostas a uma variedade de meios condicionados (DMEM, DMEM enriquecido com 10% e 50% de secretoma de adipócitos (SA), secretoma de macrófagos (SM) e secretoma de macrófagos enriquecido com 10% e 50% de SA). Os resultados mostraram que *S.aureus* com resistência a meticilina (MRSA) e *K. pneumoniae* Carbapenem/ESBL contribuem para um maior fator de risco num ambiente de SM. Por outro lado, *S.aureus*, *M. smegmatis*, *K. pneumoniae* "estirpe-selvagem", *K. pneumoniae* ESBL, *K. pneumoniae* Carbapenem, *E. coli* e *P. aeruginosa* parecem depender mais de um ambiente enriquecido em SA. Ainda, as estirpes bacterianas com resistência a AmpC apresentam crescimento bacteriano elevado num ambiente enriquecido em SA. No geral, os resultados mostraram que a estirpe e painel de resistência, podem contribuir para o estado de inflamação crónico de forma diferenciada.

keywords

Obesity; Chronic Inflammation; Antimicrobial Resistance; Secretome; Macrophages

abstract

Obesity is characterized by excessive fat in adipose tissue and continues to be described as a public health problem. In addition, it is associated with the development and worsening of comorbidities such as type II diabetes, cardiovascular diseases, hypertension and metabolic syndrome. Another public health problem focuses on antimicrobial resistance (AMR). Over time, they have been gaining alarming proportions worldwide, due to the ability to acquire new mechanisms of resistance threatening the treatment of infections acquired in the community and in hospitals, resulting in a prolonged illness, disability, and death. The state of obesity entails an imbalance in the production of proinflammatory and anti-inflammatory factors that contributes to infections' susceptibility. This susceptibility translates into respiratory and skin barrier impairment, obesity-related comorbidities, slower healing and inefficient antimicrobial therapy. Bacteria's alarming ability to gain resistance has raised concerning obstacles in antimicrobial therapies in patients with chronic inflammation, such as obese individuals. This study aimed to understand the influence of an inflammatory obesity-mimicking environment in the growth of *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Klebsiella pneumoniae*, *Escherichia coli* and Pseudomonas aeruginosa strains with different antibiotic resistance. For this, the cell lines Raw 264.7 (macrophages) and 3T3-L1 (adipocytes) were collected to serve as a conditioned medium for bacterial growth. All strains were exposed to a variety of conditioned media (DMEM, DMEM enriched with 10% and 50% adipocyte secretome (SA), macrophage secretome (SM) and macrophage secretome enriched with 10% and 50% SA). Results showed Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Klebsiella pneumoniae* Carbapenem/ESBL are of greater risk factor in a SM environment. On the other hand, *S.aureus*, *M. smegmatis*, wild-type *K.pneumoniae*, *K.pneumoniae* ESBL, *K.pneumoniae* Carbapenem, *E. coli* and *P. aeruginosa* only seems to depend more on SA environment. Additionally, bacterial strains with AmpC resistance showed high risk to bacterial growth in environment enriched with SA. Overall, this study demonstrates that the kind of resistance and the environment in which each bacteria strain is present can contribute to the state of chronic inflammation.

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List of Abbreviations

AT	Adipose tissue
AMR	Antimicrobial Resistance
BMI	Body Mass Index
CAUTI	Catheter-associated Urinary Tract Infections
CFU	colony-forming unit
CLABSI	Central line-associated bloodstream infections
CVD	Cardiovascular disease
DMEM	Dulbecco's Modified Eagle Medium
ECDC	European Centre for Disease Prevention and Control
ESBL	Extended-spectrum β-lactamase
EARS-Net	European Antimicrobial Resistance Surveillance Network
HAI	healthcare-associated infection
IFN	Interferon
IL	Interleukin
INSEF	“Inquérito Nacional de Saúde com Exame Físico”
KPC	*Klebsiella pneumoniae* carbapenemase
LPS	Lipopolysaccharide
MCP-1	Monocyte chemoattractant protein-1
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
MUHO	Metabolically unhealthy obese
NTM	Montuberculous mycobacteria
NK	Natural killer
OD	Optical density
PAMPs	Pathogen-associated molecular pattern
PMN	Polymorphonuclear leukocytes
SA	Adipocyte secretome
SCAT	Subcutaneous abdominal adipose tissue
SFRP5	Secreted frizzled-related protein 5
SM	Macrophage secretome
SSI	Surgical site infections
T2DM2	Type 2 diabetes mellitus 2
TG	Triglycerides
TLR4	Toll-Like receptor 4
TNF	Tumor necrosis factor
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
UTI	Urinary tract infection
VAP	Ventilator-associated pneumonia
VAT	Visceral adipose tissue
WHO	World Health Organization

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I. Background

1. Obesity Epidemiology

According to the World Health Organization (WHO), obesity is defined by excess or abnormal accumulation of fat in adipose tissue. Due to the increased morbidity and mortality rate, this is recognized as a global epidemic (1). It is estimated that about 29% of the population in Portugal is obese (2). According to the INSEF (Inquérito Nacional de Saúde com Exame Físico) statistics from 2015, the prevalence of obesity among people aged between 25 and 74 in Portugal 28.6%, with a greater incidence among women (32.0%) than among males (25.0%). The prevalence of overweight (39.1%) was significantly higher in men (45.5%) than in women (33.2%) (3).

Due to its link to the rise in diabetes, cardiovascular disease, and cancer, obesity is viewed as a severe concern on a global scale (4).

There is an urgent need to find new strategies for the prevention and treatment of inflammation brought on by obesity, given the ineffectiveness of current therapeutic choices for obesity and their unfavorable side effects (4).

1.1. Adipose tissue (AT) and Obesity

Adipose tissue is considered an endocrine organ (5). This organ plays a key role in the regulation of energy homeostasis. According to the morphology, they can be classified into white, brown and beige adipocytes (6). In addition is made up of a variety of cell types and coordinately secrete a variety cytokines, chemokines and hormones (6).

The adipose tissue can be classified into two types: subcutaneous adipose tissue (SCAT) and visceral adipose tissue (VAT). During weight gain, excessive production of adipose tissue leads to a depletion of storage capacities. Additionally, it may result in ectopic lipid accumulation in skeletal muscle or liver tissue (7).

In obese patients, weight gain is common and there are higher amounts of triglycerides (TG) that will cause hyperplasia (increase in the number of adipocytes) and hypertrophy (increase in adipocyte size) (7). This expansion of tissue mass goes through a remodeling process characterized by the overproduction of the extracellular matrix, increased infiltration of immune cells and an elevated pro-inflammatory response (7).

Dysfunction at tissue adiposity is one of the hallmarks of obesity. In the initial phase, it is observed that the adipocytes are hypertrophic during a certain time of caloric excess (6). This in turn will secrete adipokines and these will recruit additional pre-adipocytes, which will then differentiate into mature adipocytes with the aim of “compensatory protection”

against the adverse metabolic consequences of obesity (6,8). In a situation of overloaded adipocyte recruitment and hypertrophy, ectopic locations (such as visceral deposits, liver, skeletal muscle, and pancreatic beta cells) are sites of fat accumulation leading to metabolically unhealthy obese (MUHO) (6). This is characterized by the state of inflammation, insulin resistance, and other features of metabolic syndrome. In this way, it can bring as consequence the increase of the risk of cardiovascular disease, since the accumulation of fat causes metabolic complications associated with obesity (6).

1.2. Chronic Inflammation of AT

In obesity, the presence of adipose tissue is associated with a state of chronic inflammation.

For a proper function of the immune system, it is important to find a balance between pro-inflammatory and anti-inflammatory factors. In addition, there is an increase in the number of macrophages (an increase of M1 and decrease of M2), reduced production of adiponectin and SFRP5 (secreted frizzled-related protein 5, anti-inflammatory molecules) and an increase of proinflammatory molecules (7). Among the pro-inflammatory molecules, leptin, interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) can be highlighted. For the physiological regulation of immunity, the two predominant adipokines are adiponectin and leptin (9).

Macrophages are crucial in the earlier stages of immunological defense against the presence of pathogens during infections. In addition, they have functions in the coordination of tissue development, tissue homeostasis (through the process of apoptosis), and signaling monitoring of changes that may occur in the tissue (10).

Macrophages can go through a polarization process that differentiates them into M1 and M2 phenotypes. The fight against intracellular infections is performed particularly by M1 macrophages. These macrophages are induced by T-helper (TH1) type of cytokines (such as interferon-γ (IFN-γ) or pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), and secrete cytokines (IL-1β IL-6, IL-12, IL-23, and TNF-α) (11). On the other hand, M2 macrophages have a strong phagocytic capacity, the ability to repair tissue, and immunosuppressive characteristics. They release growth factors including IL-10 and transform growth factor beta (TGF-β), extracellular matrix components, angiogenic and chemotactic factors, anti-inflammatory cytokines (IL-4 and IL-13), and other substances (11).

In obese patients, macrophages are positively correlated with body mass, adipocyte size, and expression of pro-inflammatory cytokines. The mechanisms that promote increased migration of macrophages to adipose tissue, are adipocytes' apoptosis, chemotactic

regulation, hypoxia, and the flow of fatty acids (7,12,13). According to *Saltiel* et al. (14), the polarization of anti-inflammatory type M2 macrophages to the form of M1 results in a phenotypic exchange (7,14). As a result, M1 promotes inflammation in the adipose tissue of obese individuals and causes the release of pro-inflammatory cytokines such as IL-1β, monocyte chemoattractant protein-1 (MCP-1), TNF-α, and IL-6 (15). Additionally, *Sun* et al. (13) hypothesized that the profile of tissue inflammation and the fate of adipocyte function is determined by a change in polarized macrophages. As a result, there is a significant correlation between body mass, adipocyte size, pro-inflammatory cytokine expression, and the number of macrophages (7).

The lack of adiponectin causes the displacement of macrophages from the anti-inflammatory phenotype M2 to the pro-inflammatory phenotype M1 leading to chronic polarization into M1 macrophages. In this way, through the release of cytokines, they will draw peripheral monocytes that will develop into M1 macrophages (9).

1.2.1. Cytokines

Cytokines are small proteins that are released (16) and play a role in the immune response during infection and inflammation (17). Interleukins, chemokines, interferons, and TNF-α are examples of cytokines (16).

Pro-inflammatory cytokines control inflammatory responses, whereas anti-inflammatory cytokines are made up of a group of immunoregulatory molecules that regulate the pro-inflammatory response (18).

Pro-inflammatory Cytokines

Leptin is expressed in adipose tissue (19) and plays a key role in regulating satiety, appetite, food intake, reproductive function, fertility, puberty, physical activity, energy expenditure and fetal growth (20). In obese individuals, leptin has a role to modulate natural killer (NK) cells that play a role in innate immune defense (21). Hyperleptinemia is one condition of the instability of the immune system, which occurs in obesity (19).

IL-6 is a cytokine, entailing physiological actions that regulate metabolism. The presence of this cytokine in healthy individuals has shown an increase in the elimination of glycolysis (through insulin), an increase in lipolysis, the oxidation of glucose and fatty acids and energy expenditure (22).

When IL-6 acts on adipose tissue, it increases leptin secretion, and suppresses satiety. In turn, it increases lipolysis of the tissue adipose, consequently stimulating hepatic gluconeogenesis and insulin resistance (22).

TNF-α is an acute/chronic inflammatory cytokine and is expressed in monocytes and macrophages. This cytokine plays a key role in inflammation and autoimmune disease (20). In addition, it is associated with adiposity, BMI (Body mass index), insulin levels and insulin resistance (6). Immunotherapy with these cytokine antibodies in several animal models demonstrated a reduction in inflammation, improved fatty liver disease and protected against diet-induced obesity- and insulin restriction (21).

Anti-inflammatory Cytokines

Adiponectin is secreted by adipocytes and plays a key role in obesity-related diseases such as insulin resistance/type 2 diabetes and cardiovascular disease. In addition, its serum levels decrease with obesity (23). Adiponectin appears to be negatively regulated by the pro-inflammatory cytokines TNF-α and IL-6, hypoxia and oxidative stress. An increase in proinflammatory markers of M1 macrophages and a decrease in proinflammatory markers M2 macrophages were observed in mice with adiponectin deficit (7).

IL-10 is produced by M2-like activated macrophages, B cells and T cells. It works by preventing the polarization of the M1 macrophage and certain pro-inflammatory cytokines' production, such as IL-1β, IL-6 and TNF-α (24).

There is a positive correlation between circulating IL-10 levels and the improvement of obesity and metabolic syndrome, where there is a decrease in BMI, the percentage of fat mass and an improvement in insulin resistance and inflammation of adipose tissue (24).

2. Antimicrobial Resistance (AMR)

Worldwide, antimicrobial resistance (AMR) represents a major threat to human health. A positive correlation between the high risk of inadequate treatment and infection was observed (25). This is a global threat and in WHO European Region. AMR is defined by the competence that a microorganism has to persist in the action of one or more antimicrobial agents (26). In Europe, it is estimated that AMR is responsible for 33,000 deaths every year (27). According to European Antimicrobial Resistance Surveillance Network's (EARS-NET) data from 2020, the most reported resistant reported bacterial species was *Escherichia coli* (*E. coli*) (41.3%), followed by *Staphylococcus aureus* (*S. aureus)* (21.9%), *Klebsiella pneumonia* (*K. pneumoniae)* (11.9%), *Enterococcus faecalis (E. faecalis)* (8.4%), *Pseudomonas aeruginosa* (*P. aeruginosa)* (6.2%), *Enterococcus faecium* (*E. faecium)* (5.5%), *Streptococcus pneumoniae* (*S. pneumoniae)* (2.6%) and *Acinetobacter* spp. (2.3%)(26). Compared to the previous year, there was a resistance increase in all bacterial species, except for *S. pneumoniae*. *K.pneumoniae* showed much more resistance to third-generation cephalosporins and carbapenems than *E.coli*. In addition, it was found that AMR varied and depended on the bacterial species, antimicrobial group, and geographic region (26).

2.1. β-Lactamases

The most used group of antibiotics is β-lactam. This group has in its structure a four-sided beta-lactam ring (28).

β-lactamases are enzymes that hydrolyze β-lactam antibiotics at a specific location, more specifically in the structure of the beta-lactam ring. The production of this enzyme is the most used resistance mechanism by gram-negative bacteria with β-lactam antibiotics (28). In addition, it consists of the mechanism of fundamental resistance against penicillin and cephalosporins antibiotics. These enzymes can be found on the bacterial chromosome or can be acquired by the plasmid. One example is the case of *Enterobacteriaceae* with β-lactamase chromosomal genes. *S.aureus*, *Enterococcus faecalis* and *Enterococcus faecium* are gram-positive bacteria that possess this enzyme (28).

For its classification, it is necessary to consider the molecular structure (classification of Amber) and its functional characteristics (Bush-Jacobi-Medeiros) (29). Cephalosporinases (group 1), serine β-lactamase and metallo (zinc dependents) β-lactamase consist of three functional groups, considering the specificity of the substrate. Taking into account the reasons composed by the primary sequence that constrict the protein molecule, there are four groups (A, B, C and D) in the Amber classification (29).

Extended spectrum β-Lactamase *(*ESBL*)*

ESBL are β-lactamases mediated by plasmids and belong to the group of serine β-lactamases in the Bush-Jacobi-Medeiros classification and group A of the Amber classification (30). In addition, they are characterized by their ability to hydrolyze penicillins and cephalosporins (31). Healthcare providers such as hospitals and community environments consist of the most common places to find ESBL-producing gram-negative pathogens. This type of enzyme is commonly found in Enterobacterales and *P.aeruginosa* (30).

AmpC β-Lactamases

AmpC β-lactamases are encoded by chromosomal genes (cAmpCs) and found in acquired plasmid mediated enzymes (pAmpC) (32). These enzymes belong to the group 1 (cephalosporinases) in the Bush-Jacobi-Medeiros classification and group C of the Amber classification (33).

The strains producing pAmpC may present multidrug-resistant phenotypes. This is due to the co-expression of multiple plasmid resistance to non-β lactams, such as quinolones, which limits the therapy options. However, Enterobacterales producing cAmpCs contain a high level of susceptibility to fluoroquinolones and aminoglycosides (32).

Carbapenemases

Carbapenamases consist of enzymes capable of hydrolyze most β-lactam antibiotics, as well as carbapenems (34). These enzymes confer resistance to carbapenem, penicillin and cephem antibiotics (35).

In addition, they can be classified into four classes of ß-lactamase: class A (such as the carbapenemase-producing *K.pneumoniae* (*bla*KPC)); class B Metallo-beta-lactamases (such as imipenemase (*bla*IMP); while class D carbapenemases (oxacillinase-group); and in class C ß-lactamase (cephamycin-hydrolyzing β-lactamase (*bla*CMY-10)) (36).

Plasmids that code for carbapenamases may have co-resistance genes that make them resistant to other b-lactam and non-b-lactam antibiotics (36). For example, *K.pneumoniae* carbapenemase (KPC), belonging to class A and cannot be inhibited by β-lactamases inhibitors such as clavulanate or tazobactam. However, they can be inhibited for example by avibactam, a non-beta-lactam (34).

2.2. Clinical relevance of bacterial infection and resistance

About 8.9 million as “healthcare associated infections” (HAI) occur every year in European intensive care hospitals and long-term care centres, according to the European Centre for Disease Prevention and Control (ECDC) (37,38).

Catheter-associated urinary tract infections (CAUTI), surgical site infections (SSI), central line-associated bloodstream infections (CLABSI), ventilator-associated pneumonia (VAP), and *Clostridioides difficile* infections are the most found HAI. Some bacterial pathogens, including *P. aeruginosa*, extended-spectrum beta-lactamase (ESBL)-producing and carbapenemase-producing Enterobacterales, *S.aureus* (including MRSA), *Mycobacterium tuberculosis*, nontuberculous mycobacteria (NTM), and others, can be found in these HAI (38).

Skin infection

The most common pathogens found in patients with cellulitis are *Streptococcus* and *S. aureus*. Cellulitis is a bacterial skin and soft tissue infection. This infection occurs in the immune system, circulatory systems, and the physical barrier of the skin when they are compromised. In a hospital environment, it is considered one of the most frequent infections to occur (39).

In this type of infection, another concern arises with the prevalence of pathogens with antimicrobial resistance, such as methicillin-resistant *S. aureus* (MRSA) (40).

Regarding the therapeutics for this type of infections, much is still unknown regarding the pharmacokinetics or pharmacodynamic characteristics of specific target organs for each of the antimicrobial agents. In this way, it becomes necessary to optimize prophylactic treatments effectively and to define properly which antibiotics to use in obese patients (40).

Respiratory tract infections

Obesity can compromise the pulmonary system since that in obese patients changes in lung mechanics can be observed (41). The reduction of lung volume, decreased compliance, ventilation and perfusion relationship and gas exchange, as well as inefficiency of the respiratory muscles, consist in alterations in the pulmonary mechanism observed in obese patients (42). In addition, the accumulation of fat in the thoracic and abdominal cavities causes the descending movement of the diagram and the external movement of the chest wall to be restricted (43).

Urinary tract infections (UTI)

It is estimated that about 11% of the general population is at risk of bacterial infection in the urinary tract, in outpatient and hospital settings. Studies have shown that obesity is a risk factor for UTI in certain conditions, such as admission to intensive care, moderate or major surgery, or after traumatic injury (44).

The mechanisms involved in the UTI susceptibility are not well defined. However, it is assumed that the reduced response of the innate immune system is involved in invasion by uropathogens (21,45). The decrease in the bactericidal functions of polymorphonuclear leukocytes (PMN) cells, together with neutrophils, which play a role in the antimicrobial defenses of the urinary tract, contributes to a reduction in the activation of TLR4 receptors present in the cell membrane, thus favoring bacterial invasion of the urinary tract (21,45).

Table 1 describes the general characteristics and type of infections that may be caused of bacteria *S. aureus*, *M. smegmatis*, *P. aeruginosa*, *E.coli* and *K. pneumoniae*.

Table 1: Summary general characteristics, local prevalence and clinical manifestations of strains

Strain	General characteristics	Local Prevalence	Clinical Manifestations	Reference
Staphylococcus aureus	Gram-positive; Commensal organism; Facultative intracellular pathogen; Virulence factors: colonization and formation of biofilms; secreted toxins.	Skin and soft tissue	Bone and joint infections; toxic shock syndrome; folliculitis; furuncles; mastitis; staphylococcal scalded skin and wound infections	(46–48)
		Respiratory tract	Pneumonia	
			Bacteremia; endocarditis, bone and joint infections	
Mycobacterium smegmatis	Gram-positive; Non-pathogenic mycobacteria; Inner cell membrane and a thick cell wall; Formation of biofilms	Environment	Bacteremia related to catheters (associated bloodstream infection in an immunocompetent patient); lymphadenitis;	(46,49,50)
Pseudomonas aeruginosa	Gram-negative; Aerobic; Bacillus; Virulence factors: pili; exotoxin A; pyocyanin; exoenzymes S, U, T, X; proteases; phospholipases C; siderophores; capsule; formation of biofilms	Environment niches	Infection (including hospital-acquired pneumonia, bloodstream and urinary tract infections) in hospitalized patients (compromised immune defenses)	(46,51–53)
Escherichia coli	Gram-negative; Facultative anaerobic; Coliform bacterium	Gut of humans and warm-blooded animals	Gastroenteritis; urinary tract infection; meningitis; hemorrhagic colitis and Crohn's disease	(46,54)
		Environment	Symptoms: severe abdominal cramps, diarrhea, hemorrhagic colitis, vomiting, sometimes fever, bowel necrosis and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, and septicemia	
		Food and untreated water		
Klebsiella pneumoniae	Gram-negative; Opportunist pathogen ubiquitous in nature; Virulence Factors: biofilm and this colonization on tissues	Environment	Blood-stream and urinary and respiratory tract infections	(46,55,56)

3. Infection and Obesity

Susceptibility to bacterial infections can occur due to increased level of neutrophils and mast cells, decreased number of eosinophils in adipose tissue, reduced capacity of bactericide PMN, the decrease in the number of activity of NK and accumulation of proinflammatory macrophages (M1) in adipose tissue (21). In addition, the physical condition associated with obesity, promotes an environment conducive to a bacterial infection.

There is clear evidence that obesity contributes significantly to infections, especially in the surgical procedure, in which obesity significantly increases skin and tissue infections. This occurs because in obesity there is a change in the function of the skin barrier, change of sebum production, and skin microcirculation (21).

Obese patients tend to crease which is favorable for friction and skin maceration, thus creating a favorable environment for bacterial and fungal growth. Folliculitis, furunculosis erysipelas, and necrotizing fasciitis are the most common bacterial skin infections in obese individuals (21). Moreover, obesity promotes other comorbidities, such as type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), hypertension, and metabolic syndrome (44). For example, T2DM wound healing is impaired, and this creates a favorable environment for fungal infections because it creates conditions for survival and proliferation, and skin folds, which in turn creates a favorable environment for the growth of bacteria (21).

4. Objectives

As already stated, obesity and antimicrobial resistance constitute health burdens with increasing evidence in the correlation between obesity and susceptibility to bacterial infections.

Taking these facts, the aim of this study was to understand how the inflammatory environment present in an adipocyte-enriched state influences the growth of clinically relevant bacteria strains. More specifically, this study aimed to dissect the role of macrophages' and adipocytes' secretome in the infection by antibiotic-resistant bacteria.

II. Materials and Methods

1. Bacterial strains and growth conditions

All bacterial strains (Table 2) were grown in Tryptic Soy Agar (TSA) medium (HIMEDIA ®, ref.:M290-500g) and Tryptic Soy Broth (TSB) medium (Liofilchem ®) for 24h at 37ºC. All clinical strains were obtained from Santa Luzia Hospital, Viana do Castelo, Portugal.

Table 2: Bacterial strains and their respective characteristics

Bacterial strains	Profile
Staphylococcus aureus	Methicillin-resistant (MRSA) ATCC 43300
	S.aureus ATCC 25923
Mycobacterium smegmatis	*M.smegmatis* CECT 3017
Klebsiella pneumoniae	*K. pneumoniae* ATCC 13883
	Extended-spectrum β-lactam-se (ESBL) producing *K. pneumoniae*
	K.pneumoniae carbapenamase (KPC)
	Extended-spectrum β-lactamase and carbapenemase producing *K.pneumoniae*
Escherichia coli	*E.coli* ATCC 25922
	Extended-spectrum β-lactamase (ESBL) producing *E.coli*
	AmpC *E.coli*
Pseudomonas aeruginosa	PAO1 ATCC 15692
	Extended-spectrum β-lactamase (ESBL) producing *P.aeruginosa*
	AmpC *P.aeruginosa*

2. Bacterial Susceptibility

The antibiotics' susceptibility profile were determined with Vitek®2 (bioMérieux®,Marcy-l'Étoile, France) automated system, using appropriate ID GN cards (bioMérieux®, Marcy-l'Étoile, France) and the respective antibiograms are shown in Support Information (SI).

3. Determination of colony-forming unit (CFU)/mL

Each strain was cultured in TSA medium for 24h at 37ºC. Afterwards, a colon was added to 3 mL of sterile TSB medium for 3 hours, at 37ºC with shaking. The optical density (OD) of bacterial suspension was measured at 600 nm (OD_{600}) using the Thermo Scientific™ Multiskan SkyHigh Microplate Spectrophotometer. OD_{600} was calculated and adjusted to 0.1. After that, serial dilutions in a factor of 10 were prepared up to 10^4 and, 10µL of each dilution was inoculated on Ø35 mm plates containing TSA medium and incubated overnight at 37ºC. The CFU/mL was determined using the formula.

$$\frac{CFU}{mL} = \frac{no.of\ colonies\ x\ dilution\ factor}{volume\ of\ culture\ plate}$$

4. 3T3-L1 cell differentiation and adipocyte secretome (SA)

To obtain adipocyte secretome (SA), a protocol already implemented by the group was followed (Figure 1).

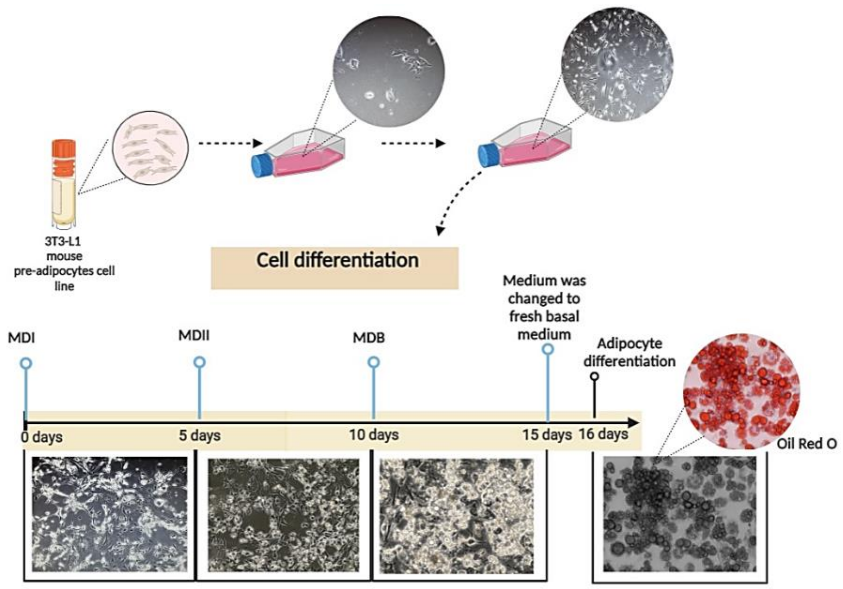

Figure 1: 3T2-L1 differentiation and adipocyte secretome (SA) production

Briefly, 3T3-L1 mouse pre-adipocytes cell line (American Type Culture Collection, ATCC® CL-173 ™) were first, expanded in Dulbecco's modified Eagle's medium (DMEM, with 4.5 g/L glucose and L-Glutamine, P0103, VWR, Biowest,Nuaillé, France) supplemented with 10% Newborn Calf Serum (NCS, Biowest) and incubated at 37ºC in a humidified chamber

containing 5% CO_2. After 24h, the medium was removed, the cells were washed with 1 mL PBS and DMEM with NCS was added. When a confluence of 70% was reached, cells were washed with PBS and the Differentiation Medium I (DMI) was added (day 0). After five days, the medium was switched to Differentiation Medium II (DMII). On day 10, the medium was switched again to basal medium (BM). On day 15, the BM was removed and new BM was added. After 24h, on day 16 adipocyte secretome was collected into 15 mL sterile tubes and stored at -80ºC.The composition of each medium is present in Table 3. In order to assess the influence of SA in bacterial growth, DMEM with increased concentrations of SA were prepared. Specifically, DMEM without SA, DMEM with 10%SA and DMEM with 50%SA.

Table 3: Culture Mediums' Composition

Culture Mediums	Composition
Differentiation Medium I (DMI)	DMEM 0,25 µM Isobutylmethylxanthine (IBMX) 2 µM Insulin 1 µM Dexamethasone 10% Fetal Bovine Serum (FBS) 1% P/S Pioglitazone
Differentiation Medium II (DMII)	10%FBS 2% µM Insulin
Basal Medium (BM)	DMEM

5. Raw 264.7 cells and macrophage secretome (SM)

The mediums described in 2.2. (DMEM, DMEM with 10% SA and DMEM with 50%SA) were used as culture medium for Raw 264.7 cells. The protocol is shown in Figure 2. Briefly, after washing with PBS, the cells, in M1 differentiation, were cultured at 37ºC in a humidified chamber containing 5% CO_2 and the macrophage secretome (SM) of each condition was then collected. The 6 mediums (DMEM, DMEM_10%SA, DMEM_50%SA, SM, SM_10%SA and SM_50%SA) were used in the following experiments as distinct conditions for bacterial growth assessment.

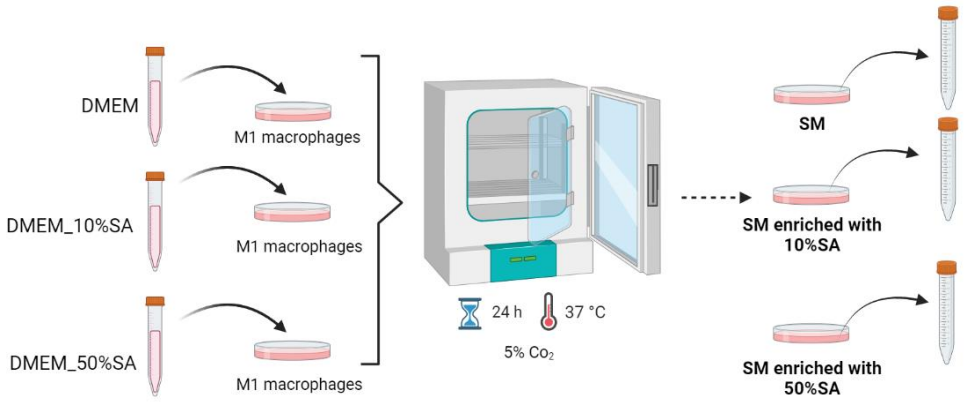

Figure 2: Preparation of conditioned media in contact with Raw 264.7 cells

6. Effect of bacterial growth curves in medium condition

After bacteria were grown in Petri dishes with TSA medium, one colony of each strain was collected and inoculated in sterile tubes with 3mL TSB overnight. The suspensions' absorbance at 600 nm (OD_{600}) was determined and adjusted to OD_{600}=0.1. After that, the suspensions were centrifuged at 1000 rpm for 5 minutes and the supernatant was removed. The pellet was resuspended in 3 mL of sterilized distilled water. Afterwards, 5 µL of each strain was inoculated in 45µL of each medium condition (obtained in 2.2. and 2.3.) in a 384-well plate. TSB was used as a control medium to ensure strain viability. All assays were performed in triplicate. The plate was incubated at 37ºC, and bacterial growth was assessed by absorbance measuring at 600 nm (OD_{600}) using the Thermo Scientific™ Multiskan SkyHigh Microplate Spectrophotometer. The kinetic protocol was used with readings every hour over a period of 5 days without shaking. At the end of the assays, all conditions were cultured in TSA plates to assess bacterial viability and cross contamination check.

7. Effect of bacterial growth of *E.coli* group in obese vs lean human serum

Blood collection tubes (BD Vacutainer, ref. 36783) were used for the collection of human blood from two male patients between 35-45 years, after each subject had given their written informed consent (This study was approved by the hospital ethics committee-227/2018-2 CHVNG/E, with Dr.Joana Rigor as the responsible investigator. No human cells were used, other than commercially available cell lines). Then, were divided based on BMI (Body mass index). Following that, blood samples were centrifuged for 10 minutes at 4ºC at 4.000 rpm, and the serum supernatant was collected. The bacterial

sample was prepared as described in 2.4. Then, 10 µL of each strain was inoculated in 90 µL of each serum and plasma sample in a 96-weel plate. DMEM was used as a control medium to ensure strain viability. All assays were performed in triplicate. The plate was incubated at 37 ºC and bacterial growth was assessed by absorbance measuring at 600 nm (OD_{600}) using the Thermo Scientific™ Multiskan SkyHigh Microplate Spectrophotometer. The kinetic protocol was used with readings every hour over a period of 5 days without shaking.

8. Statistical analysis

Statistical analysis was determined using the GraphPad Prism 9 software. The analysis was performed by comparing the mean of triplicates for each condition using the two-way ANOVA with Tukey's multiple comparisons test method for a 95% confidence interval.

III. Results

1. Effect of different medium conditions on bacterial growth curves

To evaluate how a chronic inflammatory environment can influence bacterial growth, 13 bacteria strains were selected. These strains were obtained from clinical context and chosen based on their prevalence regarding respiratory, gastrointestinal and urinary tracts. As such, the bacteria were cultured in secretome obtained from macrophages, adipocytes or both, as well as in TSB as positive control. The respective growth curves were monitored after each 24h, for a total of 5 days. As a transversal initial point, all strains were cultured in TSB until an OD of 0,1 was obtained and the respective CFU was determined (Table 4).

Table 4: Optical Density of 0,1 (OD_{600}) of bacterial strains

Bacterial strains and profile	CFU/mL
Methicillin-susceptible *S.aureus* (MSSA)	4,69 x10^7
Methicillin-resistant *S.aureus* (MRSA)	3,86 x10^7
M.smegmatis	2,31 x10^7
wild-type *K.pneumoniae*	2,27 x10^7
K.pneumoniae Carbap	3,95 x10^7
K.pneumoniae ESBL	1,75x10^7
K.pneumoniae Carbap/ESBL	3,15 x10^7
wild-type *E.coli*	1,80x10^7
E.coli AmpC	3,32x10^7
E.coli ESBL	3,57x10^7
P.aeruginosa strain PAO1	1,32 x10^7
P.aeruginosa ESBL	1,05 x10^7
P.aeruginosa AmpC	2,66 x10^7

Staphylococcus aureus

Results regarding the behaviour of both strains' growth under conditioned mediums are shown in Figure 3.

When comparing the growth rates of Methicillin-susceptible *S.aureus* (MSSA) in the different medium conditions, DMEM enriched with 10% and 50% of SA showed a decrease with statistical significance when compared to DMEM in the first 24h (p <0,05). Parallelly, when cultured in SM, the presence of 50%SA allowed this strain to remain in

the log phase up until 96h of incubation, reaching the stationary phase with a population density significantly higher (p <0,05) than any other medium conditions.

Performing the same analysis for Methicillin-resistant *S.aureus* (MRSA), the SM medium seems to be favorable for this strain's proliferation. Moreover, in SM medium enriched with 50% SA, growth rate is lower. When no SM medium is present, the enrichment of DMEM with SA provided increased density with the increase of SA percentage. Interestingly, an enrichment of 50% of SA in DMEM versus an enrichment of 50% of SA in SM medium provided similar curves up until 72h, where the population in SM showed an increased growth rate, with statistical significance (p<0,05) by day 4th.

Comparing both strains, results show that, overall, the addition of SM medium seems to have a beneficial effect on bacterial growth. Moreover, in both strains, the SM medium enriched with SA at 50% seems to create a phase of bacterial adaptation allowing an increased growth rate before the 72h time-point.

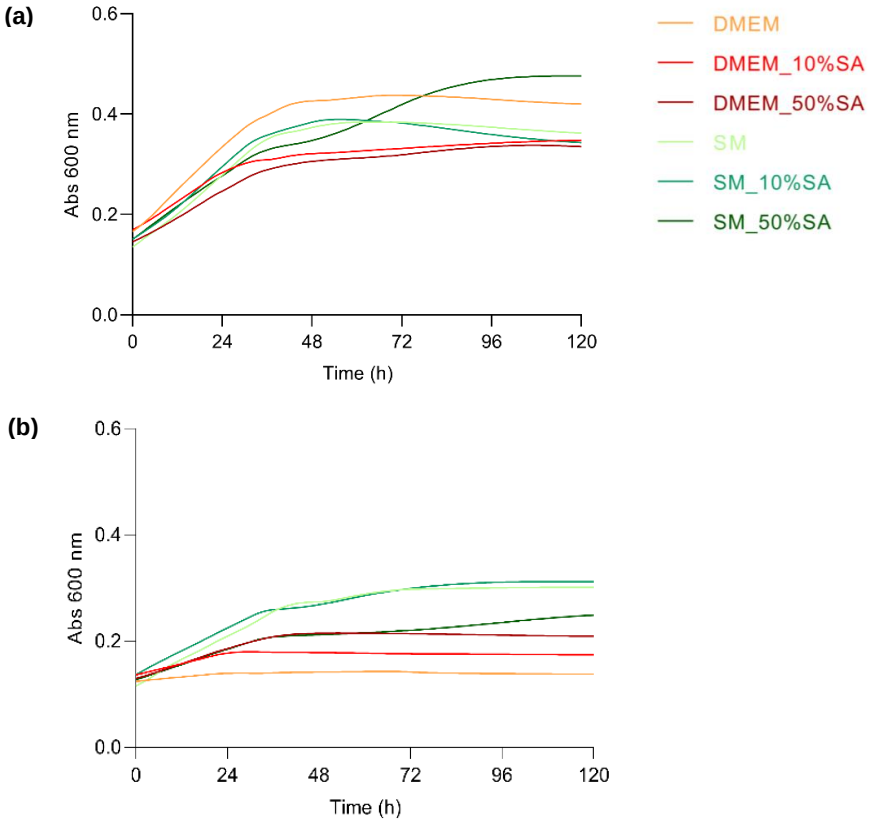

Figure 3: Growth curve of Methicillin-susceptible *S. aureus* (MSSA) (a) and Methicillin-resistant *S.aureus* (MRSA) (b) over 5 days. DMEM. Dulbecco's modified Eagle's medium; SA: adipocyte secretome; SM: macrophage secretome.

Mycobacterium smegmatis

In Figure 4 is shown the growth curves for this strain. The conditions of the DMEM medium enriched with 10% and 50% of SA reached stationary phase after 24h of incubation, remaining at constant rate for the following 26h. After that time, growth rates started to increase up until the last time-point measured.

In SM medium, the stationary phase was only reached after 32h. When comparing growth rates in different medium conditions, SM medium enriched with 50% of SA seems to benefit bacterial proliferation in the first 48h. However, the population in DMEM medium showed an increased growth rate, with statistical significance (p <0,05) until the 5th day.

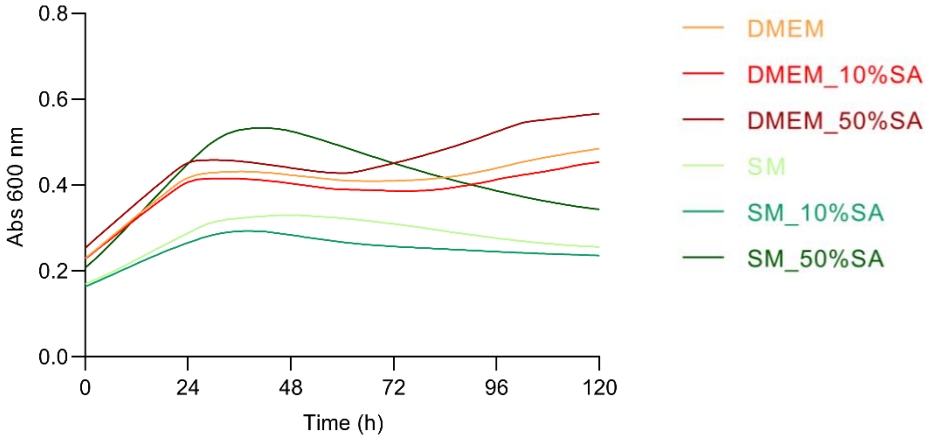

Figure 4: Growth curve of *M.smegmatis* over 5 days. DMEM: Dulbecco's modified Eagle's medium; SA: adipocyte secretome; SM: macrophage secretome.

Klebsiella pneumoniae

Comparing the growth rates of wild-type strain in the different medium conditions (Figure 5a), the bacteria showed the maximum growth in DMEM medium enriched with 10% and 50% of SA. However, no statistically significant difference between these two SA concentrations were observed. The same trend was observed for SM with different SA concentrations.

In case the *K.pneumoniae* Carbap, results for SM medium followed the same trend seen in the wild-type strain of this group. However, DMEM medium showed a decrease in bacterial growth in DMEM medium enriched with 10% and 50% of SA (Figure 5b).

K.pneumoniae ESBL showed similar growth rate to wild-type strain in the first 24h, with DMEM medium and DMEM medium enriched with 10% and 50% of SA favoring this

strain's proliferation (Figure 5c). However, unlike the wild-type strain, SM medium with 50% of SA showed an increased growth compared SM medium and SM medium with 10% of SA, with a statistical significance (p<0,05).

Concerning *K.pneumoniae* Carbap/ESBL (Figure 5d), the growth behavior showed patterns similar to the ones observed in *K.pneumoniae* Carbap. DMEM medium enriched with 10% and 50% of SA showed lower growth rate with no statistical significance between different SA concentrations. Inversely, SM medium showed an increased growth rate of 33%, regardless of SA percentage. DMEM medium versus SM medium showed a statistical significance (p<0,05) from 2th day.

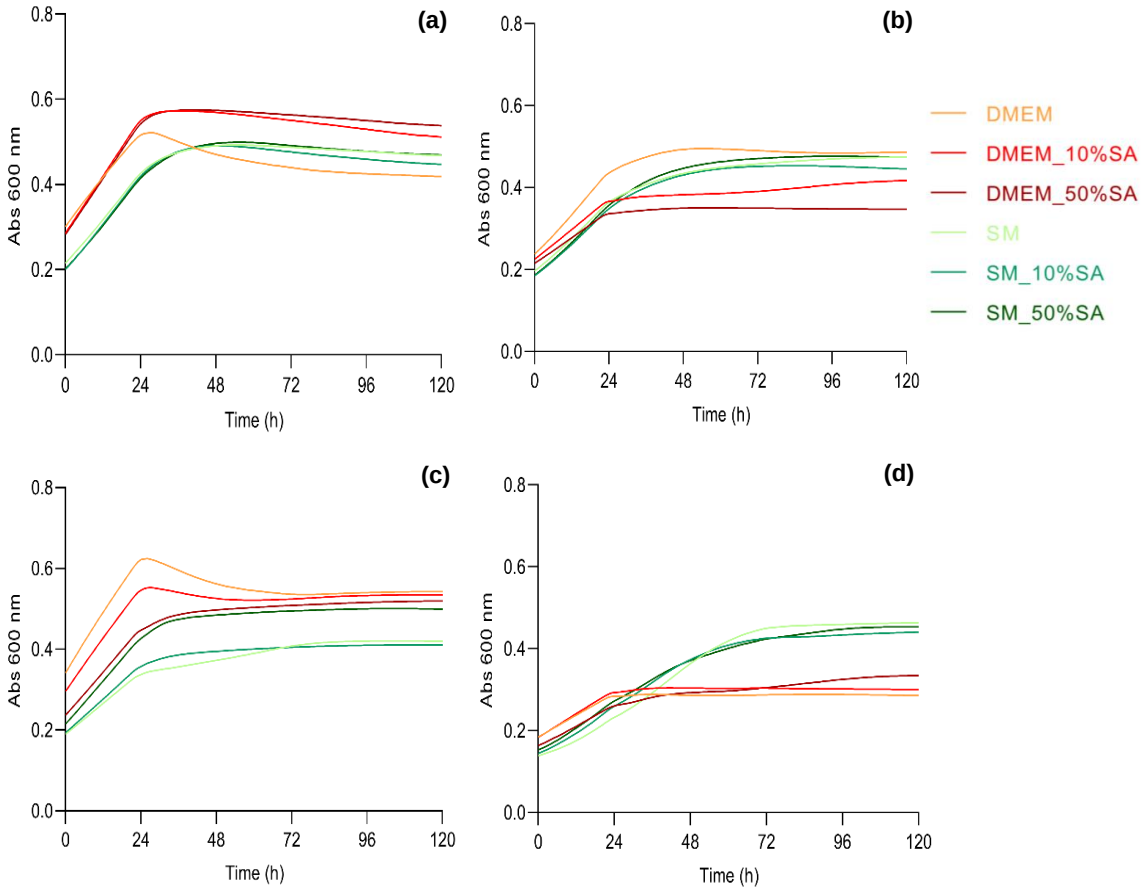

Figure 5: Growth curve of wild-type *K.pneumoniae* (a), *K.pneumoniae* Carbapenem (b), *K.pneumoniae* ESBL (c) and *K.pneumoniae* Carbapenem/ESBL (d) over 5 days. DMEM: Dulbecco's modified Eagle's medium; SA: adipocyte secretome; SM: macrophage secretome.

Escherichia coli

For the *E.coli* group, comparing the growth rate of wild-type strain in different medium conditions (Figure 6a), DMEM medium showed 30% increased growth density in comparison to SM medium. DMEM medium enriched with 10% SA showed no statistically significant difference from DMEM but DMEM enriched with 50% SA showed a 20% decrease in population density. In SM medium, the presence of SA promoted a favourable growth rate, increasing with the increase of SA concentration.

For *E.coli* ESBL (Figure 6b), DMEM medium showed a decrease of 15%, with statistical significance, in bacteria growth compared DMEM enriched with 10% and 50% of SA in the first 24 h (p<0,05). Parallelly, when inoculated in SM medium, the addition of SA by 10% allowed this strain to extend the log phase for more 20h.

In the case of *E.coli* AmpC (Figure 6c), results showed similar results to those observed in the wild-type strain of this group, with DMEM medium enriched with SA showing significantly higher population density than in SM medium. However, unlike for the wild-type strain, *E.coli* AmpC, the SM medium enriched with 50% of SA favored bacterial growth, showing growth rate close to those observed for DMEM with SA.

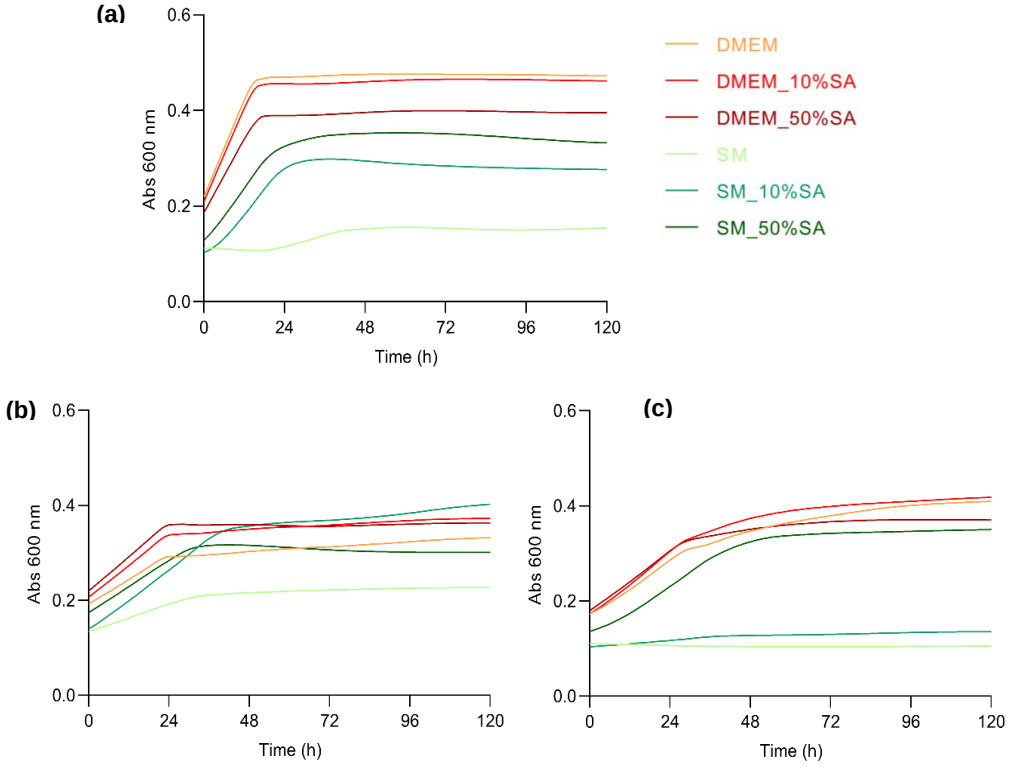

Figure 6: Growth curve of wild-type *E.coli* (a), *E.coli* ESBL (b) and *E.coli* AmpC (c) over 5 days. DMEM: Dulbecco's modified Eagle's medium; SA: adipocyte secretome; SM: macrophage secretome.

Pseudomonas aeruginosa

For strain PAO1 (Figure 7a), bacteria growth in all DMEM conditions showed similar trend, with population density higher for SM conditions at 24h time-point. In particular, SM medium enriched with SA provided a more favourable condition for growth density.

In *P.aeruginosa* ESBL (Figure 7b), similar growth trend was observed, with SM mediums, regardless SA percentage, and DMEM with 50%SA showing higher growth density. In contrast, DMEM enriched with 10% of SA showed lower bacterial growth, with statistical significance compared to the other mediums at 24h (p <0,05). Out of all mediums, DMEM with 50% SA and SM with 10%SA were the most favourable conditions for this strain's growth. After 36h of incubation, *P.aeruginosa* ESBL entered the death phase in all conditions, much like what was observed for PAO1.

For *P.aeruginosa* AmpC (Figure 7c), and unlike the other two strains in this group, DMEM medium, regardless the SA concentration allowed this strain to maintain in a stationary phase from 24h time-point and up until day 5. SM medium, in comparison to DMEM medium, allowed this strain to maintain in log phase for an additional 24h, with 20% increase in growth density for SM enriched with SA. However, unlike what was observed for DMEM medium, in SM mediums the death rate began by the 50h time-point, showing statistical significance compared to DMEM medium (p <0,05) at the 72h.

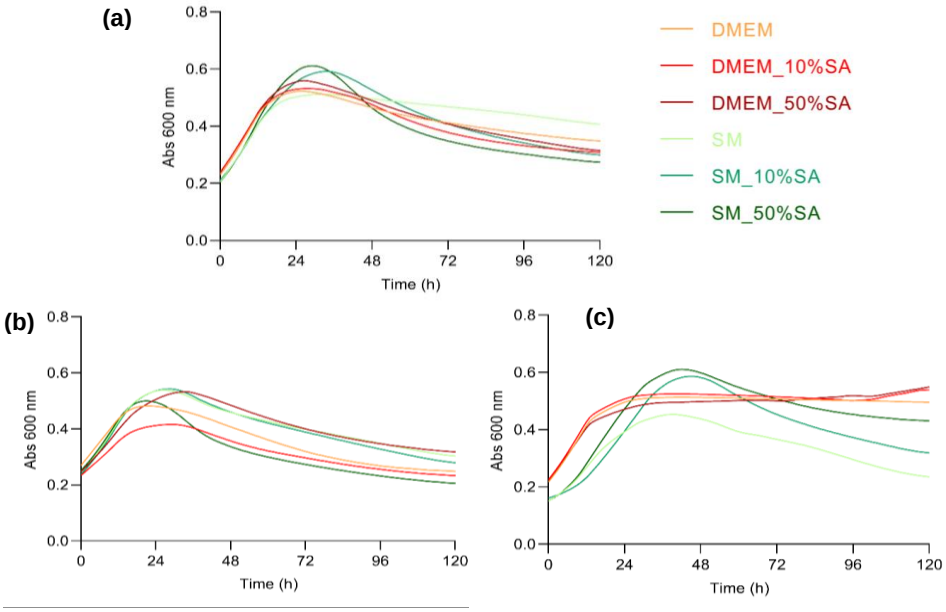

Figure 7: Growth curve of *P.aeruginosa* strain PAO1 (a), *P.aeruginosa* ESBL (b) and *P.aeruginosa* AmpC (c) over 5 days. DMEM: Dulbecco's modified Eagle's medium; SA: adipocyte secretome; SM: macrophage secretome.

2. Effect of bacterial growth of *E.coli* group in obese vs lean human and serum

Next, and considering results observed for *E.coli* strains, human serum from individuals representative of an obesity state and a lean state were selected as new nutritional mediums. The assay was conducted using the same protocol previously stated.

Regarding wild-type *E.coli*, results showed that serum from obese patient benefit bacterial growth in the first 24h (Figure 8a). However, by the 36h time-point, growth rate in the obese medium started to decrease entering the death phase. In contrast, in serum from lean patient this strain maintained under lag phase for the first 3 days, after which the log phase started and maintained up to day 5.

In contrast to wild-type strain, growth curves of *E.coli* ESBL showed faster adaptation to the lean serum, peaking population density around 30h time-point. For the obese serum, results were similar to those observed in the wild-type strain.

E.coli AmpC provided distinct results from the other two *E.coli* strains, as in both serums a death phase can be observed.

Overall, the three strains showed similar growth profile for the obese serum, reaching the peak of the log phase around the first 24h. For the lean serum, however, while for wild-type it took three days to adapt to the medium, the clinical strains adapted at similar rate for both serums, obese and lean.

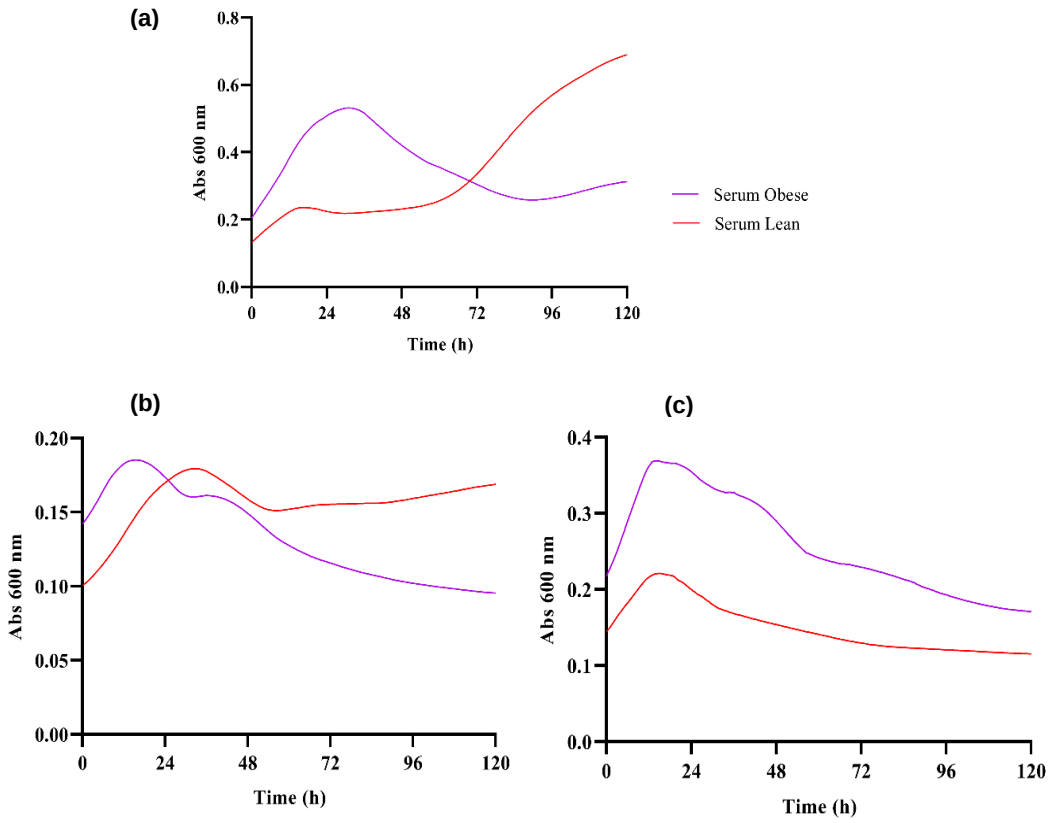

Figure 8: Growth curve of wild-type *E.coli* (a), *E.coli* ESBL (b) and *E.coli* AmpC (c) over 5 days in human serum obtained from obese or lean individuals.

IV. Discussion

Obese individuals are susceptible to developing HAI, such as skin and soft tissue infections; bacteriemia; urinary tract infections and mycosis; respiratory infections, and infections at the surgical site (44). This can occur due to the state of chronic low-grade inflammation, hyperglycemia, hyperinsulinemia, and hyperleptinemia (21). The adipose tissue, with the function of energy storage, is constituted of adipocytes, macrophages, and blood vessels. When weight gain occurs, there is hyperplasia and hypertrophy of adipocytes, as well as chemotactic regulation, hypoxia, and fatty acid flow, which can lead to adipocytes apoptosis. In turn, these four mechanisms will provide increased migration of macrophages in adipose tissue (7).

The alarming ability of bacteria to gain resistance has raised concerning obstacles in antimicrobial therapies in patients with chronic inflammation, such as obese individuals.

Thus, this study aimed to understand how the inflammatory environment, such as the one present in an obesity state, influences the growth of bacteria strains with clinical relevance. Considering that gram-positive bacteria have an outer homogeneous layer of peptidoglycan while the gram-negative bacteria display lipopolysaccharides (LPS) instead, it was hypothesized if this structural difference would influence the extent of the infection. Therefore, among the 13 strains under study, there were two gram-positive, and three gram-negative strains with distinct antibiotic resistance profiles.

The present study started by differentiating 3T3-L1 mouse adipocytes cells, to obtain the adipocyte secretome (SA). After some literature research, the induction of the cell line Raw 264.7 (macrophages) was performed through a polarization process by LPS induction, at a concentration known in the literature (10 ng/mL), to promote polarization into M1 macrophages (57). Then, DMEM medium and DMEM medium enriched with 10% and 50% of the SA were used as a medium for the cells to mimic the obesity environment as well as macrophage secretome (SM) and also conditioned with 10% and 50% of SA. In this secretome it was hypothesized the presence of IL-1β, IL-6, IL-12, IL-23, and TNF-α, since macrophages M1 release these cytokines (11).

The secretome of the cell lines Raw 264.7 (macrophages) and 3T3-L1 (adipocytes) were collected to serve as conditioned media for bacterial growth. The strains were exposed to conditioned media with increased content (DMEM, DMEM with 10% and 50% SA, as well as SM, also conditioned with 10% and 50% of SA). These different conditions were used to mimic different degrees of obesity states as well as the immune response to an inflammatory environment. Bacteria growth curves assessment was performed by absorbance measurement in a kinetic mode for a period of 5 days.

When comparing Methicillin-susceptible *S.aureus* (MSSA) with Methicillin-resistant *S.aureus* (MRSA), MRSA seemed to be more adapted to a chronic inflammatory environment than *S.aureus*, as shown in Figure 3. MRSA showed a higher growth in SM medium enriched with 10% and 50% of SA. Results reveal that SM medium and SA contribute positively to the bacterial growth of MRSA promoting its adaptation to an inflammatory environment. In a study it was shown that IL-1β promotes the formation of *S.aureus* biofilms along with increased neutrophils adjacent to biofilm-coated pumps (58). Considering the presence of IL-1β cytokine in macrophage secretome, MRSA could be adapted to a chronic inflammatory environment due to biofilm formation.

The *M.smegmatis,* a non-pathogenic mycobacterium, seems to be better adapted to SA enrichment medium than to a SM medium. However, comparing all conditions, SM medium enrichment with 50% of SA seems to enhance the growth of *M.smegmatis* until 40h. Then, a phase of death was observed and that may be due to the depletion of nutrients in the medium. Evidence was demonstrated regarding macrophages infected by *M.smegmatis* that showed a decrease in the production of cytokines such as TNF-α, IL-1β and IL-6 when compared to the control (59). Such evidence supports this study results, where lower bacterial growth in the SM medium and SM medium enriched with 10% of SA was observed. In general, the microenvironment of the adipocyte secretome is favorable to the growth of this strain.

In the case of *K.pneumoniae,* results showed wild-type strain seems to be insensitive to the presence of SA, while SM seems to affect the growth rate. In the case of *K.pneumoniae* Carbapenems showed higher population in the mediums with SM, regardless the percentage of SA, while the ESBL strain presented higher population in the mediums with SA without SM. When considering both resistances (carbapenems and ESBL), results showed a greater risk factor than resistance to extended spectrum cephalosporins in a SM environment. *K.pneumoniae* is a gram-negative pathogen, responsible for community-acquired pneumonia in individuals, that has been acquiring antibiotic resistance over the years (60). One of the main characteristics of *K.pneumoniae* strains is the colonization and formation of biofilms. These abilities confer protection against antibiotics and host immune system (60). In addition, the response to environmental fatty acids entails implications for the metabolic and pathogenic potential of *K.pneumoniae* (61). One study explored the ability of *K.pneumoniae* to assimilate and respond to exogenous fats. This found that *K.pneumoniae* is able to assimilate exogenous fatty acids into its membrane phospholipids, as well as effects of fatty acids in various phenotypes important for their survival and virulence (61). In addition, biofilm formation

seems to be affected depending on supplemented fatty acid (61). Siderophore production has been described as a cause of inflammation and bacterial spread during lung infection (62). The specific combination of siderophores secreted by *K.pneumoniae* can affect tissue localization, systemic dissemination and host survival during infection (63). This study suggest the *K.pneumoniae* with resistance Carbapenemase, strain possess higher adaptability to a SM environment perhaps by forming biofilm, allowing the observed bacterial growth.

E.coli is a gram-negative responsible for HAI pneumonia, urinary tract infections and diarrhea (46,54). This study showed that, in SM medium, the presence of SA medium counteracts the inhibitory effect of SM medium for wild-type *E.coli*, probably by serving as the main carbon source. In contrast, *E.coli* ESBL is less sensitive to SM medium, still benefiting from the presence of SA medium. In the case of *E.coli* AmpC, SA medium favors bacterial growth. Interestingly, SM medium enriched with 50% of SA is the one that best mimics the condition of obesity. This can occur due to the inflammatory response and high fat rate. Our results corroborate a study on the impact of exposure to pro-inflammatory cytokines on the virulence of an Uropathogenic *E.coli*, where it was found that TNF-α, IL-1β, IL-6, IL-8 and IFN-γ induced increased bacterial growth (68). Considering that, in the SM medium, there is the presence of cytokines such as IL-1β, IL-6 and TNF-α, these appear to serve as stimulating growth factors. In addition, the presence of fat also contributes to this growth, especially in strains wild-type and *E.coli* AmpC, which may induce chronic inflammation.

For *P.aeruginosa*, our results showed PAO1 and *P.aeruginosa* ESBL showed that different mediums do not seem to interfere with growth of these strains. In contrast, for *P.aeruginosa* AmpC results showed that this strain, in the presence of SA, represents a higher risk over time. The fact that it was facing an environment rich in nutrients allows this strain with this resistance to remain viable for longer periods of time, unlike in SM medium. *P.aeruginosa* is a gram-negative bacteria and an opportunistic implicated in skin and lung diseases (64). This strain also causes sepsis, frequently colonizing the lung and gut, displaying alarming antibiotic resistance (53), associated with the ability to activate several mechanisms to survive the host, such as the formation of biofilms (65). Biofilm formation is associated with bacterial infections, which are characterized by severe and progressive chronic inflammation (66). In addition, glucose is shown to have multiple effects on bacterial growth and biofilm formation, as a source of carbon and a metabolite (67). This information corroborates with the results obtained for the strain *P.aeruginosa* AmpC, from which the environment rich in SA provided greater bacterial growth.

Regarding the SM medium, the cytokines present in the medium seem to be contributing to the decrease of bacterial growth. Future studies are required to understand this association.

In order to understand if these results would translate similarly in real samples, *E.coli* strains were studied in the human serum of individuals representative of the state of obesity and a state of lean. It was observed that obese serum allowed higher growth rate in the first 36h of infection in wild-type strain, while the remaining strains reached higher growth rate before 24h. Overall, an obese state seems to be favorable to an infection context. The results obtained corroborate the association between obesity and bacterial infection, especially in the first hours of infection. Several studies have suggested that, in both children and adults, obesity is associated with increased risk of infection (69). Obesity is linked to altered cytokine synthesis, decreased antigenic response and decrease in function of NK cells, dendritic cells and macrophages. The way adipose tissue is distributed can influence the risk of infection due to the immunomodulatory effects of the secretion of cytokines and adipocytokines (69).

When comparing the patterns between gram-positive and gram-negative strains, at first glance the presence of the peptidoglycan layer does not seem to promote any particular adaptation to the surrounding environment.

Overall, *S.aureus*, *M.smegmatis*, wild-type *K.pneumoniae*, *K.pneumoniae* ESBL, wild-type *E.coli*, *E.coli* AmpC and *P.aeruginosa* AmpC, commensal bacteria appear to be favored by obesity, MRSA and *K.pneumoniae* Carbapenem/ESBL by inflammation, and PAO1 and *P.aeruginosa* ESBL by both, suggesting that obesity is indeed a factor for the development of bacterial infections in various contexts.

V. Conclusion

In general, this preliminary study showed an influence of an obesity-mimicking environment and bacterial resistance. It is likely that this influence is due to not only by the variation on the nutrient availability but also by the virulence factors that contribute to the persistence of certain bacterial infections in individuals with the condition of obesity.

Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Klebsiella pneumonia* with resistance to carbapenems are of greater risk factor in an inflammatory environment. On the other hand, *Mycobacterium smegmatis*, *Escherichia coli* and *Pseudomonas aeruginosa* seem to benefit more of a lipid and glucose-rich environment. Additionally, bacterial strains with AmpC resistance showed higher risk of infection in an adipose-enriched environment.

In clinical practice, treatment against infections caused by these bacterial strains is still conducted empirically, following the guidelines. It is only when the treatment is not responsive that the antibiogram becomes part of the diagnosis. The present study represents a valuable contribution regarding standard clinical practice since, at least for patients with obesity it could be highly beneficial to surpass the empirical guidelines and start with an antibiogram to avoid persistent infection from bacterial agents with high virulence factors. This change in clinical practice could help avoid the complications associated with persistence infection and even contribute to a more personalized medical practice in the long run, where treatment is adapted to the patient's specific needs, such as it is seen in cancer therapy with the use of antibodies.

Nevertheless, more studies are needed in order to achieve such goals, namely:

i. Understanding if there is a key component in each of the studied mediums that promote bacterial growth;
ii. Understand the mechanisms of virulence;
iii. Perform the same experiments with key antibiotics to understand if the susceptibility of the strains in the mediums where low growth was observed has changed;
iv. Perform co-cultures with macrophages and the studied bacterial strains to study how the infection in real-time influences both immune response and bacterial survival.

These studies will allow to further understand the role of key molecules in the inflammatory process during a bacterial infection and contribute to finding alternatives to current infection treatments.

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Support Information

Nome do Microrganismo	KLEPNEP	*Klebsiella pneumoniae*
Marcadores de Resistência	ALERTA	Microrganismo Alerta
	CARBA	Produtor de carbapenemase de classe A

Nome do Teste	**Nº Isol**	**Resultado**
Quantificação (UFC/mL)		>100.000
Ex.Bact.Cultural		POSITIVO
NMIC-502	1	Concluído
ATB Gram Negtivos (Enterobacteriaceae)	1	COMPLETO

Fármaco	**CIM/Conc**	**SIR**
Amicacina	<=4	S
Amoxicilina-Clavulanato (f)	>32/2	R
Ampicilina	>8	R
Aztreonam	>16	R
Cefalexina	>16	R
Cefepima	16	R
Cefixima	>2	R
Ceftazidima	>8	R
Ceftazidima-Avibactam	4/4	S
Ceftriaxona	>4	R
Cefuroxima	>8	R
Ciprofloxacina	>1	R
Colistina	<=0.5	X
Ertapenem	>1	R
Fosfomicina com G6P	<=16	S
Gentamicina	1/16	S
Imipenem	8	R
Levofloxacina	>2	R
Meropenem	>8	R
Piperacilina	>64	R
Piperacilina-Tazobactam	>64/4	R
Tobramicina	<=1	S
Trimetoprim-Sulfametoxazol	20/320	R

S(Sensível); R(resistente)

Nome do Microrganismo	KLEPNEP	*Klebsiella pneumoniae*
Marcadores de Resistência	ALERTA	Microrganismo Alerta
	ALERT1	Produtor de carbapenemase
	ESBL	Beta-lactamase de Largo Espectro

Nome do Teste	Nº Isol	Resultado
Pesq. De Aerobios		POSITIVO
BACTEC 9000 Lytic/10 Anaerobic/F		Positivo
Pesquisa de Anaerobios		NEGATIVO
NMIC/ID-402	1	Concluído

Fármaco	CIM/Conc	SIR
Amicacina	<=4	S
Amoxicilina-Clavulanato (f)	>32/2	R
Ampicilina	>8	R
Cefepima	>8	R
Cefotaxime	>4	R
Ceftazidima	>8	R
Cefuroxima	>8	R
Ciprofloxacina	>1	R
Colistina	<=1	X
Ertapenem	>1	R
Fosfomicina com G6P	32	S
Gentamicina	>4	R
Imipenem	>8	R
Levofloxacina	>2	R
Meropenem	>8	R
Piperacilina	>16	R
Piperacilina-Tazobactam	>16/4	R
Tobramicina	>4	R
Trimetoprim-Sulfametoxazol	>4/76	R

S(Sensível); R(resistente)

Nome do Microrganismo	KLEPNEP	*Klebsiella pneumoniae*
Marcadores de Resistência	ALERTA	Microrganismo Alerta
	CARBA	Produtor de carbapenemase
	ESBL	Beta-lactamase de Largo Espectro

Nome do Teste	Nº Isol	Resultado
Ex.Bact.Cultural		POSITIVO
NMIC-502	1	Concluído
NMIC/ID-402	1	Concluído

Fármaco	CIM/Conc	SIR
Amicacina	<=4	S
Amoxicilina-Clavulanato (f)	>32/2	R
Ampicilina	>8	R
Aztreonam	>16	R
Cefalexina	>16	R
Cefepima	8	R
Cefixima	>2	R
Cefotaxime	<=1	S
Ceftazidima	>8	R
Ceftazidima-Azibactam	1/4	S
Ceftriaxona	>4	R
cefuroxima	>8	R
Ciprofloxacina	>1	R
Colistina	<=1	X
Ertapenem	>1	R
Fosfomicina com G6P	64	R
Gentamicina	<=1	S
Imipenem	8	R
Levofloxacina	>2	R
Meropenem	4	I
Piperacilina	>64	R
Piperacilina-Tazobactam	>64/4	R
Tobramicina	<=1	S
Trimetoprim-Sulfametoxazol	<=1/19	S

S(Sensível); R(resistente)

Nome do Microrganismo	PSEAER06	*Pseudomonas aeruginosa*

Nome do Teste	Nº Isol	Resultado
NMIC/ID-402	1	Concluído

Fármaco	CIM/Conc	SIR
Amicacina	<=4	S
Amoxicilina-Clavulanato (f)		R
Ampicilina		R
Cefepima	>8	R
Cefotaxime		R
Ceftazidima	>8	R
Cefuroxima		R
Ciprofloxacina	>1	R
Colistina	<=1	X
Ertapenem		N
Imipenem	2	S
Levofloxacina	>2	R
Meropenem		S
Piperacilina	16	S
Piperacilina-Tazobactam	16/4	S
Tigeciclina		R
Tobramicina	<=1	S

S(Sensível); R(resistente)

Nome do Microrganismo	PSEAER02	*Pseudomonas aeruginosa*

Nome do Teste	**Nº Isol**	**Resultado**
NMIC/ID-402	2	Concluído

Fármaco	**CIM/Conc**	**SIR**
Amicacina	<=4	S
Amoxicilina-Clavulanato (f)		R
Ampicilina		R
Cefepima	2	S
Cefotaxime		R
Ceftazidima	4	S
Cefuroxima		R
Ciprofloxacina	<=0.25	S
Colistina	<=1	X
Ertapenem		R
Imipenem	2	S
Levofloxacina	<=0.5	S
Meropenem	0.5	S
Piperacilina	8	S
Piperacilina-Tazobactam	8/4	S
Tigeciclina		R
Tobramicina	<=1	S

S(Sensível); R(resistente)

Nome do Microrganismo:	ECOLI	*E.coli* AmpC
Carta:	AST-N215	

Fármaco	CIM/Conc	SIR
Ampicilina	<=2*	*R
Amoxicilina-Ácido clavulânico	<=2	S
Piperacilina/Tazobactam	16	R
Cefuroxima	8	I
Cefuroxima Axetil	8	S
Cefpodoxima	>=8	R
Ceftazidima	>=64	R
Meropenem	<=0.25	S
Gentamicina	<=1	S
Tobramicina	<=1	S
Ciprofloxacina	<=0.25	S
Norfloxacina	<=0.5	S
Tetraciclina	(-)	(-)
Nitrofurantoína	<=16	S

S(Sensível); R(resistente); *= Modificação do AES; (-)= Não se encontra o teste de sensibilidade