From food to hospital: we need to talk about Acinetobacter spp.

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Abstract

Some species of the genus *Acinetobacter* are admittedly important hospital pathogens. Additionally, various animal and plant foods have been linked to the presence of *Acinetobacter*, including resistant strains. However, due to isolation difficulties and the lack of official standard methods, there is a dearth of work and epidemiological data on foodborne diseases caused by this microorganism. Considering that *Acinetobacter* spp. may represent a serious public health problem, especially because of their resistance to carbapenems and colistin, and because of the fact that these pathogens may transfer resistance genes to other bacteria, studies are needed to evaluate the pathogenicity of both food and clinical isolates and to search for them using control strategies, such as the adoption of more efficient disinfection measures and use of antimicrobial substances (AMS). In contrast, AMS production by strains of the genus *Acinetobacter* has already been described, and its potential for application against other Gram-negative food or clinical pathogens, reveals a new field to be explored.

Keywords Acinetobacter spp., antibiotic resistance, infections, food contamination, antimicrobial substances.

Introduction

The Acinetobacter genus is composed of unpigmented, oxidase-negative, coccobacillus-shaped microorganisms. From this last characteristic comes the etymology of the genus name (from the Greek "acinetus" meaning "which does not move," and from the Latin "bacter", meaning bacillus). So far, 63 species within this genus have been described, and most

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Article downloaded from www.germs.ro Published September 2020 © GERMS 2020 ISSN 2248 - 2997 ISSN - L = 2248 - 2997 of them are non-pathogenic environmental organisms.

Microorganisms belonging to this genus were initially considered opportunistic commensals, that is, with low virulence and little clinical significance. However, in recent decades, infections caused by *Acinetobacter* spp. have increased in frequency and severity with the expansion of intensive care units, more frequent use of mechanical ventilation equipment and venous catheters, and especially the increased use of antibiotics.²⁴

Most reported infections involving Acinetobacter are associated with the species A. baumannii, followed by A. calcoaceticus and A. lwoffii. 5.6 Other species, such as A. haemolyticus, A. johnsonii, A. junii, A. nosocomialis, A. pittii, A. schindleri and A. ursingii, have occasionally been reported as pathogens. However, both clinical data analysis and animal model studies show that A. baumannii is the most virulent species. 6.7

Although A. baumannii is not considered a community pathogen, it can cause community-acquired bronchiolitis and tracheobronchitis in children and immunocompromised individuals. Pneumonia associated with this species has also been reported⁸ in conjunction with underlying conditions such as alcoholism, diabetes mellitus

and smoking in tropical regions of Australia and Asia.⁹

A. baumannii is implicated in invasive procedure-related bloodstream infections, such as catheter use, and in patients undergoing neurosurgery. This species is a serious problem as it can cause meningitis and other infections, resulting in a high mortality rate. While it is known that A. baumannii can be transmitted from one patient to another by formulas, sinks, doors, feeding tubes and even medical equipment, the origin of the infection remains unknown in many cases. 9,10

As mentioned earlier, A. baumannii is one of the major pathogens associated with infections and often exhibits antibiotic resistance. Recently, it has been reported that rates of A. baumannii infection have been rising, even exceeding those of Pseudomonas aeruginosa, which is ranked first among non-fermenting pathogenic bacteria amongst China's large population. 11,12

A. baumannii's ability to resist different antibiotics is giving clinical recognition to this species, especially after the emergence of multidrug resistant (MDR) strains and even panresistant strains. 12,13 The frequency and severity of public health problems associated with MDR strains has caused the Infectious Diseases Society of America (IDSA) to include A. baumannii among the ESKAPE (acronym for Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa, and Enterobacter) species, which cause the majority of nosocomial infections in the United States and worldwide and can bypass the action of various antibiotics. 14,15

Recently, ESKAPE group pathogens have been listed by the World Health Organization (WHO) within the 12 bacteria for which search for new antibiotics needs to be urgently pursued. ^{16,17} Among the bacteria on this list are those of critical priority, such as A. baumannii resistant to carbapenem antibiotics or CRAB (carbapenem-resistant Acinetobacter baumannii). Carbapenems are bactericidal antimicrobials, with proven efficacy against infection-causing bacteria that produce extended spectrum β-lactamase (ESBL). ¹⁸ The most common are

imipenem, meropenem, doripenem and ertapenem. Thus, bacteria resistant to carbapenems pose a serious threat. Based on the studies by Spellberg and Rex, 19 it is estimated that approximately 22,950 cases of CRAB infections occur in the United States annually and 75,000 cases occur worldwide, resulting in roughly 4,590 deaths in the United States and over 15,000 deaths worldwide.

CRABs currently constitute the majority of resistant A. *baumannii* strains and the WHO estimates that carbapenem resistance rates exceed 90% in some regions of the world.²⁰ Moreover, the antibiotics of choice for treating CRAB are lackluster due to some of their pharmacokinetic properties and general propensity to be resisted,²¹ such as colistin.

Colistin belongs to the class of polymyxins and is active against a wide range of Gramnegative bacteria. This antibiotic is used in both human and veterinary medicine. In humans, it is generally used to treat infections caused by multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria.²² Resistance to colistin is conferred by the mcr genes that are often found in plasmids, facilitating their spread among bacteria. It has been reported that bacteria isolated from animals and food products derived from them carry the mcr genes more frequently than bacteria isolated from humans. 22,23 This fact is probably due to the selective pressure exerted by the widespread use of colistin in veterinary practice.²³

Since it is nephrotoxic to humans, colistin has been increasingly used only as a last resort. The resistance to colistin expressed by Enterobacteriaceae family and A. baumannii was included in the World Health Organization's Global Antimicrobial Resistance Surveillance System (GLASS). With this scary scenario, it is necessary to search for new options.

Other factors contributing to the antibiotic resistance of *Acinetobacter* spp. are the production of biofilm and the ability to acquire and transfer resistance genes. Biofilms are accumulations of microorganisms, associated with a certain surface and surrounded by a self-produced polymeric matrix, that work as a cooperative consortium,

resulting in increased protection for these microorganisms.^{24,25} Biofilm production helps members of the genus *Acinetobacter* in adhesion, colonization and infection of the host epithelial cells, and contributes to the development of infections associated with invasive medical devices.²⁵

Some studies report that prevalence of antibiotic resistance in biofilms is higher than in planktonic cells or that the biofilm formation is more strongly associated with MDR isolates than with susceptible isolates. In Iran, a study of clinical isolates of A. baumannii associated with hospital infections showed that 92% of the biofilm-forming isolates were MDR.26 Similar results were observed by Bardbari and coworkers, ²⁷ aiming to determine the correlation between the ability of biofilm formation and the antibiotic resistance phenotypes in clinical and environmental A. baumannii isolates. The authors could verify a significant correlation between biofilm formation and multiple drug resistance, and also detected that clinical isolates presented a higher ability to form strong biofilms compared to the environmental isolates.

However, it is not clear that there is a quantitative correlation between biofilm formation and antibiotic resistance. Qi and colleagues,²⁸ for example, evaluated relationship between antibiotic resistance and biofilm formation by A. baumannii isolates from several hospitals in China, including MDR and extensively drug-resistant (XDR) isolates. Biofilm production was verified in more than 90% of the isolates. The authors reported that isolates with the highest level of antibiotic resistance formed the weakest biofilms, which still provides a level of protection for the isolates, while isolates with the lowest level of resistance were strong biofilm producers. The results of this study suggest that biofilm acts as a mechanism that provides better survival, especially in isolates with a level of resistance not so high.²⁸ Regardless of being directly or indirectly associated with antibiotic resistance, the biofilm production is added as another factor to the virulent potential of A. baumannii.

Gene transfer mechanisms have already been described for members of the genus *Acinetobacter*. Mobile genetic elements (MGEs), such as insertion sequences (IS), transposons, integrons, resistance islands and plasmids, have often been associated with multi-resistance to antibiotics in *Acinetobacter*. ^{11,29}

The resistance genes present in these elements can be disseminated by horizontal transfer, involving mechanisms such as conjugation, transformation and transduction.³⁰ In Gram-negative bacteria, conjugation seems to be the main transfer mechanism in *Acinetobacter* spp., and the transfer of resistance genes mediated by MGEs as transposons and conjugative plasmids has been reported in several studies.^{29,31-33}

A. baumannii, however, is the most studied Acinetobacter species and shows a considerable capacity to acquire foreign drug resistance genes, becoming a species with great genetic diversity and capable of overcoming the pressure of antibiotic selection.²⁹ This high plasticity of A. baumannii provides the accumulation of several determinants of resistance, thus contributing to the high incidence of multidrug resistance.^{29,32,34} Undoubtedly, the mechanisms underlying gene transfer are essential factors for the spread of antibiotic-resistant Acinetobacter from both clinical and food environments.

Acinetobacter spp. relation with food

Deficient attention to hygiene practices during food production or processing can cause contamination by different pathogenic or spoilage bacteria, among them, members of the *Acinetobacter* genus. Some studies suggest that proteolytic and lipolytic enzymes produced by *Acinetobacter* spp. and other bacteria from dairy foods could beneficially contribute to the taste, odor or texture of the product. ^{35,36}

However, other reports describe the species of *Acinetobacter* as potential pathogens and are concerned about their detection in foods, including samples of bovine milk, goats and dairy products. The presence of pathogenic *Acinetobacter* species in meat for human consumption has been reported by Marí-Almirall

and coworkers; 40 although the authors did not find multiresistant strains in their study, they report the risk that this may pose to public health, as these foods may act as potential reservoirs for the spread of Acinetobacter in the human population. Klotz and colleagues, 41 for instance, isolated carbapenem-resistant A. indicus strains in cattle and made an important observation: despite the low pathogenicity of this species, these isolates may contribute to the dissemination of resistance genes to other bacterial species and also to the environment through manure, contributing the contamination of vegetables used for human consumption.

Acinetobacter spp. can also be easily introduced into the hospital environment through kitchens or by food brought in by visitors, a behavior observed in Portugal⁴² and that also occurs in Brazil. Some studies report the presence of Acinetobacter spp. in hospital kitchens and in common food, ^{39,43-46} however the evidence that these microorganisms are foodborne pathogens has not yet been proven. ^{38,42}

Some reports raise suspicions of this transmission. A. *baumannii* has recently been associated with a case of enterogenic sepsis, initially diagnosed as acute gastroenteritis by symptoms such as nausea, diarrhea and vomiting, not initially associated with *Acinetobacter*.⁴⁷ There are other studies that also report the association of *Acinetobacter* with gastroenteritis.^{48,49} However, because it is not caused by a classic food pathogen, there is a possibility that this type of *Acinetobacter* infection occurs more often than has been reported, but its mode of transmission is still controversial.

In previous work by our group, we argued that the scarcity of infection data generated by Acinetobacter spp. from food intake may be a result of the difficulty of isolating these microorganisms from food sources since standard methods are lacking.³⁸ The food route, therefore, needs to be considered as a source of dissemination of Acinetobacter strains, including those resistant to antibiotics. This concern is corroborated by Cho and coworkers,⁵⁰ who suggest that when these strains enter food or

community-based clinical settings, they may acquire new antibiotic resistance genes and result in new, particularly aggressive strains.

In 2019, a study conducted in the Czech Republic also highlights the possibility of transmission of pathogenic *Acinetobacter* strains from food to humans. The authors investigated the occurrence of plasmids carrying the *mcr*-4.3 gene, which confers resistance to colistin, in isolates of *A. baumannii* from frozen turkey liver imported from Brazil and from a clinical sample. The comparative analysis highlighted the common origin of plasmids, suggesting that meat imported from Brazil could be a route of entry for colistin-resistant *Acinetobacter* to Czech Republic.⁵¹

It is also noteworthy that although they are not held as classic food pathogens, strains of *Acinetobacter* spp. can be extremely harmful to immunocompromised people, wherein simpler foods such as milk, meats and even vegetables can serve as an out-of-hospital reservoir for these bacteria.

Can we take advantage of *Acinetobacter* food isolates?

Today, food safety is a key concern for consumers, regulators and the food industry. The increased morbidity caused by foodborne diseases, along with their economic implications, contribute to ongoing efforts to produce safer food and the development of new, desperately needed antimicrobial agents.⁵²

Like other organisms, bacteria are also capable of producing antimicrobial peptides, which act in a competitive niche against their competitors. These compounds can be purified and subsequently used by the food industry as tools to protect against bacteria that cause spoilage in their products, thereby extending their shelf life and maintaining product characteristics without changing the sensory properties of foods. 44,54 These substances are essential for the food industry particularly since antibiotics cannot be used in these products. 55

Bacteriocins are ribosome-synthesized peptides and the main known antimicrobial

substances (AMS). They are produced by some bacteria that can inhibit the growth of other undesirable microorganisms. The bacteriocin like inhibitory substances (BLIS), on the other hand, are AMS also produced by bacteria that inhibit microorganisms in the same way as bacteriocins, but which have not yet had their active component characterized.⁵³

Little has been described about the production of AMS by bacteria of the genus Acinetobacter and studies reporting this feature are associated with food. In 2015, our group detected three representatives of the Acinetobacter baumannii-calcoaceticus complex, capable of producing antimicrobial substances against the indicator strains Escherichia coli ATCC25922 and Salmonella enterica ATCC19214.⁴⁴ To our knowledge, this was the first report of AMS production by this bacterial group.

Recently, in another study, our group found that five utensil isolates associated with the preparation of infant milk formulas in a hospital, three from Acinetobacter baumannii-calcoaceticus (JE3, JE6 and JE4) and two from Enterobacter cloacae (ME1 and BIE1), inhibited food pathogens such as Bacillus cereus and Salmonella enterica serotype Typhi, as well as Klebsiella pneumoniae, Proteus mirabilis and P. vulgaris. The JE6 isolate, however, was able to inhibit a larger number of indicators, showing efficacy in inhibiting antibiotic multidrug Acinetobacter baumannii-calcoaceticus and Shigella dysenteriae isolates in addition to resistant and multiresistant strains isolated from dairy products.56

Mary and coworkers⁵⁷ also described AMS production by a strain of Acinetobacter baumannii isolated from raw buffalo milk, which showed inhibitory activity against Staphylococcus epidermidis, S. aureus, Shigella flexneri and Yersinia enterocolitica.

Even recognized as classic pathogen, Acinetobacter can present potential for biotechnological application in the food area. Pathogens such as Staphylococcus aureus, for example, produce a range of well-characterized bacteriocins (aureocins and staphylococcins) that have action against several Gram-positive

bacteria, highlighting their action against other staphylococci and against *Listeria monocytogenes*. ^{58,59} The evaluation of the potential applications in foods, as alternatives to chemical preservatives, could also be performed using partially or totally purified AMS preparations, instead of the AMS-producing strains.

Resistant bacteria have been frequently isolated from different types of foods, evidencing the role of the food chain on the transmission of antibiotic resistance, and the production of antimicrobial substances by isolates Acinetobacter begins to emerge as a new tool these against microorganisms. Although preliminary, studies involving the production of AMS by Acinetobacter isolates are promising, since the majority of bacteriocins produced by Grampositive bacteria, and which are the most studied, hardly inhibit Gram-negative bacteria.⁵⁶ In addition to MDR isolates, the AMS produced by Acinetobacter spp. also inhibited important classic foodborne pathogens such as S. enterica, S. dysenteriae and E. coli, encouraging the studies of these substances against pathogens, especially those associated with food, which may also have important clinical implications.

Conclusions

Undoubtedly, A. baumannii is one of the major pathogens associated with nosocomial infections and opportunistic infections. Even with the use of broad-spectrum antibiotics and the popularization of procedures and interventions, it is still difficult to prevent their spread in the hospital.

However, some authors raise the issue that Acinetobacter may be an opportunistic food pathogen, as its association with food has increased steadily and antibiotic resistant strains have also been detected. The scarcity of data on infections generated by Acinetobacter spp. from food intake make more accurate research difficult.

In line with what was raised by Wang and colleagues, ¹² only with rapid detection of antibiotic resistance, with strict guidance on the rational use of antibiotics in clinical practice and with the adoption of efficient disinfection

measures, will it be possible to reduce the emergence and spread of antibiotic resistant A. baumannii strains in the hospital setting.

Similarly, some studies have shown that the production of AMS by *Acinetobacter* is a feature that should be explored, especially in the area of food, as it may contribute to the control of pathogens and in the future even strains of the genus *Acinetobacter* that are resistant to antibiotics.

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