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MICROBIOLOGY

Occurrence and antimicrobial resistance of bacteria in retail market spices

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ABSTRACT: *This study aimed to evaluate the microbiological quality and the transmission of multidrug-resistant bacteria in different spices sold in town fairs (local food markets) in the municipalities of Recôncavo Baiano. Samples of black pepper, oregano, and cinnamon were collected over a period of six months and investigated for coliforms at 45 °C, Staphylococcus spp., Staphylococcus aureus, Bacillus spp., Bacillus cereus, Escherichia coli and Salmonella spp. The contamination in the black pepper samples (log 4.66 CFU g^{-1}) was higher (P>0.05), than those of cinnamon (log 2.55 CFU g^{-1}) and oregano (log 2.49 CFU g^{-1}), particularly for B. cereus. E. coli (89%) and Salmonella spp. (67%) were isolated only from black pepper. B. cereus and S. aureus showed greater resistance to β-lactams (penicillin, oxacillin, and cefepime), with approximately 40% of the strains with a multiple antimicrobial resistance (MAR) index of 0.33 (i.e., resistant to three antimicrobials). E. coli was more resistant to ampicillin and Salmonella spp. to nalidixic acid, ampicillin, and ceftriaxone. Salmonella spp. had a MAR index ranging from 0.16 to 0.91 (i.e, resistant to up to 11 antimicrobials), and E. coli to up to 0.58 (i.e., resistant to 7 antimicrobials). In conclusion, the spices sold in the town fairs of Recôncavo Baiano are of low microbiological quality, with the presence of pathogens, of which some display high resistance to antimicrobials that are commonly used for treating foodborne illnesses.*
Key words: *spice plants, antibacterial activity, multiresistance.*

Ocorrência e resistência antimicrobiana de bactérias em especiarias comercializadas no varejo

RESUMO: *Este estudo teve como objetivo avaliar a qualidade microbiológica e a veiculação de bactérias multirresistentes em diferentes especiarias comercializadas em feiras livres nos municípios do Recôncavo Baiano. Foram analisadas amostras de pimenta-do-reino, orégano e canela durante seis meses e pesquisados coliformes a 45 °C, Staphylococcus spp., Staphylococcus aureus, Bacillus spp., Bacillus cereus, Escherichia coli e Salmonella spp. A contaminação nas amostras de pimenta-do-reino (log 4,66 UFC g^{-1}) foi maior (P>0,05), quando comparado com as amostras de canela (log 2,55 UFC g^{-1}) e orégano (log 2,49 UFC g^{-1}), principalmente para B. cereus. E. coli (89%) e Salmonella spp. (67%) foram isoladas apenas na pimenta-do-reino. B. cereus e S. aureus apresentaram maior resistência aos* β*-lactâmicos (penicilina, oxacilina e cefepime), com cerca de 40% das cepas com índice MAR de 0,33 (resistência a 3 antimicrobianos). E. coli foi mais resistente a ampicilina e Salmonella spp. ao ácido nalidíxico, ampicilina e ceftriaxona. Salmonella spp. apresentou índice MAR variando de 0,16 a 0,91 (até 11 antimicrobianos), e E. coli até 0,58 (7 antimicrobianos). Com isso, as especiarias comercializadas nas feiras livres do Recôncavo Baiano apresentam baixa qualidade microbiológica, com presença de patógenos e elevada resistência a antimicrobianos comumente usados no tratamento de enfermidades transmitidas por alimentos.*
Palavras-chave: *plantas condimentares, atividade antibacteriana, multirresistência.*

INTRODUCTION

Worldwide, spices are used in food preparation due to their flavoring properties. Spices are rarely free from microbial contamination, with the exception of those that undergo industrial processing. One of the major problems associated with the marketing of spices in the outdoor retail trade is the lack of adequate infrastructure and poor sanitary conditions (FOGELE et al., 2018). Despite the low water activity, which inhibits microbial multiplication, dry herbs and spices are natural products that can be contaminated with various microorganisms, including pathogenic and toxigenic species (SZÉKÁCS et al., 2018).

Although, several guidelines are available to meet the standards required for the production, processing, and use of spices (BRASIL, 2001; EC,

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2004), these products register the highest number of outbreak notifications of foodborne disease, according to the European rapid alert system for food and feed (RASFF, 2019). In Brazil, spices are among the 16 foods most highly involved in food-borne diseases, with an occurrence rate of 0.9%, which can be considered low, due to low notification. Among the microorganisms most involved in the outbreaks are *Escherichia coli, Salmonella* spp., *Staphylococcus aureus, Bacillus cereus*, and coliforms (BRASIL, 2019).

The increase in microbial resistance has had serious consequences for public health, restricting therapeutic options to combat the development of pathogenic bacteria (SEMRET & HARAOUI, 2019), including bacteria that spread through the food chain. Accordingly, the consumption of raw spices might contribute to the spread of resistant strains, because in the human gastrointestinal tract, resistance genes can be transferred to the commensal microorganisms of the host microbiota. The objective of this study was to evaluate the microbiological quality and potential spread of multidrug-resistant strains through spices sold in town fairs, in the municipalities of Recôncavo Baiano.

MATERIALS AND METHODS

The samples were collected from February to July 2018 at town fairs in Cruz das Almas, Santo Antônio de Jesus, and Cachoeira, in the Recôncavo da Bahia. It was selected spices that were consumed uncooked and are important to regional cuisine. Accordingly, we collected nine samples of black pepper (*Piper nigrum* L.), cinnamon (*Cinnamomum* sp.), and oregano (*Origanum vulgare* L.), marketed in bulk. At each fair, 100 g of the spice from the stalls of five vendors was obtained, for assessing the abundance of *Staphylococcus* spp., *S. aureus*, *Bacillus* spp., *B. cereus,* coliforms at 45 ºC as well as the presence of *E. coli* and *Salmonella* spp. The analyses were performed using the techniques recommended in the Bacteriological Analytical Manual (BAM) described by SILVA et al. (2010). The selected microorganisms are of food importance and are commonly reported to be implicated in food outbreaks, according to the Ministry of Health of Brazil (SINAN, 2018).

Antimicrobial susceptibility test was performed using the disk diffusion method (CLSI, 2014), with 32 strains of *E. coli*, 20 of *Salmonella* spp., 72 of *B. cereus,* and 34 of *S. aureus*. The following nine antimicrobials were selected against Gram-positive bacteria: penicillin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), sulfazotrim (25 µg), cefepime (30 µg), erythromycin (15 µg), clindamycin (2 µg), oxacillin (1 µg) and chloramphenicol (30 µg). Twelve antimicrobials were selected against Gram-negatives bacteria, as follows: nalidixic acid (30 µg), ceftriaxone (30 µg), imipenem (10 µg), sulfazotrim (25 µg), tetracycline (30 µg), ampicillin (10 µg), ceftazidime (30 µg)), amikacin (30 µg), aztreonam (30 µg), chloramphenicol (30 µg), gentamicin (10 µg) and nitrofurantoin (300 µg).

The multiple antimicrobial resistance (MAR) index was calculated using the following formula: MAR index = a/b, where (a) is the number of antimicrobials, to which the isolate was resistant and (b) refers to the number of antimicrobials to which the isolate was exposed (KRUMPERMAN, 1983).

The values of the variables [most probable number (MPN g^{-1}) and colony forming unit (CFU g^{-1})] of the microbiological analyses were transformed into log (x + 1) and the data were submitted to analysis of variance in a completely randomized design. The means were compared using the Tukey test ($P<0.05$). Univariate analyses were performed using the Sisvar Software program, version 5.6. Principal component analysis (PCA) was also performed using cluster analysis, according to the agglomerative algorithm of Ward's method combined with Euclidean distance measurement using SPSS Statistics for Windows, version 25.0 (IBM CORP, 2017).

RESULTS AND DISCUSSION

There was no statistical difference in the microbial count of spices between the municipalities, which may be associated with common environmental factors in the three municipalities, such as temperature of 23 ºC to 24 ºC and humidity ranging from 60% to 90% (SEI, 2012).

The contamination in black pepper was higher ($P>0.05$) than in cinnamon and oregano, considering each of the tested microorganisms (Table 1). In the three spices, the *B. cereus* count was higher, mainly in pepper; although, without statistical difference for *Bacillus* spp. The coliform group at 45 ºC had the lowest average count (Table 1).

According to Brazilian legislation, the acceptable limit for coliforms at 45 ºC, in spices, is log 2.69 MPN g^{-1} (BRASIL, 2001); whereas, for *B. cereus,* it is log 4 CFU g^{-1} (EC, 2004). Of the spices studied, only black pepper had counts exceeding these limits. Although, *B. cereus* is part of the microbiota in herbs and spices (BANERJEE & SARKAR, 2004), its presence at > log 5 CFU g^{-1} can cause various diseases due to the presence of diarrheal and emetic toxins (FOGELE et al., 2018).

Staphylococcus spp. and *S. aureus* also showed significantly higher counts in black pepper

Table 1 - Average microbial count (log x + 1) CFU g^{-1} and MPN g^{-1} of bioindicators in positive spice samples collected from town fairs in Recôncavo Baiano, Brazil.

Microorganisms	------------------------Spices------------------------			
	Black pepper	Cinnamon	Oregano	Overall average
Bacillus spp.	5.88±0.37 Aa	3.02±0.26 Bab	2.96±0.24 Ba	3.95 ab
B. cereus	6.61±0.16 Aa	3.53±0.24 Ba	3.41±0.19 Ba	4.52 a
Staphylococcus spp.	3.64±0.56 Abc	2.37±0.31 Bb	2.30±0.22 Ba	2.77 c
S. aureus	4.45±0.29 Ab	2.66±0.17 Bab	2.80±0.34 Ba	3.30 bc
coliformes a 45 ºC	2.73±0.38 Ac	1.19±0.12 Bc	1.00±0.00 Bb	1.64 d
Overall average	4.66 A	2.55 B	2.49 B	

Means followed by the same uppercase letter in the lines and lowercase in the columns do not present a significant difference according to the Tukey test at 5% probability.

(P>0.05) compared to cinnamon and oregano (Table 1). The members of the genus *Staphylococcus* produce thermostable toxins responsible for food poisoning. They can develop in foods with low water activity (0.83 to 0.86) and produce toxins at 0.86 aw (THANH et al., 2018).

E. coli and *Salmonella* spp. were isolated from 89% and 67% of the black pepper samples, respectively. The presence of microorganisms is attributed to an ability to adapt to natural antimicrobial substances in pepper, such as the alkaloid piperine (ALDALY, 2010). It is believed that the absence of bacteria in cinnamon and oregano is due to the antimicrobial action of bioactive cinnamaldehyde and eugenol present in cinnamon, and that of thymol and carvacrol in oregano, which modify the bacterial cell membrane, affecting its permeability and integrity (BURT, 2004; CEYLAN & FUNG, 2004).

Although, Brazilian legislation specifies the absence of *Salmonella* spp. in 25 g of spices (BRASIL, 2001), the commercial sale of black pepper contaminated with this pathogen is highly common in Brazil (MICHELIN et al., 2016). Therefore, it is necessary to make producers aware of the good practices in pepper production. This is particularly important in the post-harvest stages, for producers using manure or animal biofertilizers, when the peppercorns are at a greater risk of contamination by enterobacteria (MICHELIN et al., 2016). The risk of selling *Salmonella* contaminated spices is also raised due to the wide diversity of its serotypes, as mentioned by VAN DOREN et al. (2013), who reported the presence of 11 different serotypes in paprika samples.

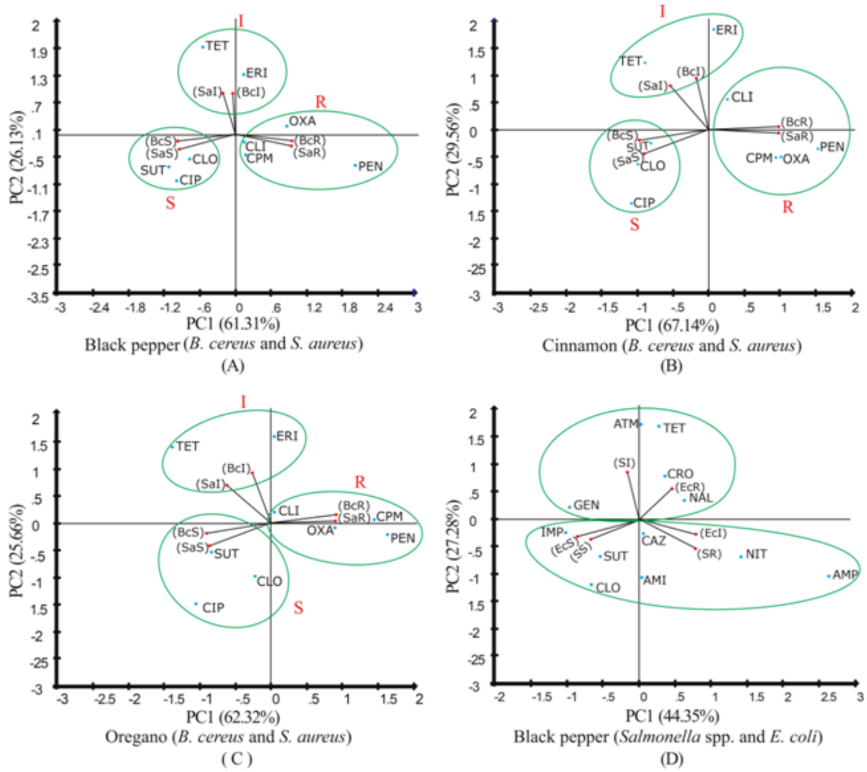
Bacteria with a high antimicrobial resistance profile were observed in the three spices. Of the 158 strains tested, 67% (105) showed resistance to at least one antimicrobial. The high prevalence of resistant strains may be due to the residual load of drugs for human and animal use released into the environment (SEMRET & HARAOUI, 2019).

Figure 1 shows the dispersion diagrams, positioning of scores and factor loads, and superposition of the midpoints of the antimicrobial profile of the four microorganisms. The behavior of Gram-positive bacteria (*B. cereus* and *S. aureus*) was similar to the antimicrobial susceptibility profile in the three spices. The resistance and sensitivity profiles constituted opposite quadrants, forming the first main component and presenting a variability of 61%, 31%, 67.14%, and 62.32% for pepper, cinnamon, and oregano isolates, respectively (Figure 1A, 1B e 1C).

The antimicrobial profiles of *B. cereus* and *S. aureus* showed that most of the isolates exhibited a resistance greater than 80% to penicillin, oxacillin, and cefepime. PARK et al. (2009) and BANERJEE & SARKAR (2004) verified the high resistance of *B. cereus* and *S. aureus* to β-lactams, respectively.

In the intermediate profile, a higher percentage was reported for tetracycline (50%) and erythromycin (70%), forming the second main component, and quantifying for the explained variability of the isolates of pepper (26.13%), cinnamon (29.56%), and oregano (25.66%) (Figure 1A, 1B and 1C). This observation suggested that; although, the antimicrobial resistance profile of each bacterial species is different, the behavior of the microorganisms, *B. cereus* and *S. aureus,* was similar across the different spices tested.

The total variability in the percentage of antimicrobial activity for *Salmonella* spp. and *E. coli* in black pepper added 71.63% for the two main



PEN – penicillin, CPM – cefepime, ERI – erythromycin, TET – tetracycline, CLI – clindamycin, OXA – oxacillin, SUT – sulfazotrim, CLO – chloramphenicol, CIP – ciprofloxacin, BcR – *B. cereus* resistance, SaR – *S. aureus* resistance, Bcl – *B. cereus* intermediate, Sal – *S. aureus* intermediate, BcS – *B. cereus* susceptible, SaS – *S. aureus* susceptible.
AMP – ampicillin, CAZ – ceftazidime, ATM – aztreonam, NAL – nalidixic acid, CRO – ceftriaxone, IMP – imipenem, NIT – nitrofurantoin, AMI – amikacin, GEN – gentamicin, EcR – *E. coli* resistance, SR – *Salmonella* resistance, EcI – *E. coli* intermediate, SI – *Salmonella* intermediate, EcS – *E. coli* susceptible, SS – Salmonella susceptible.

Figure 1 - Scattering, positioning of the scores, and factor loads of the superimposition of the antimicrobial profile of *Bacillus cereus*/*Staphylococcus aureus* (A, B, and C) in pepper, cinnamon, and oregano, and *Salmonella* spp./*Escherichia coli* (D) in pepper. R = resistant, I = intermediate, S = sensitive.

components, which was less than that observed for *B. cereus* and *S. aureus*. This highlighted the difference in behavior between *Salmonella* spp. and *E. coli* in their susceptibility profile. The resistance of *Salmonella* spp. to antimicrobials (56% resistant) was higher that of *E. coli* (7/32 or 23% resistant). Principal component analysis showed that the first main component (44.35%) was formed by EcI, EcS, SR, and SS and the second main component (27.28%) was formed by EcR and SI (Figure 1D).

These results showed how spices are subjected to different sources of contamination along the production chain, conferring harmful pathogens with high microbial resistance. The presence of food pathogens in spices, especially in black pepper, puts the health of consumers at risk and has relevant economic implications, reinforcing the need for continuous surveillance and the adoption of strict controls at all levels of the food production chain (VINHA et al., 2017).

All 20 isolates of *Salmonella* spp. showed resistance to ampicillin, ceftriaxone, and nalidixic acid. This is worrisome considering the resistance to first-line antimicrobials such as ceftriaxone, a third-generation cephalosporin, and ampicillin, a β-lactam widely used in localized or systemic infections caused by *Salmonella*. The evolution of resistant *Salmonella* strains reflects the use of antimicrobials in the treatment of bacteria after pandemics (CAMPIONI et al., 2012). Other antimicrobial resistances commonly reported among the isolates were to tetracycline (17/20), amikacin (17/20), imipenem (15/20), and aztreonam (15/20).

E. coli was resistant to ampicillin (41%), aztreonam (28%), tetracycline (28%), ceftazidime (25%), and nalidixic acid (22%). Although, *Salmonella* and *E. coli* were susceptible to ciprofloxacin, they exhibited resistance to nalidixic acid, which is another first-line antimicrobial used for treating infections caused by these bacteria, and this may be the first step towards the development of resistance to ciprofloxacin (VAN DOREN et al., 2013). Thus, the presence of resistant strains, in foods with potential to spread in the human food chain, is worrying. Most spices can also be added to ready-to-eat foods, exposing the consumer to resistant bacteria, which have not been inactivated by conservation measures, such as heating (SCHWAIGER et al., 2011).

More than 50% of the strains of *Salmonella* (13/20), *B. cereus* (41/72), and *S. aureus* (28/34) displayed a high MAR index, with *B. cereus* and *S. aureus* resistant to up to six antimicrobials, *E. coli* resistant to up to seven antimicrobials, and *Salmonella* resistant to up to 11 antimicrobials (Table 2).

High antimicrobial resistance may be related to the fact that microorganisms adapt to the chemical structure of phenolic compounds present in spices, with some compounds acting on the permeability of the bacterial cell wall (COWAN, 1999). When microorganisms are exposed to synthetic antimicrobials, often with a similar chemical structure, they express similar resistance mechanisms. This implies that flaws in the spice production chain might enhance the transmission of resistant bacteria. This would affect the treatment of diseases and contribute to the worsening of potentially curable clinical conditions.

CONCLUSION

Spices sold in the town fairs of Recôncavo Baiano are of low microbiological quality. We demonstrated the presence of pathogens, such as *Salmonella* spp. particularly in the ground black pepper. Spices might contributed to the spread of *B. cereus*, *S. aureus*, *E. coli,* and *Salmonella* spp. with

Table 2 - Multiresistance of isolated strains from spices (black pepper, oregano, and cinnamon) sold in town fairs.

N° resistant strains	N° antimicrobials	MAR index	% multi-resistant strains
-----*Staphylococcus aureus*-----			
2	2	0.22	7
11	3	0.33	39
5	4	0.44	18
9	5	0.55	32
1	6	0.66	3
-----*Bacillus cereus*-----			
8	2	0.22	19
16	3	0.33	39
9	4	0.44	22
7	5	0.55	17
1	6	0.66	2
-----*Salmonella* spp.-----			
1	2	0.16	8
5	3	0.25	38
2	4	0.33	15
2	5	0.41	15
2	6	0.50	15
1	11	0.91	8
-----*Escherichia coli*-----			
1	2	0.16	14
1	3	0.25	14
2	4	0.33	28
2	5	0.41	28
1	7	0.58	14

a high index of resistance to several antimicrobials, mainly β-lactams.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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