Isolation of resistant bacteria from commercial samples of camomile (*Matricaria recutita*)

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Chamomile is widely used in traditional medicine due to the analgesic, anti-allergic, antispasmodic, antibacterial, anti-inflammatory, sedative, scarring, proliferative cancer cells. The objective of this study was to identify and determine the resistance profile of bacteria isolated from samples of commercial chamomile (*Matricaria recutita*). In a flask containing 22.5 mL of sterile distilled water were added 2.5 g of each sample. Then 1µl of the solutions were seeded in specific media, seeded plates were incubated at 35 °C for 24 hours. Subsequently identifications were performed. The methodology used for antimicrobial susceptibility testing was Bauer et al. (1966) and the classification was made according to the CLSI. The tested material were isolated and identified as *Staphylococcus aureus* (6) *Staphylococcus* coagulase negative (4), *Pantoea agglomerans* (1), *Enterobacter cloacae* (1) and *Serratia ficaria* (1). The microorganisms were resistant to at least one of the antibiotics tested,and was observed profile of multidrug resistance in some cases.

Keywords Camomile; resistant**;** *Staphylococcus aure*us; *Staphylococcus* coagulase negativa; *Pantoea agglomerans*; *Enterobacter cloacae*; *Serratia ficaria*.

1. Introduction

The treatment of human diseases from medicinal plants (or their derivatives) is an ancient practice that is expanding. The products based on medicinal plants handled about 30 billion dollars in 2000 [1]. In the United States and the United Kingdom, herbs are used by a large and growing percentage of the population [2, 3]. At a meeting held in 2004 by the WHO, it was observed that the use of herbal medicines continues to expand rapidly throughout the world [4]. Medicinal plants are used by up to 90% of the poor population of Northeast Brazil, and serve as the primary access to health care for many communities [5, 6].

Chamomile is one of the medicinal plants known and most versatile. Its consumption in the form of tea is accounted for more than one (1) million cups per day [7]. In many countries both chamomile flowers, and as the crude drug chamomile tea bags are available in supermarkets and pharmacies [8]. In Brazil is a medicinal plant with the largest cultivation area and the greater involvement of small farmers, emphasizing the Paraná as largest producer [9, 10, 11].

Known since ancient times, by the Egyptians, Greeks and Romans, due to its medicinal properties, cosmetics, ornamental and aromatic chamomile is a herbaceous plant originally from Europe and Northern Asia. Consumption in Iran has a long history in folk medicine. It is recognized as an official drug in 26 countries and is listed on the American and British Pharmacopoeia. In Brazil, located in the National Medicinal Plants of Interest to SUS - RENISUS. Some countries produce chamomile for the international market, they are: Argentina, Egypt, Bulgaria, Hungary, Spain, Czech Republic, Germany, Brazil, Chile and Peru [12, 13, 14, 15, 16, 17, 18]. Chamomile is widely used in traditional medicine due to analgesic, anti-allergic, antispasmodic, antibacterial, anti-inflammatory, sedative, healing, cancer cell antiproliferative properties [19, 20, 21, 22, 8, 23].

With increased consumption also increases the responsibility of regulatory agencies and manufacturers aiming to be assured the quality and therapeutic efficacy [24]. Although the market for natural products is promising, and its demand is increasing, the lack of quality from raw material to finished product is one of the common problems in this field. Among these problems is the improper storage. In addition to generating losses of active product, storage can facilitate the diverse agents contamination, which, in turn, can cause damage to the health of consumers [25, 26]. The World Health Organization considers the lack of safety of these products an important public health problem [4].

Thus, the evaluation of their sanitary quality is mandatory step. Furthermore, the therapeutic efficacy may also be compromised by decomposition of components caused by the action of microorganisms [27, 28]. In a study conducted with samples of medicinal herbs collected from public markets of Lisbon (13 of these Chamomile - *Matricaria recutita* L.) was observed the presence of *Penicillium* sp. in 100% of samples [29]. In Brazil, the situation is no different. In Maranhão, the research of foreign elements in medicinal plants revealed that 86% of commercial samples had impurity as other organs of the plant itself or another, and parasites living or dead insects, soil, sand and stone. Research on fungi and bacteria contamination revealed in 81.5% of the material analyzed [30]. In Paraná, the samples of chamomile (*Chamomila recutita*) analyzed were contaminated by bacterial species of *Enterobacteriaceae*, in particular *Escherichia coli* [31]. In São Paulo, were evaluated 91 samples of herbal drugs, among them 3 chamomile (*Matricaria recutita*). The material in question, 51 kinds of herbal drugs showed higher microbial populations to pharmacopoeial limits. The presence of *Escherichia coli*, *Enterobacter* spp. and *Klebsiella* spp. respectively were observed in 26.2%, 52.3% and 60% of plant species investigated [32].

The first case of antibiotic resistance has been reported eight years after the start of the use of penicillin in treating infections. The situation has evolved until exist thousands of bacterial strains classified as multiresistant [33]. The genetic variation and rapid multiplication allow bacteria to develop specific mutations adapt to different environments and develop resistance to multiple antibiotics [34]. When acquiring resistance, micro-organisms maximize its ability to colonize environments less prone to bacterial growth, thereby creating a new reservoir of potential contamination and growth [35].

The objective was to identify and determine the resistance profile of bacteria isolated from commercial samples of chamomile (*Matricaria recutita*).

2. Material and methods

2.1. Selection of samples

The selection was made by applying the criteria used by the RDC No. 12 of 2001 [36], whose general instructions state that each sample unit shall be comprised of at least three units of the same batch. Thus, the analysis was performed with two brands of tea in sachets, these brands were chosen three batches and each batch, three boxes/samples.

2.2. Primary Isolation

In a flask containing 22.5 mL of sterile distilled water were added 2.5g of each sample. The whole was mechanically agitated for 15 minutes [37]. After the shaking time, starting from solutions of washes, aliquots were plated with a platinum loop calibrated (1μL) for depletion in triplicate in media: Agar Eosin Methylene Blue (EMB) Agar Hektoen enteric (HE), Salmonella Agar-Shigella (SS), sheep blood agar and Mannitol Salt Agar. The seeded plates were incubated at 35 ± 2 °C for 24 hours. After incubation, the colonies were re-isolated for identification.

2.3. Identification of isolates

All isolated colonies were stained by the Gram method which allowed the observation of morphology, arrangements, and their classification: Gram + (positive) and Gram - (negative). Then, they were targeted to specific tests for each group. Gram - were subjected to the following tests: using fermentation of glucose, lactose and sucrose; H_2S production, motility, indole production, use of sodium citrate, lysine decarboxylation; activity of ornithine decarboxylase, methyl red, Voges-Proskauer test, reduction of nitrate to nitrite, and acid production by carbon source. For Gram + tests were: catalase, coagulase, DNAse, growth and turn of mannitol salt agar [38].

2.4. Analysis of the sensitivity profile of the identified microorganisms to different antibiotics

The methodology used for antimicrobial susceptibility testing was the method of Bauer et al. (1966) [39]. Colonies were transferred from each identified microorganism in a culture to a tube with 5 ml sterile water to form a solution with turbidity tube corresponding to 0.5 McFarland turbidity. The suspension was plated on a plate containing Mueller Hinton agar. The antibiotic disks were placed on the surface of the medium uniformly sown aseptically and following the same distance. Then the plates were incubated at 35° C. After 16 to 18 hours of incubation, the plates were examined and the diameters of inhibition zones were measured and ranked according to the Clinical and Laboratory Standards Institute - CLSI [40]. To evaluate the sensitivity of enterobacterias were used discs of Ampicillin 10μg, Cephalothin 30μg, Gentamicin 10μg, Cefoxitin 30μg, Cefotaxime 30μg, Cefepime 30μg, Tetracycline 30μg and for Staphylococcus, discs of Oxacillin1μg, Penicillin G 10U, Erythromycin15μg, Chloramphenicol 30μg, Gentamicin 10μg, Tetracycline 30μg and Cephalothin30μg.

3. Results and discussion

Colonies were isolated from samples of chamomile followed for biochemical tests after confirming the presumptive identification on selective media and differential. In biochemical tests it was possible to confirm the presence of 10 staphylococcal and 3 of enterobacteria strains.

3.1. Identification and resistance profile of *Staphylococcus*

According to the tests used to identify microorganisms isolated, six have been identified such as *Staphylococcus aureus*, i.e.: they are Gram positive cocci, in clusters, catalase positive, ferment mannitol, and have the enzyme DNAse and present the enzyme coagulase; and four were identified as coagulase negative *Staphylococcus* (SCN), ie.: they are Gram positive cocci in clusters, catalase positive and coagulase negative.

As the resistance profile, all *S. aureus* were resistant to penicillin, two to chloramphenicol, one to cephalothin, three to oxacillin, four to erythromycin and one to tetracycline. There was no resistance to gentamicin. It is noteworthy that

the microorganism A2 may be classified as multiresistant since it only has a sensitivity to gentamicin, and tetracycline. Microorganisms were considered multidrug resistance those wich have resistance to two or more groups of antibiotics [41]. Must also highlight the presence of three organisms (A1, A2 and A5) oxacillin resistant (ORSA). Regarding the resistance profile of *Staphylococcus* coagulase negative (SCN), three microorganisms were resistant to oxacillin, two to penicillin and erythromycin, and one to cephalothin. One of the microorganisms was sensitive to all antibiotics tested (Table 1).

Table 1 Resistance profile of *Staphylococcus*.

Microorganism/ antibiotics	A1	A2	A3	A4	A5	A6	SCN1	SCN2	SCN3	SCN4
Chloramphenicol	S	R	S	S	S	R	S	S	S	S
Cephalothin	S	R	S	S	S	S	S	S	R	S
Oxacillin	R	R	S	S	R	S	R	R	R	S
Gentamicin	S	S	S	S	S	S	S	S	S	S
Penicillin	R	R	R	R	R	R	R	S	R	S
Erythromycin	S	R	S	R	R	R	R	S	R	S
Tetracycline	S	S	S	R	S	S	S	S	S	S

Legend: S - sensitive, R - resistant, A - aureus, SCN - *Staphylococcus* coagulase negative

The importance of pathogens such as *Staphylococcus sp*. in raw foods is linked to your power enterotoxigenic with consequent gastrointestinal disorder when the ingestion of contaminated food. It is noteworthy that the micro-organism is thermolabile and can be destroyed after the normal process of cooking. However, the enterotoxin produced previously in food is heat resistant and can resist to pasteurization and ultra pasteurization. The staphylococcal enterotoxins are extracellular proteins of low molecular weight, water soluble and resistant to proteolytic enzymes in the digestive system, remaining active after ingestion [42, 43]. *Staphylococcus aureus* can produce more than one type of toxin which can cause symptoms of poisoning, accompanied mainly by vomiting and diarrhea [44]. This species is most prevalent in outbreaks of staphylococcal food poisoning, however, the *S. intermedius* and *S. hyicus* also produce enterotoxin [45].

In the literature there are reports of isolation of *S. aureus* of different foods, including foods ready for consumption [46]; animal products such as meat [47], milk [48] and jerked beef [49], fruits [50], and teas. Study evaluated the bacterial contamination of powdered preparations based on medicinal plants and observed that 65.33% were contaminated with *Staphylococcus aureus* [51].

In a research, starting from samples of raw milk were isolated 201 strains of *S. aureus*, which were submitted to tests of resistance to antibiotics of these 88 strains were resistant to penicillin, 90 to ampicillin, 24 to tetracycline, and 40 to chloramphenicol [52]. These results corroborate those of the present study because there was greater resistance to penicillin and some isolates showed resistance to chloramphenicol and tetracycline.

Despite the belief that usually coagulase negative species are not object of importance in the epidemiology of staphylococcal poisoning, searches exhort holdings towards investigation of other species that produce coagulase [53]. The production of enterotoxins by Staphylococcus coagulase negative as *S. capitis*, *S. conhnii*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. saprophyticus*, *S. schleiferi*, *S. warneri*, *S. xylosus* and *S. chromogenes* was observed in several studies conducted under laboratory conditions. This suggests that SCN may be the casual agent in potential of food poisoning [54].

Coagulase negative staphylococci can be found in many foods such as meat goats [55], salamis [56], fermented sausages [57], cheese curd [58], Cheddar cheese [59], cheese Graviera typical of Greece [60] and beer Italian [61].

Faria et al. (2009) [62] reported the standards of antibiotic resistance of coagulase negative staphylococci isolated from a wastewater treatment plant for drinking water, distribution network of drinking water, responsible for supplying water to consumers. The highest rate of resistance were to erythromycin, and also was being found tetracycline resistance. In the present work it was observed resistance to erythromycin.

3.2. Identification and resistance profile of Enterobacterias

In biochemical tests it was possible to confirm the presence of *Enterobacteriaceae* and identification of three species: *Pantoea agglomerans*, reduce nitrate to nitrite, urea negative, motility positive and Vosges-Proskauer positive doesn't have the enzyme lysine and ornithine decarboxylase and doesn't ferment sucrose; *Enterobacter cloacae* , reduce nitrate to nitrite, citrate positive, Voges-Proskauer positive,has enzyme ornithine decarboxylase and mobile and *Serratia ficaria*, reducing nitrate, citrate fermenter, urea negative, indole negative, esculin positivepresents no lysine and ornithine enzymes decarboxylase. For the resistance profile, *Pantoea agglomerans* and *Serratia ficaria* were resistant to ampicillin, *Enterobacter cloacae* showed resistance to cefoxitin, cephalothin and ampicillin (Table 2).

Table 2 Resistance profile of *Enterobacteriaceae*.

Microorganism/ antibiotics	*Pantoea agglomerans*	*Enterobacter cloacae*	*Serratia ficaria*
Cefepime	S	S	S
Cefoxitin	S	R	S
Cephalothin	S	R	S
Tetracycline	S	S	S
Cefotaxime	S	S	S
Gentamicin	S	S	S
Ampicillin	R	R	R

Legend: S – sensitive, R – Resistant

Species belonging to the genus *Pantoea* are isolated from plants, as epiphytes (growing on or attached to the plant) and endophytes (growth within the plant), soil, water, food, animals and humans [63, 64, 65, 66, 67, 68]. Analyzing contamination of different vegetables and resistance profile of the microorganisms was observed the presence of *Enterobacter cloacae*. The resistance to tetracycline was most common (43%), streptomycin (37%), chloramphenicol (29%) co-trimoxazole (9%) and gentamycin (4%) [69]. *Serratia ficaria* is an enterobacteria part of the ecosystem of the fig tree. From the fig tree, the bacteria can be spread by insects and ants. This could explain the spreading isolated from human respiratory route and the oral contamination. The isolation is rare in clinic and role as a pathogen is not clear, but infections caused by *S. ficaria* in patients with poor health can have serious consequences [70, 71, 72].

4. Conclusions

The microorganisms isolated and identified are potential pathogens and represent additional risk for having developed and/or demonstrated resistance to some of the antibiotics tested. Highlighting the presence of multiresistant microorganisms and oxacillin resistant staphylococci (ORSA).

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