Thi-Qar Medical Journal (TQMJ): Vol.(14), No.(2), 2017
Email:utjmed@utq.edu.iq Web Site: https://jmed.utq.edu.iq

Evaluation of antibacterial activity of cinnamon and ginger extracts against vancomycin-resistant *Staphylococcus aureus* isolated from nose of food handlers in restaurants and cafeterias

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Abstract:

Background: Food handlers are the main source of *Staphylococcal* food poisoning in developed countries.

Objective: The aim of this study was to determine the prevalence of the nasal carriage of vancomycin resistant *Staphylococcus aureus* among food handlers in restaurants and cafeterias, and to evaluate of antibacterial activity of cinnamon and ginger extracts against these bacteria.

Material and Methods: A total of 100 nasal swabs were collected from healthy food handlers and analyzed for *Staphylococcus aureus* ,methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin *resistant Staphylococcus aureus* (VRSA) using standard methods. Aqueous and ethanolic extracts of each plant were prepared by using a Soxhalet apparatus and the antibacterial activities of these extracts against VRSA were determined by using agar well diffusion method.

Results: of 100 nasal swabs, 30 (30%) isolates were *Staphylococcus aureus* , among which 25 (83.3%) isolates were MRSA, and 20 (80%) isolates among MRSA were VRSA. Both plant extracts showed potent antibacterial activity against VRSA. Cinnamon extracts showed higher antibacterial activity when compared with ginger extracts , whereas ethanolic extract of each plant was more effective than that of aqueous extract.

Conclusion: This study revealed a relatively high prevalence rate of VRSA nasal carriage among food handlers. Cinnamon and ginger are rich source of antimicrobial compounds

which can be used as natural food preservatives and to treat infections caused by these bacteria .

Keywords: Food handlers, *Staphylococcus aureus*, MRSA, VRSA, Nasal carriage, Cinnamon, Ginger and Antibacterial activity.

INTRODUCTION:

Foodborne diseases are one of the major health problems throughout the world that can be affected about 30% of the population in developed countries each year . *Staphylococcus aureus (S. aureus)* is the most foodborne pathogen which naturally colonizes the mucous membranes of anterior nares of healthy people. About 10-50% of people are carrier of both methicillin resistant and sensitive *S. aureus* (MRSA and MSSA) [1] .

Nasal carriage of *S. aureus* among food handlers working in restaurants and cafeterias are the main source of food contamination and food poisoning [2].Therefore, the Staphylococcal food poisoning considered as the most economically important diseases resulted from ingestion of food containing enterotoxins produced by some species of staphylococci which characterized by sudden onset of symptoms, including; nausea, vomiting, abdominal cramps, and diarrhea [3,4].

Staphylococcus aureus infections may lead to high morbidity and mortality because their ability for developing resistance quickly and successfully to antibiotics via a mechanism that involves acquisition and transfer of antibiotic resistance plasmids, as well as possession of intrinsic resistance mechanisms .Therefore , multidrug resistant *S. aureus* has been recognized as a persistent nosocomial and community acquired pathogen responsible for a several diseases, including endocarditis, osteomyelitis, toxic-shock syndrome, pneumonia, food poisoning and carbuncles. These infections can occur in wounds ,skin, burns, eyes, bones, heart or blood and other sites of the body [5].In addition, this bacteria has a capacity to evolve different mechanisms of resistance against most antimicrobial agents like methicillin and vancomycin which continuously develop parallel with the drugs developed against it [6,7,8]. Vancomycin resistant *S. aureus* (VRSA) is clinical isolate of methicillin resistant *Staphylococcus aureus*

(MRSA) which shows *in vitro* non susceptibility to vancomycin [9]. Increasing failure of chemotherapeutics and antibiotic resistance exhibited by *S. aureus* especially VRSA has led to screen several medicinal plants for their potential antimicrobial activity [10].

Plants have been a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection. Herbs and spices are well known for their preservative and medicinal value as well as for boosting the flavor, color and aroma of the food. Therefore, the global food industries used spices as natural food preservatives instead of chemical preservatives due to increase occurrence of pathogenic strains resistance against these chemical compound[11,12].

Cinnamon (*Cinnamon Zeylanicum*) is a tropical evergreen tree grows in India and East Asia, used for the preparation of different types of chocolate, beverages, spicy candies and liquors . In addition cinnamon has been employed in traditional herbal medicine to treat a variety of health conditions including, diabetes , gastrointestinal disorders and urinary infections, it has also been used as antimicrobial, anti-inflammatory, antioxidant , antimutagenic and as neuroprotective agent [13,14,15,16].

Ginger (*Zingiber officinale*) is one of the world's best known spices belongs to Zingiberaceae family, grows in Southeast Asia and cultivated in other countries like India. Ginger commonly used as flavoring agent and cooking spice. It has a several pharmacological activities such as antibacterial, antioxidant, anti-inflammatory, antidiabetic, anticarcinogenic and hepatoprotective activities, it has also been recommended to improve gastrointestinal illnesses , immunologic dysfunction and hypertension[17,18,19,20].

MATERIALS AND METHODS:

Collection and preparation of plant extracts:

Cinnamon barks and ginger rhizomes were purchased and collected from the local market of Al-Nasiriya city, Iraq. The plants were cleaned and washed well in sterile distilled water and dried at room temperature, then milled into fine powder using an electric blender. One hundred grams (100g) of each powdered plant material were extracted separately with 500 mL of ethanol (70%) in a Soxhlet apparatus for 8hours.This extraction procedure was

also used for aqueous extract. Both extracts were filtered by Whatman No.1 filter paper . Then, the filtrates were evaporated under reducing pressure using rotary evaporator at 40°C and the final dry extracts were stored at 4°C in pre sterilized air tight flasks until use to prepare 100 mg/mL from each extract [21,22].

Sample collection and bacterial identification:

A total of one hundred nasal swabs were collected from healthy food handlers male who work in different restaurants and cafeterias in Nasiriya center, Iraq, from February to June, 2015. The Sterile cotton swab sticks previously moistened with sterile normal saline and carefully inserted in both anterior nares of each person and gently rotated at least 5 times. All samples were directly inoculated on Mannitol salt agar and incubated at 37 ºC for 24 - 48 hours. The identification of the *S. aureus* isolates was dependent on mannitol fermentation, Gram stained morphology, catalase, coagulase and DNase tests[23,24].

Detection of methicillin resistance *S. aureus* by disc diffusion method:

All *S. aureus* isolates were tested for methicillin (5 µg) and oxacillin (1 µg) susceptibility by modified Kirby-Bauer method on Mueller-Hinton agar (MHA). The bacterial suspension was prepared in sterile saline by selecting the isolated colonies produced by overnight incubation on nutrient agar plate(NA), the cell turbidity was adjusted to 0.5 McFarland standards. Subsequently, a sterile swab was dipped into the suspension and streaked on the Muller Hinton agar plate and allowed to dry for few minutes, the antibiotic discs were then placed onto the plates and incubated for 48 hours at 35°C . The zone of inhibition around the disc was measured and interpreted according to the Clinical and Laboratory Standards Institute chart. The strains were considered resistant to both drugs if the zone size was ≤10 mm.[25,26,27].

Detection of vancomycin resistance *S. aureus*:

VRSA among MRSA isolates were detected by both agar disc diffusion and broth dilution methods (MIC).

In disc diffusion method, Mueller-Hinton agar plates were inoculated with the bacterial suspension which was previously adjusted to 0.5 McFarland standards. Afterward, a 30 µg

vancomycin discs were aseptically placed on the surface of the agar plates within 15 minutes after inoculation, and incubated for 24 hours at 37°C. The zone of inhibition around the disc was measured and interpreted according to the Clinical and Laboratory Standards Institute chart [25,26,27].

In broth dilution method, the minimum inhibitory concentration (MIC) of vancomycin was determined using Muller Hinton Broth. 0.5 McFarland equivalent bacterial suspension was prepared in normal saline and spotted onto Muller Hinton plates containing different concentrations of vancomycin and incubated for 24 hours at 37°C and checked for any visible growth. *S. aureus* strains were considered resistant if had MIC of ≥16 µg/ml [2٧,2٨].

Evaluation of antibacterial activity of plants:

The modified agar well-diffusion method was used to evaluate the antibacterial activities of the extracts. 0.1mL of the standardized inoculums (1.5 x 10^8 CFU/ml) of vancomycin resistant *S. aureus* (VRSA) was aseptically spread onto the surface of sterile Mueller Hinton agar and left to dry for 30 min. Wells of 8 mm in diameter were made into agar plates containing the bacterial using a sterile stainless steel borer and filled with 50 µl of each extracts. The prepared plates were left at room temperature for 30 minutes allowing the diffusion of the extracts into the agar, then incubated at 37 °C for 24 hours. The antibacterial activity was evaluated by measuring the diameter of inhibition zone produced by the extracts against tested bacteria [29].

Statistical analysis:

All the experimental results of were expressed as mean using Microsoft Excel 2010 software.

Ethical Consideration:

Informed consent was obtained from food handlers who work in restaurant and cafeterias after explanation the concept and dimensions of study to them

.

RESULTS:

In this study, out of total 100 nasal swabs collected from healthy food handlers working in the restaurants and cafeteria, 30 (30%) isolates were identified as *S. aureus* based on morphology, Gram stain, mannitol salt agar fermentation , DNase ,catalase and coagulase tests. Among these isolates, 25 (83.3%) were detected as MRSA based on their resistant to methicillin and oxacillin by disc diffusion method, and 20 (80%) isolates of MRSA were detected as VRSA based on their resistant to vancomycin by disc diffusion method and on MIC in broth dilution method (Table 1).

Table 1 :The prevalence of *Staphylococcus aureus,* MRSA and VRSA among the food handlers working in the restaurants and cafeteria.

No.	Bacteria	Number of isolates	%
1	*S. aureus*	30 (out 100 isolates)	30
2	MRSA	25 (out of 30 S. aureus)	83.3
3	VRSA	20(out of 25 MRSA)	80

Cinnamon and ginger extracts showed potent antibacterial activity against VRSA. The aqueous and ethanolic extracts of cinnamon exhibited antibacterial activity with an inhibition zone diameter 15 mm and 18 mm respectively, while aqueous and ethanolic extracts of ginger showed antibacterial activity with an inhibition zone diameter 14 mm and 16 mm respectively (Table 2).

Cinnamon extracts showed higher antibacterial activity when compared with ginger extracts , whereas alcoholic extract of each plant was more effective than that of aqueous extract .

Table 2:Antibacterial activity of cinnamon and ginger extracts against VRSA.

Mean diameter of inhibition zones (mm)		
Extract **Plant**	Aqueous extract	Ethanolic extract
Cinnamon 100 mg/mL	15	18
Ginger 100 mg/mL	14	16

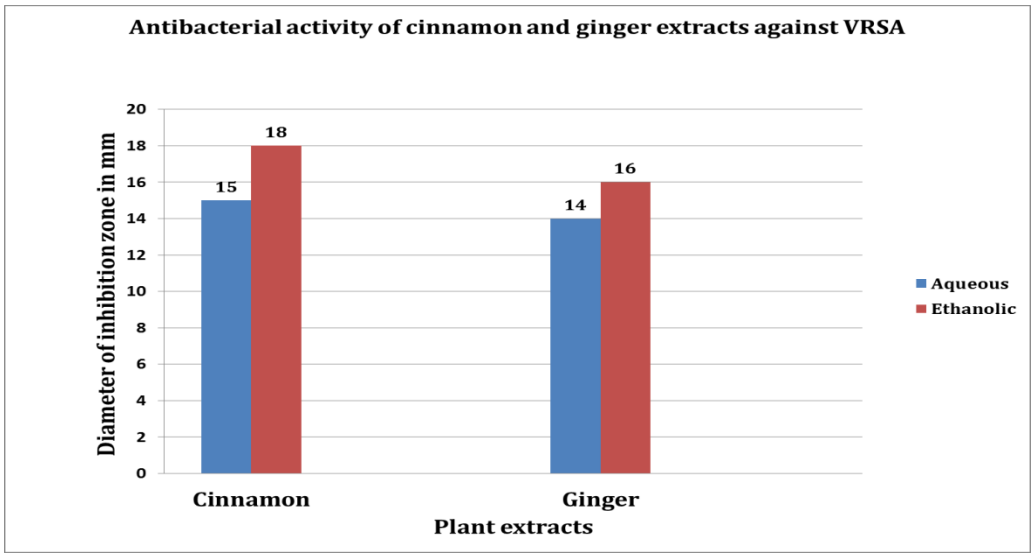

Figure 1: Antibacterial activity of cinnamon and ginger extracts against VRSA.

DISCUSSION:

Food poisoning is one of the major health problems in the world, occurs mainly in developing countries due to poor hygiene and sanitary precautions. Food handlers especially those working in restaurants and cafeteria are considered as the main sources of *Staphylococcal* food poisoning because they contaminate foods by their hands with *S. aureus* especially vancomycin resistant *S. aureus* which is carried in their nasal cavities . Therefore, treatment of infections caused by VRSA are often very difficult due to cross-resistance of these bacteria with a large group of antibiotics. Nevertheless, it seems reasonable to explore new sources of natural plants with antibacterial activity against them[3,12].

In this study, out of total 100 nasal swabs collected from healthy food handlers working in the restaurants and cafeteria , only 30 isolates(30%) were positive for *S. aureus*. The prevalence rate of nasal carriage of *S. aureus* in our study was higher than those observed in similar studies among food handlers working in Ethiopia (20.5%) [30], Turkey (23.1%) [31], USA (23%) [32], Saudia Arabia (8.5%) [33] and Kuwait (26.6%) [34]. Nevertheless, it was lower than those obtained in studies conducted in Chilean (65%) [35] , Botswana (57.5%) [36], **N**igeria (60%) [24] and Kuwait (53.2%)[37], while it was nearly similar to that of 29%, 31% ,30.1%, 29.24 % which had been reported in

Brazil, Egypt , Sudan , Iraq respectively [38,39,40,41].

In present study, 25 (83.3 %) of the 30 *S. aureus* isolates were found to be MRSA , and 20 (80 %) of the 25 MRSA isolates were found to be VRSA. Nearly similar results were obtained by other authors, they found that 28(28 %) of nasal swabs from food handlers working in Samara city restaurants were positive for *S. aureus*, all of these isolates were resistant to methicillin(100%), and only 5(17.85%) of the MRSA isolates were resistance to vancomycin [42].

Other study demonstrated that high percentage of *S. aureus* carriers was found among food handlers who worked in restaurant in Makkah, Saudi Arabia, it showed that all nasal swabs of food handlers were positive for *S. aureus*, also showed about 43% of these isolates were MRSA and 100% were VRSA [43]. Other study showed 69.35 % of collected samples were *S. aureus*,49.04 % of which were found to be MRSA, and 9.8% of MRSA were resistant to vancomycin [44]. All of these results are compatible with the of present study, while the difference in the percentages of nasal carriage of *S. aureus* ,MRSA and VRSA among food handlers is due to several factors such as variation in countries, season of sample collection per year, size of samples and excessive use of antibiotics .

In this study, both aqueous and ethanolic extracts of cinnamon bark exhibited antimicrobial activity against VRSA, these results agree with the results of another study which revealed that aqueous and alcoholic extracts of cinnamon bark had considerable antimicrobial activity against VRSA of urine samples from patients with urinary tract infection [45]. A study conducted in Pakistan has been shown that ethanolic extracts of cinnamon possess strongest antibacterial activity against VRSA of pus/wound samples from patients admitted to different hospitals of Pakistan[44].

In addition, the antibacterial activity of cinnamon was also tested in previous study against VRSA isolated from wounds, blood, respiratory and urinary tract of infected patients by agar diffusion method. The results showed that cinnamon oil exhibited antibacterial activity against tested bacteria [46],these results are in accordance with present findings.

The main mode of antibacterial action of cinnamon is damage of bacterial cell membrane, which has

been attributed to the presence of some active constituents in their essential oils and extracts, including cinnamaldehyde , eugenol , eugenyl, linalool , benzoic acid, benzaldehyde , cinnamic acid and saponins [47,48,49,50].

The cinnamaldehyde is highly electro-negative aromatic aldehyde interfere in biological processes involving electron transfer and react with nitrogen-containing components like proteins and nucleic acids, and therefore inhibit the growth of the microorganisms[51]. It is also exhibited antibacterial activity by their ability for inhibition the production of an essential enzyme by the bacteria and damage bacterial cell wall, this could be explained by their hydrophobicity which allowed them to partition the lipids of the bacterial cell membrane, turning them more permeable and leading to leakage of ions and other cell constituents [52,53,54]. Cinnamaldehyde is also known to inhibit amino acid decarboxylase activity [55] and bacterial acetyl-CoA carboxylase which responsible for major antibacterial activity[56]. In addition , cinnamaldehyde leads to inhibition of N- 3- oxohexanoyl-Lhomoserine lactone (3- oxo-C6-HSL) and AI-2 ,which has the potential to affect bacterial quorum sensing (QS) regulated processes [48,50].

Previous studies reported that eugenol and eugenyl acetate are the major bioactive constituents of cinnamon bark oil. These volatile phenolic compounds are known to be either bactericidal or bacteriostatic agents, depending upon the concentration used [51], they have ability to destroy the membranes of bacterial cells, leading to leakage of intracellular material and cell death [57,58]. Eugenol also limits the growth of microorganisms by inhibiting the production of certain enzymes needed for growth [59].

Previous study demonstrated that cinnamon significantly decreased the production of enterotoxin A and enterotoxin B of *S. aureus* [60,61]. Furthermore, the essential oils result in immediate reduction of bacterial population and might be more effective against food borne pathogens and spoilage bacteria when applied directly on food ready to be used [50].

In this study, both aqueous and ethanolic extracts of ginger(zingiber) rhizome exhibited antimicrobial activity against VRSA, these results are in accordance with the results of another study which reported that aqueous and

ethanolic extracts of zingiber rhizome showed highly significant antibacterial activity against VRSA with the zone of growth inhibition 15 mm and 30 mm respectively [62]. they also agrees with another study demonstrated that alcoholic extract of zingiber had strong antibacterial activity with 17 mm of inhibition zone against VRSA of ear, nose and throat swabs from patients attending ENT clinic in Nigeria [63]. Our findings are also compatible with those of previous study which revealed that ethanolic of zingiber rhizomes extract had antibacterial activity against multiple-drug resistant *S. aureus* (VRSA) from patients with urinary tract and pus infections[64]. Hydro-alcoholic extracts of zingiber rhizomes extract possess strong antibacterial activity against vancomysin resistant *S. aureus* with growth inhibition zone 16 mm [65].

The antibacterial activity has been attributed to the presence of some active constituents in the ginger rhizomes extracts like, gingerol, zingiberene, β-bisabolene, α-farnesene, shogaol, β-sesquiphellandrene and α-curcumene [22].

Gingerol and other phenolic compound which are protein denaturing agents lead to swelling and rupture of the bacterial cell membrane by changing their permeability, which in turn caused damage of cytoplasmic membrane and release of cell materials, like nucleic acid, metabolites and ions. In addition, most of phenolic compounds are metal chelators that attach to the active site of the metabolic enzymes, reducing enzyme activities and therefore slowing bacterial metabolism and reproduction [66].At the same time, the β-bisabolene, α-farnesene and sesquiphelandrene of ginger compounds cause disruption of the cytoplasmic membrane and coagulation of the cell contents[67].

Several studies indicated that the antimicrobial potency of ginger mainly caused by the presence of oxygenated mono- and sesquiterpenes, hydrocarbons. These compounds attack the cell walls and cell membranes and affecting their permeability leading to release of intracellular constituents (e.g. ribose, Na glutamate) , also interfere with membrane functions including electron transport, nutrient uptake, protein, nucleic acid synthesis and enzyme activity leading to inhibition of bacterial pathogens[68,69].

Other authors have suggested that antimicrobial components of the ginger extracts (terpenoids and

alkaloids) interact with enzymes and proteins of the microbial cell membrane causing its disruption to disperse a flux of protons towards cell exterior which inhibit enzymes necessary for amino acids biosynthesis and induces bacterial death[70,71].

previous study reported that essential oils of zingiberaceae family can provoke depolarization of the mitochondrial membranes by decreasing the membrane potential, affecting ionic Ca^{++} cycling and other ionic channels , reducing the pH gradient, affecting the proton pump and ATP pool. They change the fluidity of membranes, which become abnormally permeable resulting in leakage of radicals, cytochrome C, calcium ions and proteins ,finally leads to bacterial death[72].

In this study, the ethanolic extracts of each plant had potent antibacterial activity than that of aqueous extracts ,which compatible with other studies demonstrated that ethanolic extracts of cinnamon and ginger possesses a higher inhibitory activity against the tested organisms than that of the aqueous extracts[71]. Because ethanol is an organic solvent and dissolve organic compounds better, hence liberate active components required for antimicrobial activity[73,74].

In conclusion, this study has revealed a relatively high prevalence rate of VRSA nasal carriage among food handlers working in the restaurants and cafeteria.Cinnamon and ginger extracts had potent antibacterial activity toward VRSA , therefor can be used as a natural food preservatives and to treat infections caused by VRSA .

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Thi-Qar Medical Journal (TQMJ): Vol.(14), No.(2), 2017
Email:utjmed@utq.edu.iq Web Site: https://jmed.utq.edu.iq

تقييم فعالية المضاد البكتيري لمستخلصات القرفة والزنجبيل ضد المكورات العنقودية الذهبية المقاومة للفانكومايسين والمعزولة من انف المتعاملين مع الاطعمة في المطاعم والكافيتريات

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الخلاصـــــــة

يعتبر الاشخاص المتعاملين مع الاطعمة مصدرا رئيسيا للتسمم الغذائي بالمكورات العنقودية في البلدان المتقدمة.

الهدف: تهدف هذه الدراسة الى تحديد مدى انتشار المكورات العنقودية الذهبية المقاومة للفانكومايسين في تجويف انف الاشخاص المتعاملين مع الاطعمة في المطاعم والكافتيريات ،وتهدف ايضا الى تقييم فعالية المضاد البكتيري لمستخلصات القرفة والزنجبيل ضد هذه البكتيريا.

طريقة العمل: تم جمع١٠٠ مسحة من تجويف انف الاشخاص المتعاملين مع الاطعمة ، وتم زراعتها في أوساط زرعية للكشف عن المكورات العنقودية الذهبية، المكورات العنقودية الذهبية المقاومة للميثيسيلين والمكورات العنقودية الذهبية المقاومة للفانكومايسين. تم تحضير المستخلصات المائية والايثانولية لكل نبات باستخدام جهاز السوكسليت و استخدمت طريقة الاكار لتحديد فعالية المضاد البكتيري لهذه المستخلصات ضد المكورات العنقودية الذهبية المقاومة للفانكومايسين.

النتائج: تم الحصول على ٣٠ (٣٠%) عزلة من المكورات العنقودية الذهبية من مجموع ١٠٠ مسحة. كان من بينها ٢٥ (٨٣.٣%) عزلة من المكورات العنقودية الذهبية المقاومة للميثيسيلين ، في حين تم الحصول على ٢٠ (٨٠%) عزلة من المكورات العنقودية الذهبية المقاومة للفانكومايسين من بين٢٥ (٨٣.٣%) عزلة من المكورات العنقودية الذهبية المقاومة للميثيسيلين..

أظهرت المستخلصات النباتية نشاطا فعالا عاليا ضد المكورات العنقودية الذهبية المقاومة للفانكومايسين. كما بينت الدراسة ان مستخلصات القرفة تمتلك فعالية عالية ضد البكتريا عند مقارنتها مع مستخلصات الزنجبيل، في حين أن المستخلص الإيثانولي لكل نبات كان أكثر فعالية من المستخلص المائي.

الخلاصة: وجدت هذه الدراسة ازدياد نسبي في معدل انتشار المكورات العنقودية الذهبية المقاومة للفانكومايسين في تجويف انف الاشخاص المتعاملين مع الاطعمة. كذلك وضحت بان القرفة والزنجبيل هما مصدرا غنيا للمركبات المضادة للميكروبات التي يمكن أن تستخدم كمواد طبيعية حافظة للأغذية ولمعالجة الالتهابات التي تسببها هذه البكتيريا.

الكلمات الرئيسية: المتعاملين مع الأطعمة، المكورات العنقودية الذهبية، المكورات العنقودية الذهبية المقاومة للميثيسيلين ،المكورات العنقودية الذهبية المقاومة للفانكومايسين ، نقل البكتريا بواسطة التجويف الأنفي، القرفة، ،الزنجبيل ،فعالية المضاد البكتيري.