The Effect of Cinnamon Oil on Multi-Drug Resistant Klebseilla Pneumoniae Strains.

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Abstract

The study was carried out to assess the effect of cinnamon oil on multi-drug resistant (MDR) strains of *Klebseilla pneumoniae* isolated from various clinical specimens. 150 specimens were collected from Al-Hakeem Teaching Hospital in Al-Najaf Governorate, 21 isolates were found to be *Klebseilla* spp. On further study at the species level only six of these isolates were found to be multi-drug resistant *K. pneumoniae*. The results of antibiotic sensitivity test showed that most of these isolates were highly resistant (resistance percentage was 50-80%) for the used antibiotics, these strains were defined as a multi-drug resistant *K. pneumoniae*. The antimicrobial effect of cinnamon oil on these isolates was tested via performing disk diffusion method, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) tests. The results showed a similarity in the antimicrobial actions between this oil and Ceftazidim, Cefotaxim, and Ciprofloxacin in 50% of cases. The study concluded that cinnamon oil could be used as an effective antimicrobial even in case of difficult treated MDR *K. pneumoniae* as well as it could be used as a safe external ointment in the treatment of infected wounds and vaginal infections.

الخلاصة

أجريت هذه الدراسة لتَقييم تأثير زيت الدارسين على بكتيريا أل *Klebseilla pneumoniae* المتعددة المقاومَة للمضادات الحياتية والمعَزولة مِنْ نماذج سريريةِ مُخْتَلِفةِ.إذ جمع 150 نموذجا من مستشفى الحكيم التعليمي في محافظةِ النجف،وتبين ان 21عزلة من هذه العزلات كَانت *Klebseilla* spps بدِراسَة أخرى لمستوى النوعَ تبين ان ستّة مِنْ هذه العزلات فقط كَانتْ من نوع *K. pneumoniae* ألمتعدّدةِ ألمقاومة للمضادات الحياتية .أظهرت نَتائِجَ اختبار حسّاسيةِ المضادات الحيوية بأنّ أغلب هذه العزلاتُ كَانتْ مقاومة جداً (تراوحت ألنسبة ألمئوية للمقاومةِ بين 50-80 %) للمضادات الحيوية المستعملةِ، وتم التاكد فيما بعد ان هذه العزلات *K. pneumoniae* المتعددة المقاومَة للمضادات الحياتية (MDR). أختبر تأثير زيت الدارسين على هذه العزلات عن طريق إجراء الاختبارات التالية طريقةِ انتشار القرص، اختبار التركيز التثبيطي الأدنى واختبار تركيز الحَدّ الأدنى المبيد للبكتريا وقد أظهرت النَتائِج تشابها في التأثير المضاد للمكروبات بين هذا الزيت والمضادات التالية Ceftazidim وCefotaxim و Ciprofloxacinفي 50 % مِنْ الحالاتِ. استنتجت الدراسة بأنّ زيت الدارسين يُمْكِنُ أنْ يُستَعملَ كمضاد فعّال للإحياء المجهرية حتى في حالة *K. pneumoniae* المتعددة المقاومَة للمضادات الحياتية بالإضافة إلى إمكانية استعمالة كمرهم خارجي آمن في معالجةِ التهاب الجروح والإصاباتِ المهبليةِ.

Introduction

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases (Tepe *et al.,* 2004). World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare. Medicinal plants and their essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal, and antioxidant properties (Burt, 2005).

Plants essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots). They can be obtained by expression, fermentation or extraction but the method of steam distillation is most commonly used for commercial production (Sylvestre *et al.,* 2006). Essential oils are complex mixers comprising many single compounds. Chemically they are derived from terpenes and their oxygenated compounds. Each of these constituents contributes to the beneficial or adverse effects (Suhr and Nielsen, 2003).

Cinnamon which is native to India and Sri Lanka (Ceylon) and now it is cultivated in many tropical countries including Mexico considers one of the most important medicinal plants. The scientific name for cinnamon is *Cinnamomum verum* which belongs to the family *Lauraceae*, Its medicinal parts include the outer bark, inner bark, leaves, and essential oil. The active principles in those parts are the volatile oils (cinnamaldehyde, eugenol, cinnamic acid, weitherhin), mucilage, diterpenes, and proanthocyanidins (Soliman and Badeaa, 2002). Most of the cinnamon extracts are safe and having little side effects. Their essential oil contains both antifungal and antibacterial activity that can be used as antibiotics and to prevent food spoilage due to bacterial contamination (Dragland *et al.,* 2003), it is also possesses anti-diabetic property (Broadhurst *et al.,* 2000). For these reasons, the present study was conducted to evaluate the ability of cinnamon oil to inhibit the growth of different multi-drug resistant *K. pneumoniae* isolates with different resistance patterns.

Materials and Methods

The present study was carried out in the Department of Microbiology, Medicine Collage, Kofa University in the period from August 2005 to April 2006. The specimens were collected from Al-Hakeem Teaching Hospital in Al-Najaf Governorate. Isolation of pathogenic bacteria from clinical specimens and identification to the species level were performed by standard methods (MacFaddin, 2000) and by using of API-20 system. The antimicrobial sensitivity test was done according Kirby-Bauer disc diffusion method standardized as per NCCLS (2002). Antibiotics were selected according to WHO model list of essential drugs. The chosen antibiotics included Amoxicillin, Ceftazidim, Cefotaxim, Nitrofurantoin, Ciprofloxacin, Clareth-romycin, Tetracycline, Trimethoprim, Amickacin, and Cephalothin. Extended spectrum β-lactamase (ESBLs) producing strains were screened by the double-disk synergy test and confirmation tests were recommended by the National Committee for Clinical Laboratory Standards (NCCLS). Results were interpreted according to NCCLS (2002) standard tables.

Cinnamon Oil Disk Preparation

The disks were prepared according to **Barry (1976)** in which empty sterilized discs (Whatman no. 6mm diameter) were impregnated with 50 µL per disk with different concentrations (1:100, 1:50, 1:25, 1:12.5, and 1:6.25 mg/ml) of cinnamon oil and kept in a sterile container in the refrigerator to be used during one month. The disks were placed on the cultured Mueller-Hinton agar surface and all petridishes were sealed with sterile laboratory parafilm to avoid eventual evaporation of the test samples. The plates were left for 30 min at room temperature to allow the diffusion of oil, and then they were incubated at 37°C for 18 h. After the incubation period, the zone of inhibition was measured. Inhibition of bacterial growth in the plates containing test oil was judged by comparison with growth in blank control plates.

The MICs were determined as the lowest concentration of oil inhibiting visible growth of *K. pneumoniae* on the agar plate. according to the method recommended by the NCCLs (2002)**.**

Results and Discussion

New and safe antimicrobial agents are needed to prevent and overcome severe bacterial infections and the problems of bacterial resistance (Harry *et al.,* 2005). Plants essential oils and extracts especially cinnamon have been used for many thousands of years, in pharmaceuticals, alternative medicine, and natural therapies. It is necessary to investigate this plant scientifically to improve the quality of healthcare.

In this study, twenty one isolates of *Klebseilla* spp. were isolated from 150 specimens collected from different sources including wound, urine, vaginal, and tonsil swabs (Table 1). Six of these isolates were fully identified for the species level and characterized as *K. pneumoniae.*

Table 1. The site of specimen collection for the chosen K. pneumoniae isolates

Strains	Site of specimen collection
1	Tonsils
2	Wound
3	Wound
4	Urine
5	Urine
6	Vagina

Antibiotic sensitivity test (AST) was performed for all strains using 10-different antibiotic disks including; Amoxicillin, Ceftazidim, Cefotaxim, Nitrofurantoin, Ciprofloxacin, Clarethromycin, Tetracycline, Trimethoprim, Amickacin, and Cephalothin. The results of AST were recorded for the 6-chosen strains,which showed a high degree of resistance
in most of the isolates. The total resistance for all strains ranged from (50% -80%) (Table 2). Amoxicillin, Tetracycline, Clarethromycin, and Cephalothin recorded a high resistance percentages (100%) in comparison to a lower percentages (ranged from 0-16.67%) recorded for Cefotaxim, Nitrofurantoin, Ciprofloxacin, Trimethoprim, and Ceftazidim.

Table 2. Antibiotic sensitivity test for the 6- K. pneumoniae strains

Strains	*Amox.*	*Tetra.*	*Cefot.*	*Nitrof.*	*Cefta.*	*Trime.*	*Cephal.*	*Cipro.*	*Clarth.*	*Amik.*	*Resistance percentages*
1	R	R	S	M	S	S	R	S	R	M	40%
2	R	R	S	M	S	R	R	S	R	M	50%
3	R	R	M	S	S	S	R	S	R	S	40%
4	R	R	S	M	S	M	R	M	R	R	50%
5	R	R	S	M	S	M	R	M	R	R	50%
6	R	R	R	R	R	S	R	S	R	R	80%
Resistance percentages	100%	100%	16.67%	16.67%	16.67%	16.67%	100%	0	100%	50%	

R, Resistant; M, Medium Resistance; S, Sensitive*; Amox.*, Amoxicillin*; Tetra.*, Tetracycline*; Nitrof.*, Nitrofurantoin*; Cefot.*, Cefotaxim*; Cefta.*, Ceftazidim*; Trime.*, Trimethoprim*; Cephal.*, Cephalothin*; Cipro.*, Ciprofloxacin*; Clarth.*, Clarethromycin; *and Amik.*, Amickacin.

All strains were further tested for the production of extended spectrum β – lactamase enzyme. It was found that only one strain (strain no. 6) (16.7%) was able to produce extended spectrum β–lactamase (ESBLs) enzyme. ESBLs producing strains emerge by point mutation from non-extended-spectrum precursors, these enzymes are derived through a single amino acid substitution or a few amino acid substitutions from the parental enzymes TEM-1, TEM-2, and SHV-1. These enzymes are able to resist the effect of extended - spectrum Cephalo-sporins specially 3rd and 4th generation cephalosporins (Radice *et al.,* 2001).

Disc diffusion method was used to evaluate the zone of microbial growth inhibition at various concentrations of cinnamon oil (Table 3). Results showed that zones varied from 2-26 mm in diameter, and strains 1 and 4 were highly susceptible to the oil in comparison to a lower susceptibility recorded by strains 2, 3, and 6.

Strain no. 6 which is ESBLs-producing strain as mentioned previously proved to be sensitive to the effect of cinnamon oil in which the growth was inhibited with a zone of 13 mm in diameter, this zone considers a good result if it is compared it with the results of antibiotic sensitivity test in which it formed 80% resistance for the tested antibiotics (Table 2). Depending on these results, it can be concluded that cinnamon oil antibacterial effect is almost close to the effect of clavulonic acid and aztreonam combined Cephalosporins.

Similar results were recorded by Prabuseenivasan *et al.* (2006), who found that cinnamon oil (in concentration of 1:5 mg/ml) was significantly able to inhibit the growth of many pathogenic bacteria (standard strains) with various zone diameters especially *K. pneumoniae* (27.5 mm), *Pseudomonas aeruginosa* (33.3 mm), *Bacillus subtilis* (29.9 mm), *Proteus vulgaris* (29.4 mm), and *Staphylococcus aureus* (20.8 mm).

In another study Simic *et al.* (2004), suggested that cinnamon oil was very effective against pathogens even in moderate concentrations.

Table 3. Inhibition zone diameters using cinnamon oil disks

Strain	*Diameter of cinnamon oil inhibition zones (mm)*				
	1:100 mg/ml	1:50 mg/ml	1:25 mg/ml	1:12.5 mg/ml	1:6.25 mg/ml
1	*0*	*1.5*	*8*	*12*	*26*
2	*0*	*0*	*10*	*12*	*19*
3	*0*	*0*	*5.5*	*8*	*15*
4	*0*	*2*	*9*	*12*	*20*
5	*0*	*0*	*0*	*0*	*0*
6	*0*	*0*	*2*	*6*	*13*

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of cinnamon oil were determined and compared with the results of antibiotic sensitivity test (Table 4). The results revealed that cinnamon oil showed maximum activity with MIC values ranging from 1:25 mg/ml to 1:12.5 mg/ml in most of the tested strains (1,2,3,4, and 6) while the MBC was 1:6.25 mg/ml. By comparing the results of Table 4 with the results of disk diffusion test (Table 2) the oil was found to be strongly bactericidal especially the concentration 1:6.25 proved to be a cidal concentration to almost all tested strains and its action was similar to the action of Ciprofloxacin. The oil was able to inhibit the growth of most tested strains with moderate MIC values with the exception of strain no. 5 in which it was non effective. Similar results were reported by Prabuseenivasan (2006), and Mau *et al.* (2001), who stated that cinnamon oil showed promising inhibitory activity even at low concentration and the antibacterial activity of cinnamon oil was probably due to their major component, cinnamaldehyde and their properties could be multiple.

Table 4. Determination of MIC of cinnamon oil at various concentrations

Strains	***Minimum inhibitory concentration***				
	1:100*	***1:50****	***1:25****	***1:12.5****	***1:6.25****
1	+	−	−	−	−
2	+	+	+	−	−
3	+	+	−	−	−
4	+	−	−	−	−
5	+	+	+	+	+
6	+	+	−	−	−

* mg/ml
+: growth
−: no growth

In conclusion, cinnamon oil showed antibacterial activity against five of the tested strains so it could be considerd as a good source of antibacterial agent and it can be used as antibacterial supplement in the developing countries even in the treatment of MDR *K. pneumoniae*. In addition to *in vivo* studies clinical trials would be needed to justify and further evaluate the potential of this oil as an antibacterial agent in topical or oral applications.

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