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Mohammed Younus Naji Al Atbee
University of Basrah
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Incidence of Nocardiosis among Patients withLower Respiratory Tract Infections and Its Susptibility to Antibiotics and Disinfectants

1Enas A. Bady, College of Pharmacy, University of Basra, Iraq
eabdulsahib@yahoo.com
2Kawther H.Mahdi, College of Science, University of Basra, Iraq
Khmahdi.kh@gmail.com
3Mohammed Y. Naji, College of Medicine, University of Basra, Iraq
mohammednephroclinic@gmail.com
4Dheyaa B. Al-Rubeai, Director of respiratory and chest disease centre, Basra, Iraq
Hhome60@yahoo.com

Abstract

A total of 93 patients with tuberculosis and bronchitis were recorded. The patients ranged from 10 to 98 years of age and 57 male and 36 female. Culturing of sputum samples onto Sabouraud dextrose agar was performed to the isolation of *Nocardia*. A total of 27 isolate of*Nocardia* were isolated in this study; 5 isolates were tuberculosis, 10 isolates were bronchitis, 5 isolates were tuberculosis and smoking, 1 isolate was tuberculosis, smoking and diabetes mellitus, 2 isolates were bronchitis and diabetes mellitus and 4 isolates were bronchitis and smoking.The *Nocardia* isolates were showed the highest susceptibility against ofloxacin and ciprofloxacin and the lowest susceptibility against cefotaxime and erythromycin, but resistant to oxacillin and rifampicin. Five isolatesexamined for susceptibility against hand sanitizer gels and disinfectants. The results showed that the isolateswere susceptibile against three hand sanitizer gels and nine disinfectants.

Keywords**:***Nocardia*, respiratory tract infections, antibiotics, disinfectants, sanitizer gels.

Introduction

Scientific classification of *Nocardia*according to (6):

Phylum: Actinobacteria

Class: Actinobacteria

Order: Actinomycetales

Sub order: Corynebacteraceae

Family: Nocardiaceae

Among the actinomycetes, members of the genus *Nocardia* are with the exception of mycobacteria, the most commonly implicated pathogens in human disease, mostly as opportunists (9**)**.*Nocardia* causes a variety of human infections including cutenous, pulmonary and systemic nocardiosis and most commonly presents as pulmonary disease (49).*Nocardia* is a gram- positive aerobic bacteria and partially acid –fast (21) and can formed filamentous branches that fragment into rod or coccid cells similar to fungal hyphae (47). *Nocardia* species is found world widely in the soil and water. Infections acquired by inhalation to the pulmonary routes or traumatic inoculation to the cutaneous

routes in immunocompetent and immunocompromised patients (27) such as patients with AIDS (49), elderly persons (38), people on chronic steroid therapy, those with cancer, organ or bone marrow transplantation and HIV(61), diabetes mellitus and liver cirrhosis (57),sickle cell anemia(58).*Nocardia* infection is called nocardiosis which may lead to secondary and fatal involvement with the brain, lung and meningitis alone or in combination with other organs and associated with a high mortality, especially when an appropriate antibiotic treatment is delayed (49).Pulmonary tuberculosis mimics pulmonary nocardiosisboth clinically and radio logically and many a time it is wrongly treated with anti- tuberculosis drugs (20).

Hospital and community-acquired infections constitute a serious public health problem all over the world (32). Hospital acquired (nosocomial) infections are infections developing in hospitalized patients (3). Community-acquired infections on the other hand are those acquired anywhere other than in a healthcare facility such as schools, exercise facilities, or any place where people come in contact with others or with surfaces that have been contaminated(32). Hands are regarded as a major source of transmitting infection. It has been estimated that there are not less than 10000 organisms per cm2 of normal skin. This includes both nonpathogenic resident flora as well as pathogenic transient flora (17). More than1.4 million people worldwide are suffering from infections acquired in hospitals. These nosocomial infections are also, in most cases, the result of poor hand hygiene. Thus, hand hygiene is a key component of good hygiene practices in the home and community and can produce significant benefits in terms of reducing the incidence of infection, most particularly gastrointestinal infections but also respiratory tract and skin infections (12). Decontamination of hands can be carried out by washing hands with soap or by the use of hand sanitizer gels which may be either alcoholic (ethanol, isopropanol, and/or n-propanol are used) or non-alcoholic (benzalkonium chloride or the chlorinated aromatic compound triclosan or povidone-iodineaqueous) (40).

Disinfectants are chemicals agents that destroy the growing forms of bacteria but do not destroy spore forms of microorganism. Disinfectants are applied on lifeless things resembling floor and work benches as phenols, chlorhexidine, hypochlorite and alcohol (64). The present investigation aimed to isolation of *Nocardia* from patients with respiratory infections and testing inhibition of *Nocardia* by antibiotics, hand sanitizer gels and disinfectants.

Materials and Methods

A total of 93 sputum samples were collected from patients with respiratory infections in the respiratory and chest disease center in Basra city from December 2012 to February 2013.The patients were included tuberculosis 44 and bronchitis 49, 13 were immunocompromised (with diabetes mellitus diseases) and 20 were smoking. The patients ranged from 10 to 98 years of age and 57 male and 36female.The clinical specimens from the sputum were inoculated onto Sabouraud dextrose agarfor 3 weeks at 37°C (59).The antifungal agent cyclohexamide (actidione) at 50 µg /ml was added to the sterilized media at 46°C (50) .Colonies were then stained with Gram staining and acid- fast staining by Ziehl-Neelsen method (10).Conventional and specific biochemical tests were used for the identification of *Nocardia*(27).

The isolates were tested for their sensitivity toantibiotics by using the standard disc diffusion method (6). A sterile cotton swab soaked in the bacterial suspension was used to inoculate the organisms onto the surface of Mueller-Hinton agar plates. The plates were incubated at 35°C for 48 hours. The resultant inhibition zone diameter for each disc was measured according to Kirby-Bauer method.

Seven types ofalcohol hand sanitizer gelsand ethanol 70%(prepared at the moment of use by mixing 70 ml absolute ethyl alcohol and 30 ml distilled water) were used in this study purchased

from local market and pharmacy as in table (1). The agar well diffusion assay as described by (39) was used to determine the inhibitory effect of variousalcohol hand sanitizer gels and ethanol 70% on the bacterial growth. A sterile cork-borer (5 mm diameter) was used to make wells in the set agar. A McFarland 0.5 (1X10^8 CFU/mL) standardized bacterial suspension is swabbed over the surface of a Mueller- Hinton agar plate. 100il of the alcohol hand sanitizer gels and ethanol 70% were added to each well and the plates were incubated overnight at 37°C. Antibacterial activity was recorded if a zone of growth inhibition around the well is present (45).

Table (1).Alcohol hand sanitizer gels used in the study and their ingredients.

No.	Product	Ingredients	Origin of Industrial Co.
1	FEAH	Aqua, carbomer, ethanol, aminomethylpropanol, isopropyl myristate, propylene glycol, vitamine E, fragrance.	U.A.E.
2	MARCH	Ethanol ,glycerin,vitamine	Amman
3	HYGEL	62% ethanol ,glycerin	U.A.E.
4	WORLD WORKS	62% ethanol	China
5	HiGeen	70% ethanol, glycerin,carbomer, Acrylate/c10-30 akyl acrylate cross polymer, triethano- lamine, hydroxyl propyl,methylcellulose, tocopherylacetate,cellulose,lactose .	Jordon
6	Chicco	Alcohol denat, copolymer, propylene glycol, tetrahydroxy propyl, ethylenediamine.	Italy
7	Clean	75% ethanol,carbomer	Syria
8	Ethanol 70%	Ethanol 70%	British

Eleven types of disinfectants were purchased from local pharmacy are used in this study as intable (2). The antibacterial activity was determined according to (39, 45). Results were analysed statistically by T-test.

Table (2).Disinfectants used in this study and their formulations and concentrations.

No.	Disinfectants	Formulation	Origin of Industrial Co.	Conc.

1	Poviofix	Povidone- iodine	Turkey	10 %
2	Sarttol	Dichloro-meta Xylen,IPA,soap and pine oil	Jordon	-
3	MTG	Dichlorohexadine, IPA and potassium chloride	Jordon	-
4	Flash	Dimethyle benzyl ammonium chloride and hydrochloric acid	Turkey	10 %
5	Happy	Hydrogen,peroxide, asylcator	Turkey	-
6	Formaldehyde	Formaldehyde	British	-
7	H_2O_2	H_2O_2	British	3%
8	Dettole	Chloroxylenol	Iraq	-
9	SEHAT	Sodium hypochlorite	Iran	-
10	ZODA	Sodium hypochlorite	Iran	-
11	Isopropanol	Isopropanol	British	%100

Results

Among the 93sputum samples studied, a total of 27 nocardial isolates were detected from 25 sputum samples as 11 isolates from10 samples of tuberculosis patients and16 isolates from 15 samples of bronchitis patients (table3). Five isolates from 5 patients with tuberculosis only, 5 isolates from 4 patients with tuberculosis and smoking,1isolate from1patient with tuberculosis, smoking and diabetes mellitus,10 isolates from 10 patients with bronchitis only,2 isolates from2 patients with bronchitis and diabetes mellitus and 4 isolates from 3 patients with bronchitis and smoking. Among of the 27 *Nocardia*isolates, 22 were detected in males and 5 in females(table 4).

Table (3): Clinical data of sputum samples.

sputum samples	No. of samples	No. of *Nocardia* positive samples	% of positive samples	No. of*Nocardia* isolates
Tuberculosis	44	10	22.73	11
Bronchitis	49	15	30.61	16
Total	93	25	26.9	27

Table (4): Clinical data of positive *Nocardia* groups.

Positive groups	Age	Male	Female	Total No.	No. of*Nocardia* isolates

Tuberculosis	**30-48**	**3**	**2**	**5**	**5**
Tuberculosis and smoking	**36-55**	**4**	**-**	**4**	**5**
Tuberculosis ,smoking and diabetes mellitus	**24**	**1**	**-**	**1**	**1**
Bronchitis	**12-98**	**7**	**3**	**10**	**10**
Bronchitis and diabetes mellitus	**31-45**	**2**	**-**	**2**	**2**
Bronchitis and smoking	**40-47**	**3**	**-**	**3**	**4**
Total	**12-98**	**20**	**5**	**25**	**27**

The activity of 14 antibiotics against 14 isolates of *Nocardia* is presented in (table5). The sputum isolates of *Nocardia* were recorded significant differences, the highest susceptibility against ofloxacin and ciprofloxacin and the lowest susceptibility against cefotaxime and erythromycin. The susceptibility to other antimicrobial agents was variable; 50% of the isolates weresusceptible to kanamycin, 36% to amikacin, penicillin and gentamycin, 29% to nalidixic acid and vancomycin, 21% to tobramycin and 14% to clindamycin. All the isolates were resistant to oxacillin and rifampicin. Twenty-nine per cent of the isolates showed intermediate susceptibility to erythromycin, 21% to nalidixicacid, 14% to clindamycin and rifampicin and 7% to gentamycin, tobramycin, amikacin andvancomycin.

Table (5): Susceptibility percentage of *Nocardia* isolates against antibiotics according to (10).

Antbiotics	Disk potency	%Susceptible	%Intermediate	%Resistant
Ofloxacin	**5µg**	**57.1**	**0**	**42.8**
Ciprofloxacin	**5µg**	**57.1**	**0**	**42.8**
Kanamycin	**30µg**	**50**	**0**	**50**
Amikacin	**30µg**	**35.7**	**7.1**	**57.1**
Penicillin	**10U**	**35.7**	**0**	**64.2**
Gentamycin	**10µg**	**35.7**	**7.1**	**57.1**
Nalidixic acid	**30µg**	**28.5**	**21.4**	**50**
Vancomycin	**30µg**	**28.5**	**7.1**	**64.2**
Tobramycin	**10µg**	**21.4**	**7.1**	**71.4**
Clindamycin	**2µg**	**14.2**	**14.2**	**71.4**
Cefotaxime	**30µg**	**7.1**	**0**	**92.8**
Erythromycin	**15µg**	**7.1**	**28.5**	**64.2**
Oxacillin	**1µg**	**0**	**0**	**100**
Rifampicin	**5µg**	**0**	**14.2**	**85.7**

Table (6) below shows the significant differences of susceptibility pattern of 5*Nocardia* isolates to the hand sanitizers in the agar diffusion test. All alcohol hand sanitizer gels tested showed no inhibitory effect against *Nocardia* isolates except FEAH gel , HiGeen gel and ethanol 70% against isolate (3) with inhibition zone (15,12,13mm) respectively.

Table (6): Susceptibility pattern of *Nocardia* isolates to alcohol hand sanitizer gels.

Gel	*N.*(1) IZ(mm)	*N.*(2) IZ(mm)	*N.*(3) IZ(mm)	*N.*(4) IZ (mm)	*N.*(5) IZ(mm)
FEAH	-	-	**15**	-	-
MARCH	-	-	-	-	-
HYGEL	-	-	-	-	-
WORLD WORKS	-	-	-	-	-
HiGeen	-	-	**12**	-	-
Chicco	-	-	-	-	-
Clean	-	-	-	-	-
Ethanol 70%	-	-	**13**	-	-

- No inhibition

Table (7) below shows the significant differences of susceptibility pattern of 5*Nocardia* isolates to the 11 disinfectants in the agar diffusion test. Flash and Formaldehyde were the only products that showed inhibitory activity against all the tested isolates with the highest activity *(*66 mm) and the lowest(18 mm). Sarttol,MTG, H_2O_2 were active against some tested isolates and Happy, Dettole, Sehat and Isopropanolwere active against one tested isolate. Poviofix andZoda showed no activity against all the tested isolates.

Table (7): Susceptibility pattern of 5*Nocardia* isolates to disinfectant.

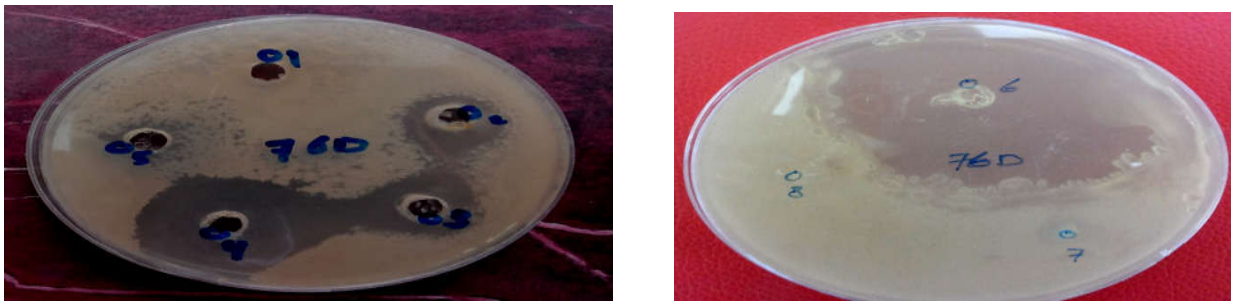
Disinfectant	*N.*(1) IZ(mm)	*N.*(2) IZ(mm)	*N.*(3) IZ(mm)	*N.*(4) IZ(mm)	*N.*(5) IZ(mm)
Poviofix	-	-	-	-	-
Sarttol	**13**	**15**	-	**17**	**20**
MTG	**12**	**15**	-	**20**	**20**
Flash	**18**	**24**	**27**	**30**	**20**
Happy	**15**	-	-	-	-
Formaldehyde	**57**	**66**	**52**	**66**	**55**
H_2O_2	**15**	**25**	**25**	-	-
Dettole	-	**20**	-	-	-
SEHAT Bleaching	-	-	**35**	-	-
ZODA Bleaching	-	-	-	-	-
Isopropanol	-	-	**25**	-	-

-No inhibition

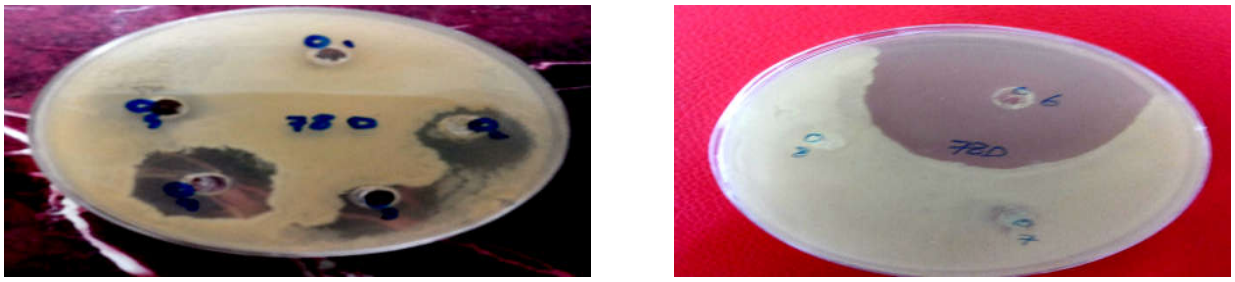

***Nocardia* isolate 2**



***Nocardia* isolate 3**



***Nocardia* isolate 4**



***Nocardia* isolate 5**

Figure (1): Inhibition zones diameter (mm) of *Nocardia* isolates against 11 disinfectants used in this study.

Discussion

Nocardia belongs to the aerobic actinomycetes that are infrequently encountered in clinical practice but are considered medically important (8, 49). Manifestations and severity of infection caused by this group are extremely variable. Their clinical presentation is often non-specific .Diagnosis is limited by the difficulty of isolation because of its slow growth and the need for invasive diagnostic procedures to obtain culture specimen (43). The microorganism was first described by Nocard in

1888 as a fungus and was further classified as an aerobic bacteria that belongs to the genus *Nocardia*, order *Actinomycetales.* (7). Nocardiosis is a worldwide disease known as an infection that affects predominantly patients with immunodepressive diseases (8).

The result of this study showed that the 27isolates were identified from a total of 93 sputum samples collected from patients with respiratory tract infections included 11 with tuberculosis and16 with bronchitis (table1). Thisresultwas in agreement with(25)whorevealed that *Nocardia*considerable occurrence among patients with pulmonary infections giving clinical symptoms similar to those occur by *M.tuberculosis*infection,therefore,must be suspicion of pulmonarynocardiosis in immunocompromised patients, especially when the clinical and radiological picture mimics pulmonarytuberculosis but sputum is negative for acid fast bacteria and patient is not responding to anti_tuberculosis drugs(20**)**. Among the 27isolates, 3were detected from diabetes mellitus patients(immunocompromised), this result was agreed with previous study that reported case of pulmonary infection with*Nocardia* in Britain in woman suffered from diabetes mellitus and liver cirrhosis probably contributed to impaired cell mediated immunity(57).

Out of the 27 *Nocardia*isolates, 22were detected in males (7 with smoking) and 5 in females. Several investigators have reported that *Nocardia*infections were more frequently recognized in males than in females (14, 29). This higher incidence of nocardiosis in males may be attributed to factors such as smoking, which was more frequently seen in males than in females in our area. The other factor may be that male and female sex hormones differently affect the growth or virulence of*Nocardia* (33).

Also, 27 *Nocardia*isolates in this study showed the highest susceptibility against quinolones such as ofloxacin and ciprofloxacin. Twenty-seven isolates in this study were resistant to oxacillin and rifampicin.The other antimicrobials showed the variable effects against 27 *Nocardia*isolates (table5).These results were in agreement withprevious studies. All *Nocardia*isolates which identified from pulmonary infections in Sudan were sensitive to ciprofloxacin**,** amikacin, clindamycin, gentamycin, Tobramycin and vancomycin, but resistance to oxacillin (24). Amikacin one of the most important antimicrobials agents effective against *Nocardia* (1, 42). Some antimicrobials such as amikacin, gentamicin and tobramycin have an effect on more than 66% of the*Nocardia* strains isolated from clinical specimens but they reported that quinolones such as ciprofloxacin and ofloxacin did not show any significant activity against the *Nocardia* isolates (30).

Hand hygiene is a means of making hands free of pathogens by using water with soap, hand rub or waterless sanitizers (16). Hand sanitizers are well-adapted to the skin (54) and work by stripping away the outer layer of oil on the skin and also remove the cutaneous microflora (4). Ethanol has the record of being the oldest skin disinfectant; it acts as a permeation enhancer when applied topically to human skin. (41). Alcohol is used as the main antibacterial component of most waterless antiseptic agents due to its antimicrobial properties (15) and alcohol-hand sanitizer gel has significantly high efficacy in reducing transient micro flora on the hand (62).The antimicrobial activity of alcohols is based on its capacity to induce microbial protein denaturation. These were reported to have excellent and rapid germicidal activity against vegetative bacteria, fungi, and many viruses (40).

The result of table (6) showed that all alcohol hand sanitizer gels used in this study no exhibited any inhibitory effect against *Nocardia* isolates except FEAH gel, HiGeen gel and ethanol 70% against *Nocardia* 3 isolate with inhibition zone (15,12,13mm) respectively. Several studies suggested that, sanitizers with at least 70% alcohol kill 99.9% of the bacterial hands (55) and effective against murine norovirus and feline calicivirus (52). Alcohols cause damage to the cell membrane and rapid protein denaturation (48). However, previous studies showed that not all sanitizers are equally effective in eradicating all germs (18, 28). While some studies reported high efficacy of hand

sanitizers in reducing the microbial flora of hand, other studies failed to show such efficacy of hand sanitizers (11, 44).

Antiseptics and disinfectants are widely used in hospitals for various topical and hard surface applications. They play an essential role in the control of infection and prevention of nosocomial infections (23). There is a wide variety of active ingredients to be found in different antiseptics and disinfectants (48). A variety of commercially available disinfectants are used by the public in their homes. In addition to commercially available products, several natural products also have been used by the public or for home health care (19). In recent years; concern for the environment has resulted in a movement to eliminate or replace antimicrobials such as disinfectants with environmentally safe or "green" alternative chemicals (53). The results of table 7 showed that the disinfectants used in this study exhibited different activity against *Nocardia* isolates. Flash and formaldehyde were more effective followed by sarttol,MTG, H_2O_2 and happy, dettole, sehat, isopropanol respectively. Poviofix andzoda showed no activity against tested isolates.

Formaldehyde as a highly effective, broad spectrum disinfectant, which typically achieve sterilization by denaturing proteins and disrupting nucleic acids (26) of fungi, viruses, mycobacteria, spores (36) and was found to be very effective in controlling the bacteria when compared with antibiotics (56). Oxidizing agents are broad spectrum, peroxide based compounds that function by denaturing the proteins and lipids of microorganisms (46) and vary in their microbiocidal range, but are considered effective on hard surfaces and equipment (31). Hydrogen peroxide is considered as a bactericidal, virucidal (non-enveloped viruses may be resistant), fungicidal and at high concentrations sporicidal. Its activity against mycobacteria is limited (36). A published suggestion for treatment of jaw osteomyelitis caused by actinomycetes (*Nocardia*) includes surgical debridement of the lesion followed by surgical fistulation to allow lavage with sterile water, hydrogen peroxide and sodium hypochlorite (2).
For many years iodine has been recognized as an effective broad- spectrum biocidal agent against bacteria, yeast and molds, Actinomycetes especially *Nocardia* and rickettsia (35). Iodine, in the form of an aqueous or alcoholic solution, has been used as an antiseptic for 150 years. The precise mode of action of iodine is unknown. Iodine penetrates into the microorganisms and attacks certain groups of proteins, nucleotides and fatty acids. This leads to eventual cell death (48).*Actenomycesisraelii*were highly susceptible to the antimicrobial action of sodium hypochlorite solution (5), due to oxidative interaction with sulfydryl on certain enzymes that can be found in the cell membrane inhibited the cellular proteins' (13) and affect bacterial DNA through the formation of chlorinated derivatives of nucleotide bases (48). A hypochlorite feed design option was chosen to inactivate and separate nocardioform bacteria within a concentrated scum manhole after aeration basin surface wasting (51). Some of the disinfectants did not inhibit the growth of the tested bacteria probably as a result of low concentrations or lack of biocides in them and or noncompliance to stringent condition (good manufacturing practices) during production among other reasons. Gross contamination of hand sanitizer during manufacturing may also compromise their effectiveness and or quality and possibly lead to infection of the users eventually (63). Most species are susceptible sodium hypochlorite, formaldehyde, ethanol, propanol, hydrogen peroxide and iodine (22). Ethyl alcohol and isopropyl alcohol causes damage to the cell membrane and rapid protein denaturation. Many alcohol products include low levels of other biocides which remain after the alcohol has evaporated (48).
Some of the sanitizers examined did not inhibit the growth of the test bacteria probably as a result of low concentrations or lack of biocides in them and or noncompliance to stringent condition (good manufacturing practices) during production among other reasons. Gross contamination of hand sanitizer during manufacturing may also compromise their effectiveness and or quality and possibly lead to infection of the users eventually (63).

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الحدوثNocardiosis
بين مرضى إصابات الجهاز التنفسي السفلي وحساسيتها لبعض المضادات والمطهرات

1إيناس عبد الصاحب بادي- كلية الصيدلة- جامعة البصرة-العراق
eabdulsahib@yahoo.com
٢ كوثر هواز مهدي - كلية العلوم - جامعة البصرة-العراق
Khmahdi.kh@gmail.com
٣ محمد يونس ناجي - كلية الطب - جامعة البصرة-العراق
mohammednephroclinic@gmail.com

٤ و ضياء بخيت الربيعي - مدير مركز الأمراض التنفسية والصدرية- البصرة-العراق
Hhome60@yahoo.com

الخلاصة

ثلاثة وتسعون مريضا يعانون من إصابات الجهاز التنفسي السفلي مثل السل الرئوي (٤٤) واخماج القصبات (٤٩) سجلت من ديسمبر ٢٠١٢-فبراير ٢٠١٣ . تراوحت أعمار المرضى من ١٠-٩٨ سنة توزعت على ٥٧ ذكر و ٣٦ أنثى، زرعت عينات البلغم على وسط سابرو يد دكستروز أكار لعزل جنس النوكارديا. عزلت ٢٧ عزلة (٥ عزلات منمرضاالسلالرئوي و ١٠ عزلات من مرضاخماجالقصباتو ٥ عزلات منمرضاالسلالرئويالمدخنينو ١ عزلة منمرضاالسلالرئويالمدخنينوالمصابينبالسكرو٢ عزلة منمرضاالتهابالقصباتالمصابينبالسكرو ٤ عزلاتمنمرضاخماجالقصباتالمدخنين).اختبرتحساسيةالعزلاتتجاهالمضاداتالحيوية، الجل المعقم لليدين و المطهرات.أظهرتالنتائجإنالعزلاتذو حساسية عالية للاوفلوكساسين و السبروفلوكساسين و حساسية واطئة للسيفوتاكسيم و الارثرومايسي وكانت مقاومة للاوكزاسلين والريفامبسين. أظهرت العزلات حساسية تجاه٣ أنواع من الجل المعقم لليدين و ٩ أنواع من المطهرات.

الكلمات المفتاحية : نوكارديا واصابات الجهاز التنفسي و المضادات الحيوية و المطهرات و الجل المعقم