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Susceptibility of *Legionella pneumophila* Isolated from Air Conditioners to Various Types of Antibiotics in Khartoum State, Sudan

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Abstract

The aim of this study was to evaluate the susceptibility of *Legionella pneumophila* isolates obtained from water samples of evaporative coolers conditioners in Khartoum State to various types of antibiotics. The number of isolated Legionella was 222 (42.3%) from a total of 525 samples. The samples were analyzed by conventional methods as well as a Real Time-PCR. Twenty isolates were subjected to disc diffusion test method. *Legionella pneumophila* isolates were found to be susceptible to the following antibiotics: ciprofloxacin (100%), azithromycin (85%), erythromycin (65%) and tetracycline (50%). *Legionella pneumophila* was resistant to Amoxicillin / clavulanic acid. Cephalexin, cefotaxim and vancomycin showed only15% sensitivity. From this study it could be concluded that Legionella *pneumophila* was more susceptible to ciprofloxacin followed by azithromycin than the other tested antibiotics.

Key words: *Legionella pneumophila, Antibiotics, Sensitivity Test.*

المستخلص

هدفت هذه الدراسة الي تقيم قابلية جراثيم الفيلقة الرئويه التي تم عزلها من مياه المكيفات بولاية الخرطوم لبعض انواع المضادات الحيوية. تم عزل 222 (42.3%) من جملة 525 عينة. تم تحليل العينات بواسطة الطرق التقليدية اضافة الي اختبار البلمرة المتسلسل الكمي اللحظي.تم اخضاع عشرون عزلة لاختبار الحساسيه للمضادات الحيوية.جراثيم الفيلقة الرئوية وجدت لها قابلية للمضادات الحيوية التالية: بيروفلوكساسين (100%)، ازيثرومايسن (85%) وارثرومايسين (65%) و تتراسيكلين (50%). باكتريا الفيلقة الرئوية كانت مقاومة لاموكسيسيلين/ حمض الكلافيونيلك. ابدت المضادات الاخري ; كالسيفالكسين وسيفوتاكسيم وفانكومايسين حساسية فقط بنسبة (15%). يستخلص من هذه الدراسة أن جراثيم الفيلقة الرئوية لها قابلية عالية للسيبروفلوكساسين يليه الازيثرومايسين أكثر من غيرهما من المضادات الحيوية تحت الاختبار.

كلمات مفتاحية : *باكتريا الفيلقة الرئوية، المضادات الحيوية، اختبار الحساسية*

Introduction

On a study by Yu *et al.,* (2002), who described 48 species of the genus *Legionella*, 90% of them confirmed to cause clinical cases due to *Legionella pneumophila*. *Legionella pneumophila* is pathogenic bacteria associated with aquatic habitat of natural and artificial environments, which are considered the causative agent of Legionnaires' disease and Pontiac fever (Fraser *et al.,* 1977). Cooling towers are the most common source of Legionellos is (Nguyen *et al.,* 2006). Legionellosis is characterized by symptoms ranging from flu – like symptoms with fever, dry cough and headache to severe pneumonia (WHO, 1990). There has been no report of human to human transmission of *Legionella* (Abu Khweek *et al.,* 2013) and inhalation or aspiration of contaminated aerosols is the most common route of infection (Cianciotto, 2001). In the Sudan 222 *Legionella* isolates were obtained from evaporative coolers air conditioners in different sites of Khartoum state by Elsanousi and Elsanousi (2017). Also a previous study was done in the Sudan to detect *Legionella pneumophila* among patients with chest infection using immunological assay, revealed (68) (22.7%) positive results of 300 cases. (Rabih *et al.,* 2014). A fatal pneumonia due to *Legionella pneumophila* was diagnosed for the first time in a young calf in northern Italy. The pathological and histological findings were very similar to those observed in human legionellosis (Fabbi *et al.,* 1998). *Legionella pneumophila* multiply intracellularly in human macrophages to avoid phagosome – lysosome fusion. There are similarities in the processes by which *Legionella* infects protozoa and mammalian phagocytic cells (Horwitz, 1993). The ability of *Legionella pneumophila* to multiply within the alveolar macrophages is a major virulence determinant. This intracellular niche affords protection from the host's immune response and some antibiotics. Macrolides and quinolones are the treatment of choice for Legionellos is (Roig and Rello, 2003). Erythromycin is the first treatment of Legionellosis, but erythomycin resistant strains can easily be obtained *in vitro* (Dowling, McDevittand Pasculle, 1985; Fraser *et al.,* 1977). Clarithromycin and azithromycin showed more effectiveness *in vitro* activity and they have better intracellular and tissue penetration than erythromycin (Stout and Yu, 1997).

Materials and Methods

A total of 525 water samples were collected from evaporative cooler air conditioners in Khartoum State .Bacterial isolation and identification were performed according to Barrow and Feltham, (2003). Real time PCR technique was used for the confirmation of the purified isolates (Sacace Biotechnologies, Italy). Antibiotic susceptibility test was done for twenty selected isolates of *Legionella pneumophila* against the following eight available antibiotics: erythromycin 15 µg (Hi media Laboratory, India), amoxicillin/clavulanic acid (20/10) µg, vancomycin 30 µg, cephalexin 30 µg, cefotaxim 30 µg, tetracycline 30 µg, ciprofloxacin 5 µg and azithromycin 15 µg(Bioanalyse- Ltd- Turkey). This test was done using disc diffusion sensitivity test method according to Cheesbrough, (2000). A plate of BCYE- α medium was dried and covered with a diluted suspension of the organism and the plate

was allowed to dry for several minutes, then the antibiotics discs were gently placed onto the surface of the medium using sterile forceps. The plates were incubated at 37 °C for 18 – 24 hours. Zones of inhibition were measured in millimeter using a ruler and defined according to the chart within the ranges. Results interpretation was done in accordance with the zone size interpretative chart of the manufacturer (Bioanalyse - Ltd- Turkey and Hi media Laboratory, India).

Results

Leginella positive results were 222 (42.3%) recovered from525 collected samples. Twenty isolates of *Legionella pnumophila* gave variable results of susceptibility to selected antibiotics. The isolates showed results as follows: 20 (100%) were susceptible to ciprofloxacin and (100%) were resistant to amoxicillin/ clavulanic acid, 17 (85%) were susceptible to azithromycin, 13 (65%) to erythromycin and 10 (50%) to tetracycline but they gave narrow zones of inhibition to: Cephalexin, cefotaxim and vancomycin, which were evaluated as 3 (15%) sensitive strains for each of them. Table (1) summarized the antibiotics sensitivity test.

Table (1): Percentage of susceptibility of *legionella pneumophila* to different antibiotics.

Antibiotics	Percentages of susceptible isolates	Percentages of resistant isolates
Ciprofloxacin	100%	0%
Azithromycin,	85%	15%
Erythromycin	65%	35%
Tetracycline	50%	50%
Cephalexin	15%	85%
Cefotaxim	15%	85%
Vancomycin	15%	85%
Amoxicillin/ clavulanic acid	0%	100%

Discussion

Patients with Legionnaires' disease require early treatment with appropriate antibiotics which can penetrate intracellular cells such as macrolides or fluoroquinolones (Edelstein and Cianciotto, 2010; WHO, 2007).These antibiotics are active against intracellular *Legionella* spp, which can survive and proliferate in human macrophages(Bruin *et al.*,2013; Onody, *et al.,* 1997).In this study, all the isolates were found susceptible to ciprofloxacin with a wide zones of inhibition, while all of them were resistant to amoxicillin/ clavulanic acid. Most of the isolates were susceptible to azithromycin, erythromycin and tetracycline but were resistant to cephalexin, cefotaxim, and vancomycin. This result agrees with previous published results by Pedro-Botet and Yu (2009), who demonstrated that the Flouroquinolones have the ability of higher intracellular inhibition to *Legionella* spp than erythromycin. Our study was also similar to the result

obtained by Dedico and Venkatesan, (1999) and Erdogan *et al.,* (2010), who demonstrated that the newer macrolides (such as azithromycin and fluoroquinolones (e.g. ciprofloxacin, levofloxacin) are recommended for the treatment of Legionnaires' disease They have a superior activity in *in vitro* and greater intracellular penetration. Tetracyclines are effective but β - lactam antibiotics are not effective (Jawetz, *et al.,* 2013). This assumption was confirmed by our study which revealed complete resistant of all tested isolates to amoxicillin/ clavulanic acid. Fifty percent of the isolates were sensitive to tetracycline and 85% of the isolates were resistant to cephalexin, cefotaxim.

Conclusion

It concluded according to this *in vitro* study that *Legionella pneumophila* was more susceptible to ciprofloxacine followed by azithromycin than the other tested antibiotics.

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