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Keywords: legionella pnemophila, buffer charcoal yeast extract agar (BCYE).

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Detection of Legionella Pneumophila in Water Air Condition in Khartoum at 2019

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ABSTRACT

Legionellae's disease affect humans causing mild to severe pneumonia, specifically in immune compromised patient. The aim of this study was to identify the causative agent and to determine the frequency of Legionella Pneumophila in water air condition.

One hundred from water samples were collected from different condition by cotton swab during November to December, environmental samples were isolated and cultured on special media Vuffer Charcoal Yeast Extract Agar (BCYE) and biochemical tests were done include: gram stain, indole, motility, citrate utilization, urease, litmus milk decolorization kligler Iron Agar Antibiotic. Sensitivity test to detect resistance of susceptibility. 22 of 100 water sample were growth the organism was gram negative bacilli sensitive to Erythromycin, Colistin, gentamycin, Amoxicillin and coloromphinoal.

This study include that legionella pneumophila exist in water air condition in Sudan with high concentration in winter further research detect during all four season.

Keywords: legionella pneumophila, buffer charcoal yeast extract agar (BCYE).

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I. INTRODUCTION

Legionella are gram-negative rods whose natural habitat is water, there are more than 50 genetically defined species of which much the most important is Legionella Pneumophila. This

species can be subdivided on the basis of deoxyribonucleic acid (DNA) relationships into three species. [1]

Legionella pneumophila caused Legionnaires' disease and Pontiac fever. 50 valid published species and subspecies and total of 71 serologic types of legionella have been isolated from either human specimens, environmental sources or both, The people who are most likely at risk to catch infection are over age 50. The risk is greater for people who suffer from health conditions such as malignancy, diabetes, lung disease or kidney disease. Other risk factors include immunosuppressive therapy and cigarette smoking. Legionnaires' disease has occurred in children and particularly confined to newborns receiving respiratory therapy, children who have had recent operations and children who are immunosuppressed, People with HIV infection and AIDS do not seem to contract Legionnaires' disease with any greater frequency than the rest of the population, however, if it is contracted, the disease is likely to be more severe compared to other cases. [2]

L. pneumophila (and other legionellae) causes pneumonia both in the community and in hospitalized immunocompromised patient. The genus is named after the famous outbreak of pneumonia among people attending the American Legion convention in Philadelphia in 1976 (legionnaires's diseases). [3]

The organism has been isolated from fresh water sources and various plumbing fixtures. Although recently discovered, L. pneumophila has been found in abundance in aquatic habitats worldwide, including chlorinated hot tubs. [4]

The organism survives in such harsh conditions because it is able to parasitize amoebae living in the water. *L. pneumophila* is not passed directly from person to person but is inhaled as aerosols from environmental sources. Legionellosis is the general heading used to include all of the diseases caused by this organism (i.e., Legionnaires' disease a pneumonia like disease, Pontiac fever a milder, short term flu like illness, and a variety of other systemic infections). Although capable of surviving extra cellular, it is classified as an intracellular pathogen because of its ability to survive and multiply inside phagosomes of pulmonary macrophages. The multiplying bacteria eventually kill the macrophages, spread, and repeat the process. The number of Legionella associated infections reported in the United States annually is 1,500 to 1,800. The CDC estimates that 10,000 to 20,000 cases go unreported each year. [5]

Legionellae are found primarily in aquatic habitats and thrive at warm temperatures; these bacteria are capable of surviving extreme ranges of environmental conditions for long periods; studies have shown that *L. pneumophila* can survive for up to 14 months in water with only a slight loss in viability. [4]

Legionella spp have been isolated from the majority of natural water sources investigated, including lakes, rivers, and marine waters, as well as moist soil. 8 Organisms are also widely distributed in man-made facilities, including air-conditioning ducts and cooling towers; potable water; large, warm-water plumbing systems; humidifiers; whirlpools; and technical-medical equipment in hospitals. [6]

There are a number of bacteria that grow only within amoebae and are closely related phylogenetically based on 16S rRNA gene sequencing to *Legionella* species; these organisms are referred to as "Legionellalike amoeba pathogens" (LLAPs). Several LLAPs have been assigned to the *Legionella* genus. One LLAP has been isolated from the sputum of a patient with pneumonia after the specimen was incubated with the amoeba *Acanthamoeba polyphaga*. Serologic surveys of patients with community-acquired

pneumonia suggest LLAP may be occasional human pathogens. [6] pneumonia affects the lungs, Legionnaires' disease is accompanied by symptoms that affect other areas of the body, About half the victims experience diarrhea and a quarter have nausea and vomiting and abdominal pain. In about 10% of cases, acute renal failure and scanty urine production accompany the disease. Changes in mental status, such as disorientation, confusion and hallucinations also occur in about a quarter of cases, In addition to Legionnaires' disease, *L. pneumophila* legionellosis also includes a milder disease, Pontiac fever. Unlike Legionnaires' disease, Pontiac fever does not involve the lower respiratory tract. The symptoms usually appear within 36 hours of exposure and include fever, headache, muscle aches and lethargy. Symptoms last only a few days and medical intervention is not necessary. clarithromycin and azithromycin) are more active against *L. pneumophila*, erythromycin has been replaced. Alternative regimens include doxycycline frequently.

II. MATERIAL AND METHOD

Study design

This was Cross-sectional study.

Study area

The study was in Khartoum Omdurman in location contain water air conditions.

Study population

The study was in water air conditioners.

Study period

The study was commenced between November 2019 and January 2020.

Sample size

100 convenience non probability sample was collected.

Data collection

was collected 100 sample by swabs then culture on Buffered Charcol Yest agar.

Ethical consideration

The Permission to sample was not used for another purpose than research.

III. EXPERIMENTAL WORK

3.1 Collection of Specimen

Sample was collected from water from air condition.

3.2 Specimens processing

Specimens were cultured as soon as possible on special media Bufferd charcoal yeast extract agar incubated at 37c for 2-3 days aerobically, after growth biochemical tests were done gram satin, indole, citrate, urase motility, kligler Iron Agar litmuse milk decolorization and Antibiotic Sensitivity test .

3.3 Biochemical identification

The environment isolate were subjected to different biochemical tests for their identification.

3.4 Indoletest

Indole test was made by incubate in tryptophan water for 24 hours at 37c, in the next day added Kovac's reagent which contains 4 (p)-dimethylaminobenzaldehyde. Reacted with the indole to produce a red colored compound. To detect break down the amino acid tryptophan with the release of indole.

3.5 Motility test

Motility test was made by single stab into the center of the semi solid medium after incubation at 37c for 24 hours movement away from the stab line or hazy appearance throughout the medium indicates a motile organism.

3.6 Citrate utilization test

Citrate test was made by culture in media contain citrate incubate at 37c for 24 hours, citrate use to identified bacteria has ability of an organism to use citrate as its only source of carbon. Colored is utilized from green to blue.

3.7 Urease test

Urase test was mad by culture in media contain urea incubate at 37c for 24 hours, urase use to detect bacteria produces urase enzyme. colure a changed to pink colure.

3.8 Litmus milk decolorization test

Litmus milk decolorization test was made by culture for up to 4 hours in a tube containing litmus milk. Reduction of the litmus milk is indicated by a change in color of the medium from mauve to white or pale yellow. tis test is used to identify bacteria has ability to reduce litmus milk.

3.9 Kligler iron agar

KIA was made by inoculate a tube of kligler iron agar used a sterile straight wre stab first the butt and then streak the slop, incubate at 37c for 24 hours. KIA use to identify bacteria has ability to ferment lactose and glucose .

3.10 Antibiotic Sensitivity test

Used Erythromycin, Gentamicine, Chloroamphinicol , Colistin and Amoxicillin.

III. RESULTS

Colonial morpholog

100 swabs were collected from water of air condition. All sample were cultured on buffer charcoal yeast agar.

After incubation period of 2 to 3 days, the colonies were appeared small and gery some of them were creamy to white, The colonies were shiny and glistening in appearance and convex were observed on BCYE medium .

22 of the grown culture colonies on BCYE medium were found suggestive of Legionella pneumophila; while the remaining 78 cultures did not show any growth.

Microscopic examination of smears were the organisms were Gram negative rods.

22 positive 22%
78 negative 87 %

Result of Biochemical Identification Indoletest

Brown ring negative –ve

Motility test

Line appease motile

Citrate utilization test

Colure was changed Blue colure positive +ve

Urase test

Colure was not changed negative –ve

Kligler iron agar

red / yellow

Litmus milk decolorization test

Colure was changed to white colour positive

Antibiotic sensitivity test

Legionella Pnemophila was sensitive to all antibiotic.

IV. DISCUSSION

Legionella pneumophila caused several diseases effect in human and cause Atypical pneumonia it could cause severe disease for immunocompromized patient.

A study was carried out to evaluate the prevalence and detect of bacteria of the legionella pneumophila in water air condition in Sudan.

100 of swabs sample were collected and cultured on Buffer Charcoal Yeast Agar and incubated for 2 days at 37°C, 22% of samples was positive which is high concentration a previous research did in 2014 was revealed just 6.7% positive result.

Collection of date and culture were done on winter and water air conditional was not used may that high level has been appeared.

Biochemical test was reveal Legionella Pneumophila positive in citrate test and litmus milk decolorization test and it is motile bacteria, and negative in indole test, urease test also reveal reaction of bacteria over Kligler Iron Agar Water air condition revealed other bacteria as a contamination.

Limitation

- This study was in one of four season.
- After air condition took long time in condition not sterile and renewable.
- Swab cannot take long time before been culture.

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