

SUSCEPTIBILITY OF SOME LEGIONELLA SPECIES TO EIGHT ANTIMICROBIAL AGENTS⁺

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Abstract :

Water samples were collected (96 samples) from cooling towers, five species of *Legionella* were identified, these are, *Legionella pneumophila* (75 isolate), *Legionella dumoffii* (58 isolate), *Legionella jordanis* (52 isolate), *Legionella oakridgensis* (48 isolate), *Legionella micdadei* (48 isolate), the number of isolates from the total samples .

Legionella species in the study were susceptible to Gentamicin, Streptomycin, Rifampin, chloramphenicol, Erythromycin, and resistance to Ampicillin, Penicillin and Cephalothin except *Legionella micdadei* it was sensitive to Ampicillin, Penicillin and Cephalothin and this species does not produce beta lactamase, while the other species produce beta lactamase enzyme.

المستخلص:

تم جمع (٩٦) عينة من الماء من أبراج التبريد، وتم عزل وتشخيص خمسة أنواع من اللجيونيليا وهي *Legionella pneumophila* (٧٥ عزلة)، *Legionella dumoffii* (٥٨ عزلة)، *Legionella jordanis* (٥٢ عزلة)، *Legionella oakridgensis* (٤٨ عزلة)، *Legionella micdadei* (٤٨ عزلة)، عدد العزلات هذه بالنسبة لعدد العينات الكلية (٩٦).

أنواع اللجيونيليا في الدراسة أظهرت استجابتها للمضادات الحيوية Gentamicin ، Streptomycin ، Rifampin ، Erythromycin ، Chloramphenicol ومقاومة للمضادات الحيوية Ampicillin ، Pencillin ، Cephalothin ماعدا النوع *Legionella micdadei* فكانت حساسة للمضادات الحيوية الثلاثة الأخيرة وأن هذا النوع (*Legionella micdadei*) كانت غير منتجة لانزيم Beta- Lactamase بينما الأنواع الأخرى قد أنتجت انزيم Beta-lactamase .

Introduction:

The genus *Legionella* is composed of aerobic, non spore forming, flagellated, Grams negative bacteria, the genus was named and classified when the main vector of a particularly virulent outbreak of pneumonia at an American legion convention in 1976 was isolated *Legionella pneumophila* in humans, this particular strain causes two known disease- Legionnaires disease and Pontiac fever. [1,2]

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legionnaires disease is characterised by severe pneumonia, gastrointestinal symptoms, and untreated case can lead to confusion mental disorientation and death .[3]

The mortality rate of legionnaires can reach 15%, Pontiac fever is little-understood syndrome characterised by flu-like symptoms occurring after exposure to *Legionella*.

Pneumonia due to *Legionella* species are quite similar .[4] ,the disease can be identified only by identification of the causative-bacterium or by serological techniques.

There are 39 known species of *Legionella* and 60 identified serogroup of which *Legionella pneumophila* is the main cause of illness in human, accounting for approximately 90% of reported cases of legionnaires disease . [5]

Legionella is spread on water droplets called aerosols and these are then inhaled by people near by, this leads to respiratory infection, *Legionella* can't be spread by person to person contact . [6]

Fortunately Erythromycin, Rifampin, Chloramphenicol appear to be efficacious for legionnaires disease . [7]; [4]

Materials and Methods:

Samples Collection: Ninety six water samples were collected from cooling towers.

Media:

Buffered charcoal- Yeast Extract (BCYE) agar and this media composed of. ACES buffer (N-2-acetamido-2-aminoethane sulphonic acid) 10g, KOH (pellets) 2.8g, Activated charcoal 1.5g, yeast extract 10g, α -ketoglutarate 1 g, L-systine HCL 0.4g, ferric pyrophosphate 0.25g, Agar 17g, Distilled water L. This medium was made as described by . [8]

Muller Hinton Agar (M.H.A). was used for susceptibility test.

Nutrient agar, Gelatin agar, urea agar, Blood agar, and Feely Gorman agar, were used for growing and do some tests for identification of bacteria.

Isolation and identification of bacteria

All samples were cultured on (BCYE) agar and the colonies that appeared on this medium were purified and then identified depending on [9] ; [10].

During these tests. Gram stain, shape, motility, catalase, oxidase, gelatinase urease, ferment glucose, Hippurate hydrolysis, Nitrate reduction, produced β -lactamase, phosphatase, grew on BCYE without L. systine, grown on blood agar, Nutrient agar, Feely Gorman agar

Sensitivity test

The antibiotic susceptibility of *Legionella* species was tested by the disc diffusion method . [11] The Antibiotic disc (all from Hi-media, India) were used:

Erythromycin (E) 15 mg, Chloramphenicol (C) 30 mg, Rifampin (R) 30mg, Streptomycin (St) 10mg, Gentamicin (G) 30 mg, Ampicillin (A) 25 mg, Penicillin (P) 10mg, cephalothin (Ch) 30mg.

Antibiotic discs were placed on Muller Hinton Agar at convenient intervals, then incubated at 37°C for 24-48hr. Zones of inhibition were measured in millimeter and susceptibilities were determined according to standard table.

Results :

Table (1) shows some cultured characters and biochemical reaction of *legionella* specis.

Table (1): Charactersistics and biochemical reaction of *legionella* species

Test	1 75 isolate	2 58 isolate	3 52 isolate	4 48 isolate	5 48 isolate
Gram stain	-	-	-	-	-
Shape of Bacterial cells	Short Bacilli	Short bacilli	Short bacilli	Short bacilli	Short Bacilli
Motility	+	+	+	+	+
Catalase	+	+	+	+	+
Oxidase	±	+	+	+	+
Gelatinase	+	+	+	+	-
Urease	-	-	-	-	-
Hippurate Hydrolysis	+	-	-	-	-
Nitrate Reduction	-	-	-	-	-
Produced beta lactamase	+	+	+	+	-
Produced phosphatase	+	+	+	-	-
Grow on blood agar	-	-	-	-	-
Grow on nutrient agar	-	-	-	-	-
Grow on F.G. agar	+	+	-	-	+
Grow on BCYE agar without L- systin	-	-	-	+	-
Colony flurescence blue	-	+	-	-	-

1=*Legionella pneumophila* (75 isolate)

2= *Legionella dumoffii* (58 isolate)

3= *Legionella jordans* (52 isolate)

4= *Legionella oakridgensis* (48 isolate)

5= *Legionella micdadei* (48 isolate)

Key: + positive

- Negative

± Most strain waek positive

Five species of *Legionella* were identified

Testing for the production of beta lactamase revealed differences among a five species as reported in Table (1), *Legionella Pneumophila* and *Legionella dumoffii* produced rapidly positive beta lactamase test which had an iontense red color after 10 min of incubation. The two species of *Legionella jordans* and *Legionella oakridgensis* varied in the speeds and intensities of their beta lactamase reaction, at certain times, the positive reaction is required 30 minute to 1 hrs to developed isolates of *Legionella micdadi* did not produced beta lactamase.

Result of invitro susceptibility testing of le species are shown in Table (2) .

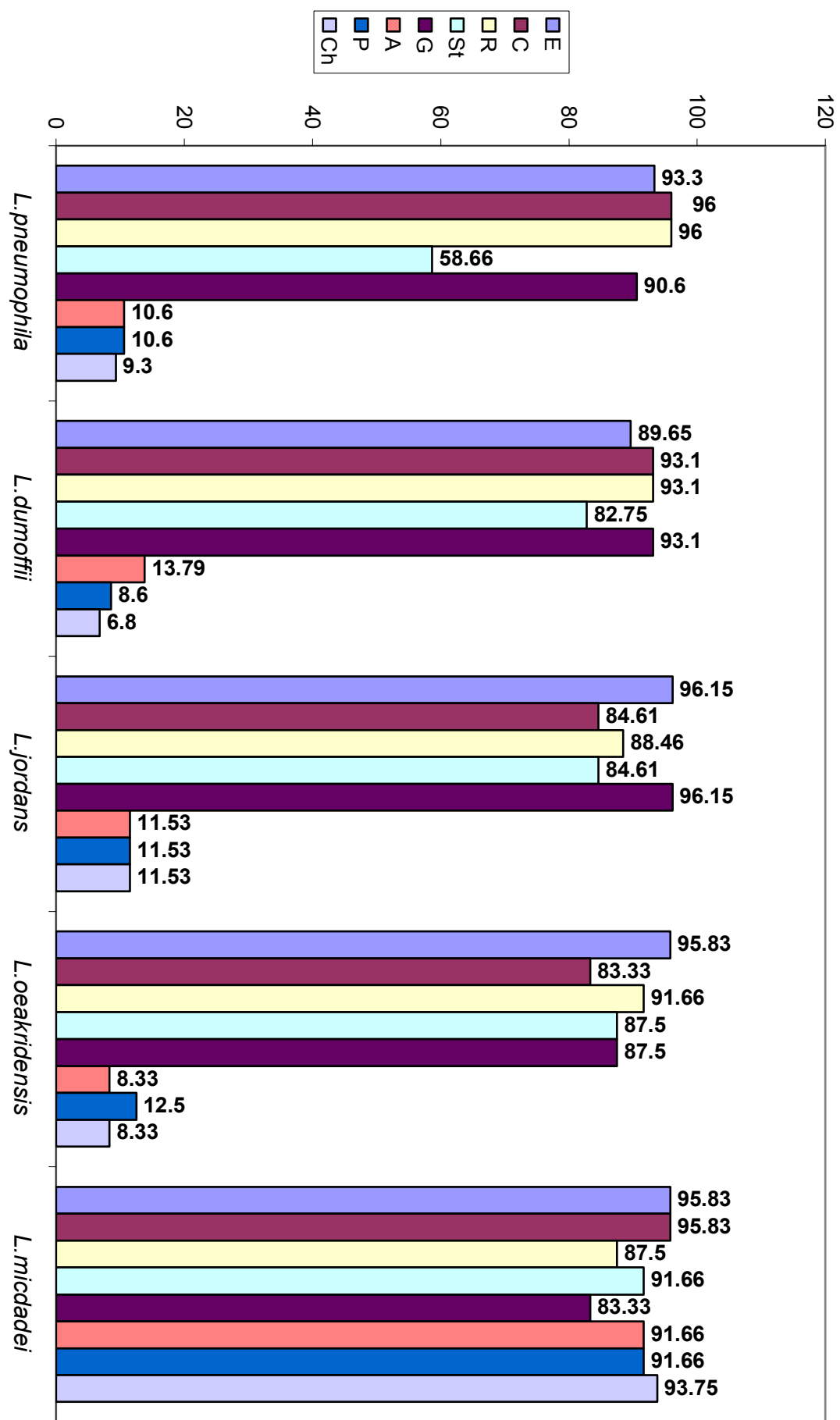
Table (2) :Antibiotic sensitivity of Legionella species isolated from 96 samples of water (cooling towers)

Legionella species	% frequency of speccies from total samples	No. Of isolated tested	E %	C %	R %	St %	G %	A %	P %	Ch %
<i>L.pneumophila</i>	78.12	75	70 93.3	72 96	72 96	44 58.66	68 90.6	8 10.6	8 10.6	7 9.3
<i>L.dumoffii</i>	60.41	58	52 89.65	54 93.1	54 93.1	48 82.75	54 93.1	8 13.79	5 8.6	4 6.8
<i>L.jordans</i>	54.16	52	50 96.15	44 84.61	46 88.46	44 84.61	50 96.15	6 11.53	6 11.53	6 11.53
<i>L.oeaktidgensis</i>	50	48	46 95.83	40 83.33	44 91.66	42 87.5	42 87.5	4 8.33	6 12.5	4 8.33
<i>L.micdadei</i>	50	48	46 95.83	46 95.83	42 87.5	44 91.66	40 83.33	44 91.66	44 91.66	45 93.75

- see material and methods for the names of antimicrobial agents
L=Legionella
S=Sensitive

The five species of *Legionella* differ in susceptibilty to Ampicillin, penicillin and cephalothin . *Legionella micidadei* is highly susceptible to Ampicillin, penicillin and cephalothin, while *Legionella pneumophila* *Legionella dumoffii*, *Legionella jordans* and *Legionella oeakridgensis* were resistente to these antibiotics (Fig 1) illusterated that.

All of *Legionella* species tested were susecptible to aminoglysocide antibiotic, all species were susceptible to Erythromycin, Rifampin, Gentumicin Chloramphenical and streptomycin, with differences between these species.



Percentage of Sensitive Strain

Discussion

Buffered charcoal yeast extract (BCYE) is the best medium for the isolation of *legionella* species, this medium is not the optimal for susceptibility testing because of its complex nature and because of inhibitory effect of BCYE agar on some antibiotic. Such as sulfonamide, polymyxin B and Tetracycline, so we used muller Hiuton agar (M.H.A) in susceptibility test .[12]

Five species of *Legionella* are identified and the frequency of these species are different, the frequency of *Legionella pneumophila* is more predominant than the other species in this study.[13]

It is found that the frequency of *Legionella pneumophila* is more than the other species and *L.pneumophila* causes approximately 90% of all documented cases of legionellosis .[14]

Legionella species are susceptible in vitro to several antibiotic including Erythromycin, Rifampin, Gentamicin, Chloramphenicol and Streptomycin and resistant to Penicillin and Cephalothin except *Legionella micdadei* is highly susceptible to Penicillin and Cephalothin because *Legionella micdadei* does not produce beta lactamase so this species is susceptible to beta lactam antibiotic, while the other species in this study produce beta lactamase and have resistance to penicillin and cephalothin, this result is documented by .[15]; [16]

Beta lactamase of *Legionella pneumophila* is more active against cephalothin than against penicillin, and this also is documented by .[17]

As noted by others [18];[17] the invitro susceptibility of *Legionella micdadei* to beta lactam and aminoglycoside antibiotic may have little clinical significance, Erythromycin to which *legionella micdadei* displays both in vivo and invitro susceptibility, had been successfully used for the treatment of *Legionella micdadei* pneumonia .[4]; [19]

Erythromycin would seem to be the best choice for therapy of infection caused by *Legionella pneumophila*, *Legionella dumoffii*, *Legionella jordanis*, *Legionella oakridgensis* and *Legionella micdadei* and this also documented by [20]

The resistance of *Legionella pneumophila*, *Legionella dumoffii*, *Legionella jordanis* and *Legionella oakridgensis* to Penicillin and cephalothin has been documented [21]; [15]; [17]

The result of this study indicates that the addition of Penicillin and Cephalothin to selective media would conceivably increase the usefulness of such media for the isolation of *legionella pneumophila*, *Legionella dumoffii*, *Legionella jordanis*, *Legionella oakridgensis*, but not for *Legionella micdadei*,

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