

UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ

FACULTAD DE CIENCIAS QUÍMICAS

LABORATORIO DE MICROBIOLOGÍA GENERAL

BIOCHEMICAL TESTS AND ANTIMICROBIAL SUSCEPTIBILITY WITH

*Klebsiella oxytoca*

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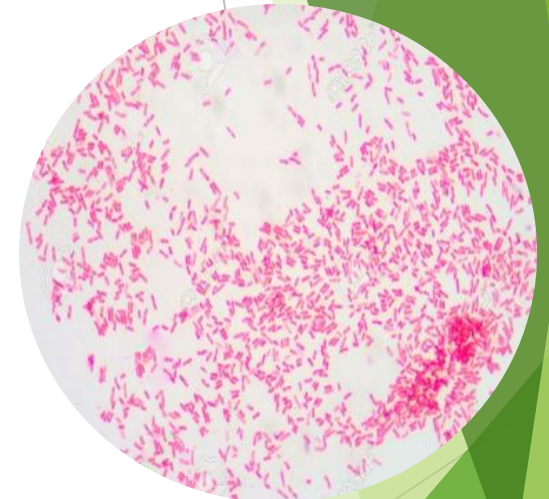
Maestra: Q.F.B. Juana Tovar Oviedo

GRUPO: 8:00 - 9:00

# INTRODUCTION

## *Klebsiella oxytoca*

- ▶ Are immobile aerobic Gram-negative bacilli belonging to the family Enterobacteriaceae.
- ▶ They are found universally in soil, water and vegetation, as well as being part of the normal intestinal flora of many animals in addition to man.
- ▶ Urinary or biliary tract infections.
- ▶ Infections polymicrobial or acquired in the hospital, especially in diabetic patients, treated with antibiotics previously or who have a previous disease of seriousness.



# INTRODUCTION CLINICAL CASE



## ► PATIENT:

Male, white, 29 years old.

Sign in for poisoning observation with Paraquat. It is given treatment with parenteral solutions and Endoxan. Three days later he started fever of  $40^{\circ}\text{C}$  and chills, associated with diarrheal stools, without blood, pujo or tenesmus. Endoxan is discontinued and sodium penicillin is started. The following day phlebitis is observed in the upper right limb and a blood culture, coproculture and biochemical test are performed.

# OBJECTIVE

- ▶ Perform the necessary biochemical tests in order to analyze and determine the proper interpretation of the results for the correct identification of a microorganism.
- ▶ Perform and determine the results of the Kirby-Bauer susceptibility test.



# METHODOLOGY

## BIOCHEMICAL TESTS

### MATERIAL

- ▶ Fat pencil or marker
- ▶ Bunsen burner
- ▶ Handles and Holders
- ▶ Racks
- ▶ Biochemical tests: A. Simmons citrate, A. Kligler iron, A. FEA, A. LIA, Middle SIM, Gelatin, Medium MIO, C. Urea, C. Malonate and Middle VP-RM.
- ▶ Reagents for biochemical tests: oxidase (tetramethyl-p-phenylenediamine hydrochloride), methyl red solution,  $\alpha$ -naphthol, potassium hydroxide, hydrogen peroxide, Kovacs reagent or Ehrlich reagent.



# METHODOLOGY

## BIOCHEMICAL TESTS

### METHOD

- 1) Gram staining
- 2) Test of oxidase
- 3) Seeds in: Test of kligler, ornithine decarboxylase, urea, Simmons citrate, LIA, FEA, SIM, methyl red, proskauer vogues and malonate.
- 4) Incubate at 37 ° C for 24h.

# METHODOLOGY

## ANTIMICROBIAL SUSCEPTIBILITY

### MATERIAL

- ▶ Fat pencil or marker
- ▶ 1 Mueller-Hinton agar box
- ▶ Sterile swab
- ▶ 1 sterile tube with 0.85% saline solution
- ▶ 24 h culture of *Escherichia coli* or *Serratia marcescens*
- ▶ Mc Farland Tube Standard 0.5
- ▶ Metal dissecting clamp
- ▶ Sensidisks

# METHODOLOGY

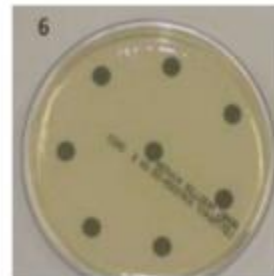
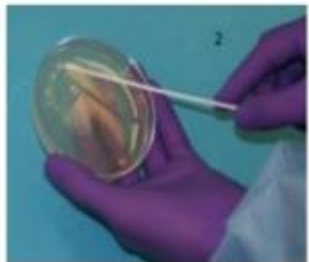
## ANTIMICROBIAL SUSCEPTIBILITY

### ► Kirby Bauer technique



Preparation of the inoculum

Inoculum intake



Incubate 37°C,  
18-24 h

Extent of inoculum

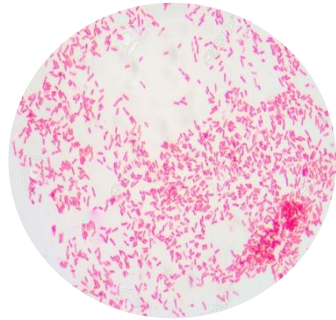
Placing antibiotic discs



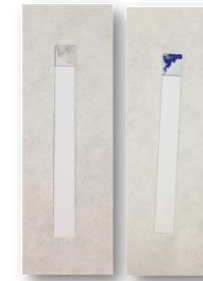
# RESULTS AND DISCUSSION

## BIOCHEMICAL TESTS

▶ Gram staining



▶ Test of oxidase  
(-)

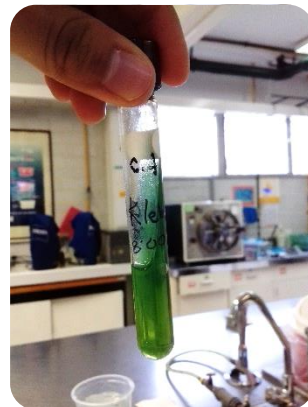


$FeCl_3$

▶ Simmons Citrate

(-)

It does not use citrate as the only source of carbon



▶ Phenylalanine deaminase (FEA)

(-)

Does not have the ability to deaminate phenylalanine



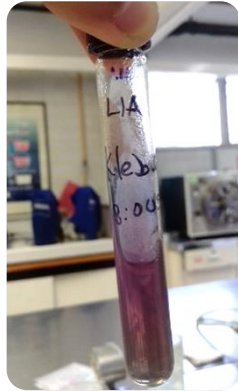
# RESULTS AND DISCUSSION

## BIOCHEMICAL TESTS

### ► LIA

(+)

It has the capacity of decarboxylation of lysine

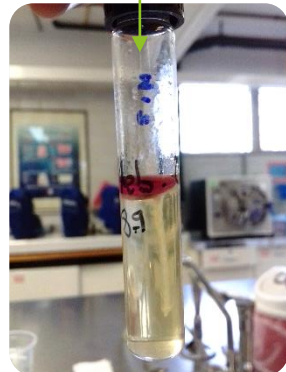


kovac

### ► SIM

(+)

It has the ability to unfold tryptophan by the enzyme tryptophanase and produce indole



### ► Kligler test

(+)

Fermentes glucose and lactose



kovac

### ► MIO

(-)

Does not contain ornithine decarboxylase  
No mobility



# RESULTS AND DISCUSSION BIOCHEMICAL TESTS

## ► Malonate

(-)

No ability to use malonate as the sole source of carbon

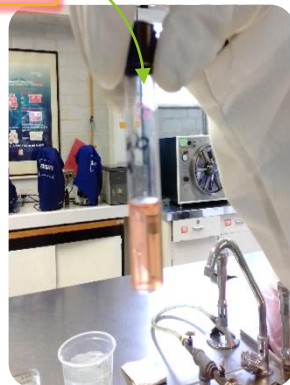


Rojo fenol

## ► Urea

(+)

It is capable of hydrolyzing urea (With enzymes)



## ► Voges proskauer

(+)

It is capable of producing the fermentation of butanediol



KOH

$\alpha$ - naftol

Rojo fenol

## ► Methyl red

(-)

Does not produce mixed acid fermentation



# RESULTS AND DISCUSSION

## BIOCHEMICAL TESTS

CUADRO 1

[Adaptado de Farmer *et al* (4)]  
**DIFERENCIACION BIOQUIMICA DE LAS ESPECIES DE KLEBSIELLA**  
 (A 24 ó 48 horas y a 36°C)

Medio diferencial	<i>Klebsiella</i>			
	<i>pneumoniae</i>	<i>oxytoca</i>	<i>ozaenae</i>	<i>rhinoscle-romatis</i>
Indol	—	+	—	—
Rojo Metilo	*	*	+	+
Voges-Proskauer	+	+	—	—
Urea	+	+	*	—
Citrato	+	+	—+	—
Malonato	**	**	—	+
Lactosa	+	+	—+	—

- † = 95% o más de las cepas son positivas
- = 0–5% de las cepas son positivas
- \*\* = 80–94.9% de las cepas son positivas
- \* = 5.1–20% de las cepas son positivas
- + = reacción es variable.

# RESULTS AND DISCUSSION

## ANTIMICROBIAL SUSCEPTIBILITY



- ▶ CRO-30 (Ceftriaxone 30 µg)
- ▶ NA-30 (Nalidixic acid)
- ▶ SXT25 (Trimethoprim / sulfamethoxazole 23.75 µg)
- ▶ CIP-5 (Ciprofloxacin 5 µg)
- ▶ AM-10 (Ampicillin 10 µg)

# RESULTS AND DISCUSSION

## ANTIMICROBIAL SUSCEPTIBILITY

Discos antibióticos	Resistente	Intermedio	susceptible
CRO-30	≤13	14-20	≥21
NA-30	≤13	14-18	≥19
SXT25	≤10	11-15	≥16
CIP-5	≤15	16-20	≥21
AM-10	≤13	14-16	≥17

- ▶ CRO-30 (Ceftriaxone 30 µg)

Diameter: 35 mm → susceptible

- ▶ NA-30 (Nalidixic acid)

Diameter: 28 mm → susceptible

- ▶ SXT25 (Trimethoprim / sulfamethoxazole 23.75 µg)

Diameter: 26.5 mm → susceptible

- ▶ CIP-5 (Ciprofloxacin 5 µg)

Diameter: 32 mm → susceptible

- ▶ AM-10 (Ampicillin 10 µg)

Diameter: 0 mm → resistant



# RESULTS AND DISCUSSION

## CLINICAL CASE

- ▶ Observing that it is a *Klebsiella oxytoca* bacterium belonging to the family Enterobacteriaceae, the patient is given treatment with:
- ▶ Gentamicin (60 mg every 8 hours)
- ▶ After ten days, the patient leaves in good condition



# CONCLUSIONS

- ▶ Based on the results by colorimetric methods, the specific biochemical activity of the bacteria was known and, at the same time, the identification of *Klebsiella Oxycota* was determined by means of the different types of biochemical tests.
- ▶ By means of the kirby-Bauer technique the different types of antimicrobial drugs from which the bacteria can be resistant or even susceptible to some specific antibiotic can be known.



# BIBLIOGRAPHY

- ▶ Boza R, Román MA. Bacteremia by klebsiella ozaenae and by klebsiella oxytoca for the purpose of five patients. Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud (INCIENSA), Clinical Laboratory, Bacteriology Section, San Juan de Dios Hospital: Costa Rica.
- ▶ Garzón J, Lemos E, Rivas R. Prevalence of extended spectrum betalactamases in Escherichia coli, Klebsiella pneumoniae and Klebsiella oxytoca of West Kennedy Hospital. Level III, Bogota. Rev. Cienc. Health / Bogotá (Colombia) 2 (2): 124-38, July-December 2004.
- ▶ García A, Mateos F. Enterobacteria. Unit of Infectious Diseases. Medina Internal Service. Complejo Hospitalario Universitario de Albacete. Albacete: Spain. Medicine. 2010; 10 (51): 3426-31.