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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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°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, α-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 vorges 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**

**Extent of inoculum**

**Placing antibiotic discs**

**Incubate 37°C, 18-24 h**
RESULTS AND DISCUSSION
BIOCHEMICAL TESTS

- Gram staining
- Test of oxidase
  
- Simmons Citrate
  
- Phenylalanine deaminase (FEA)

- It does not use citrate as the only source of carbon
- Does not have the ability to deaminate phenylalanine
RESULTS AND DISCUSSION
BIOCHEMICAL TESTS

- **LIA**
  - (+) It has the capacity of decarboxylation of lysine

- **SIM**
  - (+) It has the ability to unfold tryptophan by the enzyme tryptophanase and produce indole

- **Kligler test**
  - (+) Fermentes glucose and lactose

- **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

- Voges proskauer
  
  (+)
  It is capable of producing the fermentation of butanediol

- Urea
  
  (+)
  It is capable of hydrolyzing urea (With enzymes)

- Methyl red
  
  (-)
  Does not produce mixed acid fermentation

- KOH
- α- naftol
### CUADRO 1

[Adaptado de Farmer et al (4)]

**DIFERENCIACIÓN BIOQUÍMICA DE LAS ESPECIES DE KLEBSIELLA**

(A 24 ó 48 horas y a 36°C)

<table>
<thead>
<tr>
<th>Medio diferencial</th>
<th><strong>Klebsiella</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>pneumoniae</em></td>
</tr>
<tr>
<td>Indol</td>
<td>-</td>
</tr>
<tr>
<td>Rojo Motilo</td>
<td>*</td>
</tr>
<tr>
<td>Voges-Proskauer</td>
<td>+</td>
</tr>
<tr>
<td>Urea</td>
<td>+</td>
</tr>
<tr>
<td>Citrato</td>
<td>+</td>
</tr>
<tr>
<td>Malonato</td>
<td>**</td>
</tr>
<tr>
<td>Lactosa</td>
<td>+</td>
</tr>
</tbody>
</table>

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1. 95% o más de las cepas son positivas
2. 0–5% de las cepas son positivas
3. = 80–94.9% de las cepas son positivas
4. = 5.1–20% de las cepas son positivas
5. = 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

<table>
<thead>
<tr>
<th>Discos antibióticos</th>
<th>Resistente</th>
<th>Intermedio</th>
<th>susceptível</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRO-30</td>
<td>≤13</td>
<td>14-20</td>
<td>≥21</td>
</tr>
<tr>
<td>NA-30</td>
<td>≤13</td>
<td>14-18</td>
<td>≥19</td>
</tr>
<tr>
<td>SXT25</td>
<td>≤10</td>
<td>11-15</td>
<td>≥16</td>
</tr>
<tr>
<td>CIP-5</td>
<td>≤15</td>
<td>16-20</td>
<td>≥21</td>
</tr>
<tr>
<td>AM-10</td>
<td>≤13</td>
<td>14-16</td>
<td>≥17</td>
</tr>
</tbody>
</table>

- **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.
