استعمال المضادات الحيوية في تنظيف مزارع الكلوسترديوم

دراسة شرف: طلعت الحديد, طلعت شوام

تم دراسة تأثير التركيزات المختلفة من المضادات الحيوية الآتية:
- كورينانديوم
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- شوام
- كلوسترديوم
- كلوسترديوم

وتعد 100 ميكروجرام/ملي لي من الجنتاميسين مثمرة لتركيز تأثيري ونقيبة جميع أنواع الكلوسترديوم باختصار حيث أنها تمنع نمو جميع أنواع البكتريا المطلوبة.
USES OF ANTIBIOTICS AS AN AID FOR PURIFICATION OF CLOSTRIDIAL CULTURES
(With 2 Tables)

By
DOREYA SHARAF, A. EL-GID and M.T. SHOUMAN
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SUMMARY

The effect of six antibiotics was determined by tube dilution method on some clostridial species. The inhibitory and sublethal concentration of each antibiotic differ according to tested clostridial organism. Of all, 100 μg/ml gentamycin had in vitro the best antibacterial effect on the contaminated organisms and its use has been extended to the purification of clostridial culture.

INTRODUCTION

The determination of the drug susceptibility of anaerobic organisms is generally more complicated than for aerobic organisms. LOWBERG and LILLY (1955) and WILLIS and HOBBS (1959) using agar dilution technique stated that most pathogenic clostridia were partially inhibited, while a wide range of aerobic bacteria were completely inhibited on MacConkey plate containing 50 μg/ml neomycin sulphate except Clostridium welchii type A. SHARPAH and FERDINSON (1969) developed a new medium containing sodium sulphate, kanamycin sulphate and polymyxin B-sulphate for enumeration of Clostridium perfringens.

FARRAC (1971) and KIRKLAND (1973) stated that 100 μg/ml neomycin sulphate inhibited completely the growth of Clostridium chauvoei and partially that of Clostridium septicum, while a concentration of 25 μg/ml could be used for isolation of Clostridium welchii type A. Also GÜVEN et al. (1973) developed a selective media for isolation and purification of clostridia from Gram-negative aerobic contaminant, which contained 100-150 μg/ml neomycin and other dyes as inhibitory agents.

The purpose of the present study was to assay in vitro the effect of currently used antibiotics in purification of pathogenic clostridial cultures or vaccines.

MATERIAL and METHODS

In this investigation the following 5 clostridial species were used: Clostridium welchii types B and D, Clostridium septicum and Clostridium chauvoei, which were obtained from anaerobic section, Animal Research Institute, Dokki. In addition, 4 aerobic and facultative anaerobic bacteria were examined as coagulase positive Staphylococcus aureus, Peptostreptococcus anaerobius, Peptostreptococcus anaerobius, and B. subtilis which were isolated from contaminated clostridial cultures and identified according to BUCHANAN and Gibbons (1974). The five clostridial species were tested for their resistant as well as susceptibility to the different concentrations (5-500 μg/ml) of Kanamycin, Neomycin, Gentamycin, Erythromycin, Terramycin and Ampicillin by using tube dilution method.

Technique used:

A loopful of 3 or 4 colonies was picked from an overnight blood agar plate culture and inoculated into a tube of thioglycolate broth. The inoculated broth was incubated at 37°C for 24 hours and then diluted so that it should contain 10^5 colony forming units per ml and this dilution is used during work. These cultures were then mixed with the antibiotic and incubated at 37°C for 48 hours aerobically and anaerobically according to the type of organisms. Control tubes included with each run consisting of inoculated broth with no antibiotics as a growth control. The end point of each test was determined by subculturing each tube showing turbidity to no antibiotic containing blood agar plates. These plates were incubated aerobically or anaerobically for 48 hours. Results were recorded as:

1) Complete inhibitory concentration was read as the tube containing the highest concentration of antibiotics where there is no visible growth.

2) Sublethal Inhibitory concentration was the least concentration of any antibiotic that allows growth of clostridial organisms.

Preparation of mixtures:

The effect of the determined sublethal concentration of antibiotics which allowed the growth of some clostridial species, were tested on:

1) Clostridial suspension and mixture, of Peptococcus anerobius, Pectastreptococcus anerobius and Staphylococcus aureus.

2) Clostridial suspension and Bacillus subtilis.

These cultures and controls were incubated for 48 hours at 37°C aerobically and anaerobically. Samples were collected from each mixture and recultivated on 10% sheep blood agar and incubated aerobically and anaerobically for demonstration of the growth of clostridial species as well as the degree of inhibition of growth of other contaminant. At the same time the inoculated controls without antibiotics were recorded.

RESULT

The results showed that all species of clostridia were highly sensitive and completely inhibited by low concentration of tetracycline and ampicillin (15 and 5 µg/ml) respectively. A concentration of 200 µg/ml of gentamicin could inhibit completely all clostridial species examined. Only C. welchii types B and D resisted the action of same concentration of kanamycin. Neomycin, while 200 µg/ml erythromycin had a powerful inhibitory effect on all types of clostridia except C. chauvoei (Table 1). Partial inhibition of C. chauvoei, C. septiocom and C. oedematiens occurred at 100 µg/ml concentration of gentamicin, erythromycin, neomycin and kanamycin while C. welchii types B and D were highly resistant to the action of 100 µg/ml of neomycin and kanamycin.

In the light of these results, it was found that the sublethal concentration for growth varied according to species and type of antibiotic as it was 100 µg/ml gentamicin for all types of clostridia, 150 µg/ml neomycin for C. welchii and 100 µg/ml neomycin for other, and 150 µg/ml kanamycin and erythromycin for C. chauvoei and C. septiocom, 75-100 µg/ml for C. oedematiens and 200-250 µg/ml for C. welchii (Table 1).

Effect of antibiotics on mixtures:

The results in Table (2) showed that the concentration of 100 µg/ml of gentamicin on mixtures of clostridia and contaminant was the most effective which permitted medium growth of all species of clostridia with complete inhibition of the contaminants, either in mixtures or individually.

Addition of the appropriate concentration of neomycin and kanamycin to the media allowed heavy growth of C. welchii with complete inhibition of contaminant, while in case of other clostridial species the contaminant showed partial inhibition.

Erythromycin (in 150-200 µg/ml concentration) could purify C. chauvoei and C. septiocom cultures although the growth of contaminant was slightly affected with C. welchii and C. oedematiens.

DISCUSSION

During the last time considerable interest have been aroused by studying the effect of antibiotics on anaerobic organisms and it have been used by a number of workers as selective agents for isolating anaerobic bacteria. In the present study the effect of six types of antibiotics on five clostridial species was studied to evaluate the effectiveness of antibiotic in purifying anaerobic cultures. The results showed that gentamicin gave the best antibacterial spectrum and on mixtures of anaerobic and aerobic organisms, the growth of clostridial organisms were slightly affected while it completely inhibited the growth of the tested contaminant. This was also evident by WAIN and WELMSTEIN (1969) and NARAHARAN and BARNUM (1974), they reported that nearly all members of organisms studied were susceptible to gentamicin. Such results were in agreement with GUEN et al. (1973) and KERNOLOD (1973) who used 150 µg/ml neomycin alone or in combination with other inhibitory substances for the growth of clostridia.
Isolation and purification of *C. welchii*. It was also observed that 150 μg/ml neomycin completely inhibited the growth of *C. novyi*, *C. septicum* and *C. chauvoei*. A finding which goes hand in hand with many authors such as LOWBURY and LILLY (1955) and FARRAG (1971), a through it disagreed with WILLIS and HOBBS (1959), Ellner and O’DONELL (1971) where they recommended the use of 150 μg/ml for the improvement of growth of *C. welchii*, *C. septicum* and *C. novyi*. Considering the study of mixtures it was observed that kanamycin and neomycin could be used for purification of *C. welchii* cultures as it was highly effective on contaminants. This findings agree to large extent with KUCERS (1972). For *C. septicum* and *C. chauvoei* it was found that the best antibiotics is erythromycin which completely inhibited the growth of all contaminants with permission of clostridial organisms.

In conclusion, the results lead us to propose the addition of 100 μg/ml gentamycin for purification of clostridial cultures, since these contaminants may cause a high percentage of cultures to be contaminated and this may lead to unnecessary loss of material and extra expensive.

REFERENCES


Table (1): Sublethal and complete inhibitory concentration (μg/ml) of the used antibiotics for the growth of examined clostridia

<table>
<thead>
<tr>
<th>Tested Organisms</th>
<th>S.C. Concentrations of the antibiotics by μg/ml</th>
<th>C.I.C.</th>
<th>Erythromycin</th>
<th>Gentamycin</th>
<th>Kanamycin</th>
<th>Neomycin</th>
<th>Terramycin</th>
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</thead>
<tbody>
<tr>
<td><em>Clostridium welchii</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Types B and D</td>
<td>S.C. 75, 150</td>
<td>C.I.C.</td>
<td>100, 200, 250</td>
<td>250, 500</td>
<td>150, 250</td>
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<tr>
<td><em>Clostridium chauvoei</em></td>
<td>S.C. 200, 250</td>
<td>C.I.C.</td>
<td>100, 150, 200</td>
<td>150, 200</td>
<td>100, 150</td>
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<td></td>
</tr>
<tr>
<td><em>Clostridium septicum</em></td>
<td>S.C. 150, 200</td>
<td>C.I.C.</td>
<td>100, 200, 250</td>
<td>150, 200</td>
<td>100, 150</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clostridium oedematia</em></td>
<td>S.C. 75, 100</td>
<td>C.I.C.</td>
<td>100, 150, 200</td>
<td>100, 150</td>
<td>100, 150</td>
<td></td>
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</table>

Remarks: ug = microgram = \( \frac{1}{1000} \) mg gram
SC = Sublethal concentration
CIC = Complete Inhibitory concentration

Table (2): Effect of sublethal concentration of the used antibiotics on mixtures of the examined clostridia and the isolated contaminants.

<table>
<thead>
<tr>
<th>Examined Organisms</th>
<th>Concentration of antibiotics by μg/ml</th>
<th>Gentamycin</th>
<th>Neomycin</th>
<th>Kanamycin</th>
<th>Neomycin</th>
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<td>100, 150</td>
<td>100, 250</td>
<td>150, 200</td>
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<td>Staph. aureus</td>
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<tr>
<td>Peptococcus anerobius</td>
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</tbody>
</table>

Remarks: M = Medium growth
P = Partial inhibition of growth
C = Complete inhibition of growth
- = not tested