قســـــــم : الميكروبيولوجيا - كلية الطب - جامعة أسيوط.
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استعمال المضادات الحيوية فى تنقية مزارع الكلوستريديا

دريـــة شرف ، علـــــى الجـــــد ، طلعت شومان

تم دراسة تأثير التركيزات المختلفة من المضادات الحيويه الاتيه :
كبريتات النيومايسين - كبريتات الكانامايسين - كبريتات الجنتامايسين - الاريثروميسين الميرامايسين - الامبسيلين على خمسة عترات من الكلوستريديا هى كلوستريديم ولشياى نوع - د وكلوستريديم شوفياى - وكلوستريديم سبتكم - كلوستريديم الورمى .

وقد ثبت من التجربة أن المضادات الحيوية تختلف فى تأثيرها على كل ميكروب. كمـــا أن تركيز ١٠٠ ميكروجرام/مل من الجنتامايسين تعتبرأحسن تركيز لعزل وتنقية جميع أنـــــواع الكلوستريديا المختبرة حيث أنها تمنع نمو جميع أنواع البكتريا الملوثة .

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USES OF ANTIBIOTICS AS AN AID FOR PURIFICATION OF CLOSTRIDIAL CULTURES

(With 2 Tables)

By

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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 Ug/ml gentamycin had invitro the best antibacterial effect on the contaminated organisms and its use has been extended to the purificantion of clostridial culture.

INTRODUCTION

The determination of the drug susceptability of anaerobic organisms is generally more complicated than for aerobic organisms LOWBERRY 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 Nagler plate containing 100 Ug/ml neomycin sulphate except Cl.welchii type A. SHAHIDI and FERGUSON (1969) developed a new medium containing sodium sulphate, kanamycin sulphate and polymyxin B-sulphate for enumeration of Cl.perfringens

FARRAG (1971) and KIRROLOS (1973) stated that 100 Ug/ml neomycin sulphate inhibited completely the growth of Cl.chauvoei and partially that of Cl.septicum, while a concentration of 25 Ug/ml could be used for isolation of Cl.welchii type A. Also GUVEN et al (1973) developed a selective media for isolation and purification of clostridia from Gram-negative aerobic contaminant, which contained 100-150 Ug/ml necomycin 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 pathagenic clostridial cultures or vaccines.

MATERIAL and METHODS

In this investigation the following 5 clostridial species were used; Cl.welchii types B and D. Cl.oedematiens type B, Cl.septicum and Cl.chauvoei, which were obrained from anaerobic section, Animal Research Institute, Dokki. In addition, 4 aerobic and facultative anaerobic bacteria were examined as co-agulase positive Staph aureus, Peptococcus 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 Ug/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 cultrure and inoculated into a tube of thioglycollate broth. The inoculated broth was inculated at 37°C for 24 hours and then diluted so that it should contain 10^8 colony forming units per ml and this dilution is used during work. These cultures were then mixed with the antibiotics 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 anaerobius, Petostreptococcus anaerobius and Staphylococcus aureus.
2) Clostridial suspension and Bacillus subtillis.

These cultures and controls were incubated for 48 hours at 37°C aerobically and anaerobically. Samples were collected from each mixtures 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 terramycin and ampicillin (15 and 5 Ug/ml) respectively. A concenration of 200 Ug/ml of gentamycin could inhibit completely all clostridial species examined. Only Cl.welchii types B and D resisted the action of same concentration of kanamycin, neomycin, while 200 Ug/ml erythromycin had a powerful inhibitory effect on all types of clostridia except Cl.chauvoei (Table 1) Partial inhibition of Cl.chauvoei, Cl.septicum and Cl.oedematiens occurred at 100 Ug/ml concentration of gentamycin, erythromycin, neomycin and kanamycin while Cl.welchii types B and D were highly resistant to the action of 100 Ug/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 Ug/ml gentamycin for all types of clostridia, 150 Ug/ml neomycin for Cl.welchii and 100 Ug/ml neomycin for other, and 150 Ug/ml kanamycin and erythromycin for Cl.chauvoei and Cl.septicum, 75-100 Ug/ml for Cl.oedematiens and 200-250 Ug/ml for Cl.welchii (Table 1).

Effect of antibitics on mixtures:

The results in Table (2)showed that the concentration of 100 Ug/ml of gentamycin 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 Cl.welchii with complete inhibition of contaminant, while in case of other clostridial species the contaminant showed partial inhibition.

Erythromycin (in 150-200 Ug/ml concentration) could purify Cl.chauvoei and Cl.septicum cultures although the growth of contaminant was slightly affected with Cl.welchii and Cl.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 effectivness of antibiotic in purifying anaerobic cultures. The results showed that gentamycin 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 WAITZ and WEINSTEIN (1969); HARIHARAN and BARNUM (1974), they reported that nearly all members of organisms studied were susceptible to gentamycin. Such results were in agreement with GUVEN et al.(1973) and KERROLOS (1973) who used 150 Ug/ml neomycin alone or in combination with other inhibitory substances for

isolation and purification of Cl.welchii. It was also observed that 150 Ug/ml neomycin completely inhibited the growth of Cl.novei, Cl.septicum and Cl.chauvoei. A finding which goes hand in hand with many authors such as LOWBURY and LILLY (1955) and FARRAG (1971), a thrugh it disagreed with WILLIS and HOBBS (1959), ELLNER and O'DONELL (1971) where they recommended the use of 150 Ug/ml for the improvement of growth of Cl.welchii, Cl. septicum and Cl.noveie considering the study of mixtures it was observed that kenamycin and neomycin could be used for purification of Cl.welchii cultures as it was highly effective on contaminants. This findings agree to large extent with KUCERS (1972). For Cl.septicum and Cl.chauvoei it was found that the best antibiotics is erythromycin which completely inhibited the growth of all contaminants with permition of clostridial organisms.

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

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

Tested Organisms	Concentrations of the antibiotics by ug/ml											
	Ampicillin		Erythromycin		Gentamycin		Kanamycin		Neomycin		Terramycin	
	S.C.	C.I.C.	S.C	C.I.C.	S.C.	C.I.C.	S.C	C.I.C.	S.C.	C.I.C.	S.C.	C.I.C.
Cl. welchii types B and D	5		75	150	100	200	250	500	150	250	-	15
Cl. chauvoei	5		200	250	100	150	150	200	100	150	-	25
Cl. septicum	5		150	200	100	200	150	200	100	150	-	50
Cl. oedematiens	5		75	100	100	150	100	150	100	150	-	15

Remarks. ug = microgram = $\frac{1}{1000}$ ml 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.

Examined organisms	Concentration of antibiotics by ug/ml								
	Gentamycin	Neomycin		Kanamycin			Erythromycin		
	100	100	150	100	150	250	75	150	200
Cl.welchii types B & D	M	-	M		-	M	M	-	-
Cl.septicum	M	M	-		M	-		M	
Cl.chauvoei	M	M			M		-	-	M
Cl.oedematiens	M	M	-	M			M	-	-
Staph. aureus	C	P	C	P	C	C	C	C	C
B. subtilis	C	P	C	P	C	C	C	C	C
Peptostreptococcus anaerobius	C	P	C	P	P	C	P	C	C
Peptococcus anaerobius	C	P	C	P	P	C	C	C	C

Remarks: M = Medium growth

P = Partial inhibition of growth

C = Complete inhibition of growth

- = not tested