

Study the Susceptibility of *Bacillus cereus* isolated from Milk and Milk Products to Antibiotics

دراسة في استجابة عصيات سيريس المعزولة من الحليب ومشتقاته
للمضادات الحيوية

Abdul-Hadi A. Abd, Aysar Saadoun Abood, Nadia Ibrahim AbdelA'Al
Department of Veterinary Public Health,
College of Veterinary Medicine, Baghdad University

Abstract

Both antibiotic discs in agar and liquid broth cultures methods were used in this study. *Bacillus cereus* strain ATCC 11778 and the strain that isolated from milk locally were tested for antibiotics susceptibility. Both strains are susceptible to cephalexin, ampicillin and lincomycin in broth method to minimal inhibitory concentrations (MIC). Standard strain was sensitive to cephalexin 125µg/ml, ampicillin 1mg/ml and lincomycin 100µg/ml while local strain is sensitive to cephalexin 250µg/ml, ampicillin 10mg/ml and lincomycin 1mg/ml. The following antibiotics tetracycline, cephalexin, neomycin, streptomycin, penicillin 10µg and ceftriaxone 30µg were used in antibiotic disc concentration method. Both strains were sensitive to Tetracycline, cephalexin, neomycin and streptomycin while both strains were resistant to penicillin 10µg and ceftriaxone 30µg. Our results showed that local strain is tougher in susceptibility to the antibiotic s than standard strain.

الخلاصة

درست طريقتنا الاقراص المحتوية على المضادات الحيوية في الوسط الزرعي و المرق الزرعي. قورنت العتر المعزولة محليا بعتره قياسية (*Bacillus cereus* strain ATCC 11778) لاختبار الاستجابة للمضاد الحيوي عليها. العترتان المحلية والقياسية استجابت للمضاد الحيوي سيفالكسين وامبسلين و لينكوميسين في طريقتي الاقراص المحتوية على المضادات الحيوية في الوسط الزرعي و المرق الزرعي. المضاد سيفالكسين استجاب بتركيز 125 مايكروغرام / مل ، المضاد امبسلين استجاب بتركيز 1 ملغم/مل والمضاد لينكوميسين استجاب بتركيز 100 مايكروغرام /مل في حالة العتره القياسية اما في حالة العتره المحلية فقد استجاب المضاد سيفالكسين بتركيز 250 مايكروغرام / مل، المضاد امبسلين استجاب بتركيز 10 ملغم/مل و لينكوميسين استجاب بتركيز 1 ملغم/مل.

استعملت المضادات الحيوية التالية تتراسايكلين، سيفالكسين، نيومايسين، ستربتومايسين، بنيسيلين 10 مايكروغرام وسفترياكسون 30 مايكروغرام في طريقة الاقراص المحتوية على المضادات الحيوية في الوسط الزرعي. استجابت كلتا العترتين للمضادات الحيوية تتراسايكلين، سلفالكسين، نيومايسين و ستربتومايسين ولكنهما قاومتا البنيسيلين 10 مايكروغرام والسفترياكسون 30 مايكروغرام. اظهرت النتائج ان العتره المحلية اكثر مقاومة للمضادات الحيوية من العتره القياسية.

Introduction

B. cereus has been associated with both food-borne illness and non-gastro-intestinal infections (1). It causes gastrointestinal distress, necrotic enteritis, liver failure, bacteraemia, endocarditis, meningitis, pneumonia and skin lesions (2-4). Polymixin pyruvate egg yolk mannitol methylene blue agar (PEMBA) plating medium for *Bacillus cereus* contains 5-bromo-4-chloro-3-indoxyl-myoinositol-1-phosphate, which changes from colorless to turquoise upon enzymatic cleavage (5). Antibiotic sensitivity testing seeks to identify the susceptibility of *Bacillus cereus* to a range of potential therapeutic agents (6). The sensitivity of *Bacillus cereus* to a particular antibiotic is determine by ascertaining the Minimal Inhibitory Concentration (MIC) or breakpoint, this is the lowest concentration (conventionally tested in doubling dilutions) of antibiotic at which *Bacillus cereus* cannot produce visible growth after overnight incubation (7).

Resistance to antibiotics can either be naturally occurring for a particular organism/drug combination or acquired resistance, where miss-use of anti-microbial results in a population being exposed to an environment in which organisms that have genes conferring resistance (either spontaneously mutated or through DNA transfer from other resistant cells) have been able to flourish and spread (8). As *B. cereus* is generally resistant to penicillin, penicillin-sensitive isolates of *Bacillus subtilis* were also included.

From the many case reports of *B. cereus* infections, the broad picture is one of resistance to penicillin, Ampicillin, cephalosporins, and trimethoprim and susceptibility to clindamycin, erythromycin, chloramphenicol, vancomycin, the aminoglycosides, and, usually, tetracycline. Ciprofloxacin was used successfully in the treatment of *B. cereus* wound infections (9). In a comparison of MIC methods, (10) found that, of five *B. cereus* strains, all were susceptible to ciprofloxacin and, with some variation between methods, to deoxycycline; all were resistant to penicillin while, to tetracycline, two were susceptible, one was resistant, and two gave variable readings.

The purpose of the study was to determine the susceptibilities of *Bacillus cereus* species to a set of antibiotics selected to have the greatest guidance value to public health authorities encountering *B. cereus*, infections in humans.

Materials and Methods

Bacillus cereus strains ATCC 11778 was a generous gift from, Biotechnology & Environmental Biology, School of Applied Science, RMIT University, Bundoora West Campus, Victoria, Australia. Milk strain was isolated from Baghdad local areas (5).

Antibiotics powders in this experiment were supplied from Arabic Industries for Antibiotics.

Sterile tips of 1ml and sterile capped 7.5 x 1.3 cm tubes / small screw-capped bottles, broth culture of test and control organisms required antibiotic in powder required sterile distilled water as a solvent for the antibiotic, suitable nutrient broth medium.

Prepare stock dilutions of the antibiotic of concentrations 1000 and 100 µg/L as required from original stock solution (10,000 mg/L). Arrange two rows of 12 sterile 7.5 x 1.3 cm capped tubes in the rack. In a sterile 30ml (universal) screw capped bottle, prepare 8ml of broth containing the concentration of antibiotic required for the first tube in each row from the appropriate stock solution already made.

Mix the contents of the universal bottle using a pipette and transfer 2ml to the first tube in each row. Using a sterile pipette, add 4 ml of broth to the remaining 4 ml in the universal bottle mix and transfer 2ml to the second tube in each row. Continue preparing dilutions in this way but where as many as 10 or more are required the series should be started again half the way down. Place 2 ml of antibiotic free broth to the last tube in each row. Inoculate one row with one drop of an overnight broth culture of the test organism diluted approximately to 1 in 1000 in a suitable broth and the second row with the control organism of known sensitivity similarly diluted. The result of the test is significantly affected by the size of the inoculums. The test mixture should contain 10^6 cfu/ml. Incubate tubes for 18 hours at 37°C. Inoculate a tube containing 2ml broth with the organism and keep at 4°C in a refrigerator overnight to be used as standard for the determination of complete inhibition. Inoculate 1ml of each test tube to nutrient agar for counting the number of bacteria grows in each tube. Determine the tube with no growth as the concentration of antibiotic.

In case of disc solid method, the Miller-Hinton agar was used to identify the zone of antibiotic inhibition. The inoculums quantity was evaluated with 0.5 McFarland standards which were swabbed on to the surface of the agar before application of the strip. The antibiotics studied were tetracycline, cephalixin, neomycin; streptomycin, penicillin 10µg and ceftriaxone 30µg, and incubation in triplicates were at $36^{\circ}\text{C}\pm 1^{\circ}\text{C}$ for 18 hours. The reading of inhibition zone was measured by metal ruler divided into 0.1 mm according the method (11).

Results

The liquid media results have shown in minimal inhibitory concentration (MIC) for Bacillus cereus strain ATCC 11778 was 125 µg/ml cephalixin, 1 mg/ml ampicillin and 100 µg/ml lincomycin while in the milk strain was 250 µg/ml cephalixin, 10 mg/ml ampicillin and 1 mg/ml lincomycin (**Table 1**). In case of antibiotic disc concentration method these antibiotics tetracycline (T) 30µg, cephalixin (CL) 30µg, neomycin (N) 30µg, and streptomycin(S) 10µg were sensitive to both strains while penicillin 10µg and ceftriaxone 30µg were resistant to both strains (**Table 2**).

Table 1. Broth method to measure the Minimum Concentration of Antibiotics

Antibiotic Concentration	B. cereus (ATCC 11778)	Antibiotic Concentration	B. cereus Local milk strains
Cephalixin 125 µg/ml	S	Cephalixin 250 µg/ml	S
Ampicillin 1mg/ml	S	Ampicillin 10 mg/ml	S
Lincomycin 100 µg/ml	S	Lincomycin 1mg/ml	S

Table 2. Antibiotic disc Concentration method for B. cereus (ATCC 11778) and B. cereus (local milk strain).

Antibiotic Concentration	B. cereus (ATCC 11778)	B. cereus (local milk strains)
Tetracycline (T) 30µg	S	S
Cephalixin (CL) 30µg	S	S
Neomycin (N) 30µg	S	S
Streptomycin (S) 10µg	S	S
Penicillin (PG) 10µg	R	R
Ceftriaxone (CT) 30µg	R	R

Note: S=Sensitive R= Resistant

Discussion

B. cereus has extensive been associated with both food-borne illness and non-gastro-intestinal infection (12). This study is vital for clinician to prescribe the exact dose of antibiotics when it is necessary to know exactly the dose of the antibiotics treatment for human being suffer from food poisoning or non-gastro-intestinal infection (13) . Both methods for antibiotics susceptibility are studied. The antibiotic discs in agar and the liquid broth cultures methods were used in this investigation. Local strain was sensitive to cephalixin 250µg/ml, ampicillin 10mg/ml and lincomycin 1mg/ml while standard strain was sensitive to cephalixin 125µg/ml, ampicillin 1mg/ml and lincomycin 100µg/ml (**Table 1**).

These results indicated that local strain which isolated from the milk of Baghdad area was more resistant than standard strain (ATCC 11778). B. cereus isolated from local milk more resistant because the farmers in this country usually use antibiotics more than other countries in treatment. Sometime the farmers used antibiotics with feed ration to protect their animals from diseases because they believed that these antibiotics are useful for growing and building their bodies (14). Agar tests for both strains were showed more accurate than the liquid broth because the probability of contamination was less than in the liquid broth. Sensitivity of antibiotics in case of disc sensitivity test was sensitive in tetracycline, cephalixin, neomycin and streptomycin while resistant in case of penicillin and ceftriaxone. These results are similar to the findings recorded by (15). This

study was summarized the antibiotics treatment and susceptibility of *B. cereus* strains had been isolated from Baghdad districts.

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