EFFECT OF SUBINHIBITORY CONCENTRATIONS OF SOME ANTIBIOTICS ON SLIME FORMATION AND CRUDE EXOPOLYSACCHARIDE PRODUCTION BY *klebsiella pneumoniae*[+]

Nisreen Sharif Alyasiri[*]

Abstract

Highly viscous *K.pneumoniae* isolated from urine of patient suffering from urinary tract infection was cultured on media containing different concentrations of Lincomycine, Metronidazole and Ciprofloxacine.

The results showed that growth of encapsulated *K.pneumoniae* in medium containing sub-MICs of Lincomycine (16-64)µg/ml progressively increased the viscous slime formation and the amount of crude exopolysaccharide execrated. There were no notable or significant differences in slime formation occurred after the addition of sub-MICs of Metronidazole (8-32)µg/ml but there was remarkable reduction in the amount of crude exopolysaccharide execrated.

On the other hand growth of encapsulated *K.pneumoniae* in media containing sub-MICs of Ciprofloxacine reduce the viscous slime formation at (4&8) µg/ml and remarkably increased the amount of exopolysaccharide execrated but only at low concentrations of sub-MICs which is 2 µg/ml. Further concentrations of sub-MICs of Ciprofloxacine (4-8)µg/ml reduced the viscous slime formation but have only a little effect on crude exopolysaccharide production.

Increasing the production of exopolysaccharide with Lincomycine and low concentrations of Ciprofloxacine without inhibiting bacterial growth may be useful in extraction of capsular exopolysaccharide for research and laboratory purposes such as vaccine production.

المستخلص

تم زرع بكتريا الـــ *K.pneumoniae* عالية اللزوجة و المعزوله من أدرار مريض يعـاني مـن التهاب المجاري البولية على أوساط تحتوي علىتراكيز مختلفـة مـن اللنكومايـسين ،مترونيـدازول و سبروفلوكساسين .أظهرت النتائج أن نمو بكتريا الـــ *K.pneumoniae* الحاوية على الكبـسولة علـى وسط يحتوي على سلسلة من التراكيز المثبطة تحت الدنيا من اللنكومايسين(١٦ - ٦٤) مايكرو غرام/مـل أدى الى زيادة مظطردة في تكوين الوحل اللزج وزيادة في الكمية المستخلصة من عديد السكريد الخارجي الخام. لم نشاهد أختلافاً ملحوظاأو مهماً في أنتاج الوحل اللزج بعد اضافة التراكيز المثبطةتحت الدنيا مـن المترونيدازول (٨ - ٣٢) مايكروغرام/مل لكن كان هناك نقصان واضح في الكمية المستخلصة من عديـد السكريد الخارجي الخام .

[+] Received on: 8/12/2004 - Accepted on: 30/5/2005
[*] Assist .Lecturer / College of Health and Medical y.

من جهة اخرى أظهرت تنمية بكتريا الـــ *K.pneumoniae* ذات الكبسولة علـــى وســـط يحتـــوي علىالتراكيزالمثبطة تحت الدنيامن السبروفلوكساسين نقصان في كمية الوحل أللزج المتكونة عند التراكيز (٤و٨) مايكروغرام/مل و زيادة ملحوظة في كمية عديد السكريد الخارجي الخام المستخلصة ولكن فقط في التراكيز المنخفضة من التراكيز المثبطة تحت الدنيا وهو(٢ مايكروغرام /مل) . اما التراكيز الاعلى (مـــن التراكيز المثبطة تحت الدنيا) للسبروفلوكساسين وهي (٤ - ٨ مايكروغرام/مل) أدت الى نقصان في تكوين الوحل اللزج ولكن كان لها تأثير بسيط على كمية عديد السكريد الخارجي الخام الـــــمستخلصة .

أن زيادة أنتاج عديد السكريد الخارجي بواسطة اللنكومايسين والتراكيز الواطئة من السبروفلوكساسين قد يكون ذو فائدة في استخلاص عديد السكريد الخارجي للاغراض البحثية والمختبرية مثل انتاج اللقاحات .

Introduction

Capsule is a complex acidic polysaccharide layer, characteristically surrounds ***Klebsiella pneumoniae*** that shields the bacterium from host defenses and it is also important virulence factor [1]. The capsular repeating subunits consist of four to six sugars and very often uronic acid (as negatively charged components) [2].

Capsules are essential to the virulence of Klebsiella pneumoniae, the capsular materials form thick bundles of fibrillous structures covering the bacterial surface in massive layers, this protect the bacterium from phagocytosis by polymorphonuclear granulocytes, on one hand and prevents killing of the bacteria by bactericidal serum factors on the other [2,3]. Reduction or loss of capsular polysaccharide by mutation results in increased susceptibility to phagocytosis and loss of virulence [1,3]. Antibiotics that reduce the production of capsular polysaccharide or exopolysaccharide may provide unique adjuct to therapy against infections caused by encapsulated bacteria. Antimicrobial agents with activities against k .pneumoniae may be more effective in the presence of less capsule. Moreover, the reduction of capsular polysaccharide may enable host defences to clear encapsulated organisms more effectively [3,4]. By this conclusion a variety of agents were tested for there abilities to reduce capsular polysaccharide production, for instance the addition of sodium salicylate to culture media significantly reduced the quantity of capsular polysaccharide produced by k. pneumoniae [1,5]. stated that growth in the presence of sub-MICs of Cefuroxime or Ciprofloxacine had little detectable effect on the Lipopolysaccharide LPS profiles; also using Subinhibitory concentrations of several Cephalosporins (Ceftriaxone, Cephalexine, Cefuroxime and Cefotaxime) reduced the capsule formation by K.pneumoniae[6,7].

On the other hand, capsular polysaccharides have the obvious vaccine candidates for several reasons. Capsules are produced by almost all k.pneumoniae strains; they represent the outermost layer of surface structures in contact with the host milieu and they have been proved to be highly immunogenic and nontoxic [2,4].

Materials and Methods

2-1- Bacteria employed in this study characterized by high viscosity, was isolated from urine of patient suffering from urinary tract infection and then identified using the Api 20E system.

2-2-Antibiotics solutions: stock solutions of Metronidazole, Ciprofloxacine and Lincomycine (Eron S.A., Coba) at concentration of 10 mg/ml were prepared as described . [8].

2-3-Minimum Inhibitory Concentration (MIC) test was determined by agar dilution method [9].

2-4-Viscosity measurements: *k .pneumoniae* was grown on media containing sub-MICs of each antibiotic ,and on media without antibiotic as control. When the antibiotic was added, ***K.pneumoniae*** produced a viscous slime. The viscosity of the slime was expressed as the slime index and was determined according to [10] as following; the bacterial inoculums were harvested with 10 ml PBS then mixed vigorously for 5 minutes. The time index was calculated by measuring the length of time the culture took to drain from a 10 ml volumetric pipette then divided by the length of time distilled water took to drain from the same pipette.

2-5-Exetraction of crude exopolysaccharide.

-Bacterial removal

After harvesting the bacterial culture, the bacteria was first harvested by centrifugation at 6000 rpm for 20 minutes at 4ºC, the clear supernatant then heated for 30 minutes at 50 ºC to kill viable bacteria and denaturate proteins [1].

-Protein removal

The bacterial suspension was centrifuged at 12000 rpm for 20 minutes at 4ºC [10]. The supernatant was transferred to new tubes then 3 volumes of (95%) ethanol were added to each volume of the supernatant. The formed precipitate was collected by centrifugation at 1000 rpm for 10 minutes. The pellet then washed with 5 ml of (100%) ethanol and allowed to dry [1].

Results and Discussion

Influence of added antibiotics on slime formation

When encapsulated *K.pneumoniae* were grown on media containing sub-MICs of Lincomycine (16,32,64)µg/ml, viscous slime formation progressively increased at the concentrations 16&32 µg/ml but further concentrations had little effect on slime formation (Fig.1).

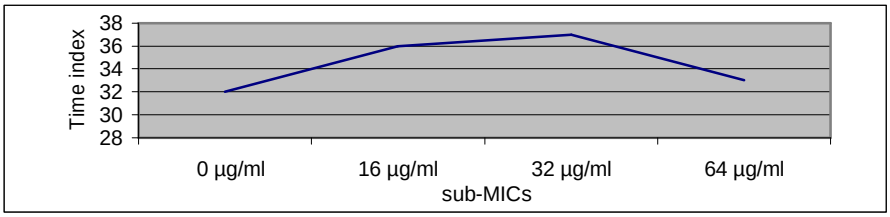

Fig. (1): Effect of sub-MICs of Lincomycine on slime formation

The addition of sub-MICs of Metronidazole (8,16,32) µg/ml showed mild effect on slime formation with no notable or significant differences in slime formation at each of the concentrations of Metronidazole (Fig.2).

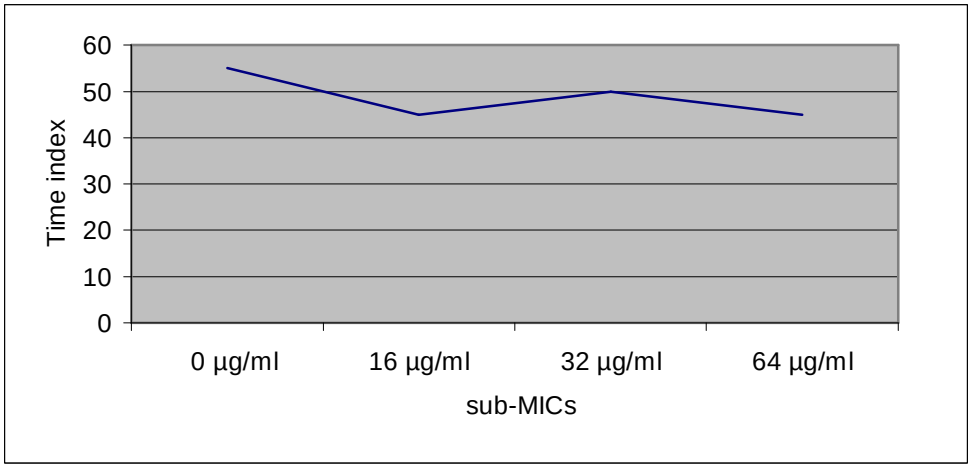

Fig.(2): Effect of sub-MICs of Metronidazole on slime formation

On the other hand growth of encapsulated ***K.pneumoniae*** in media containing sub-MICs of Ciprofloxacine (2,4,8) µg/ml reduced the viscous slime formation (Fig.3).

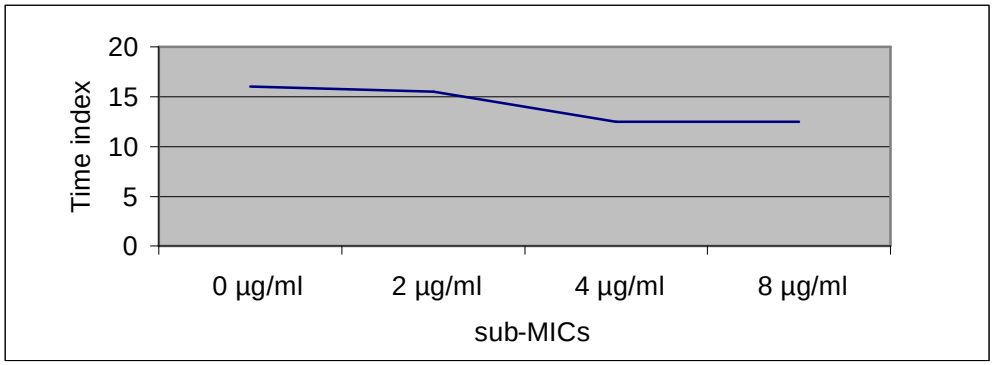

Fig.(3): The effect of sub-MICs of Ciprofloxacine on slime formation

The production of slime is considered to be a characteristic of ***K.pneumoniae*** [2]. The composition of this slime is not known, but it is thought to be a protein. It can be precipitated with ethanol or acetic acid and it gives a positive Lowry test for protein and negative Molisch and anthrone tests for carbohydrates [10].

-Influence of added antibiotics on crude exopolysaccharide production

The addition of antibiotics to the growth medium affected the amount of crude exopolysaccharide excreted. 16µg/ml of Lincomycine increased crude EPS amount about 2.5 folds and the concentrations 32&64 µg/ml increased it about 3 folds (Fig.4).

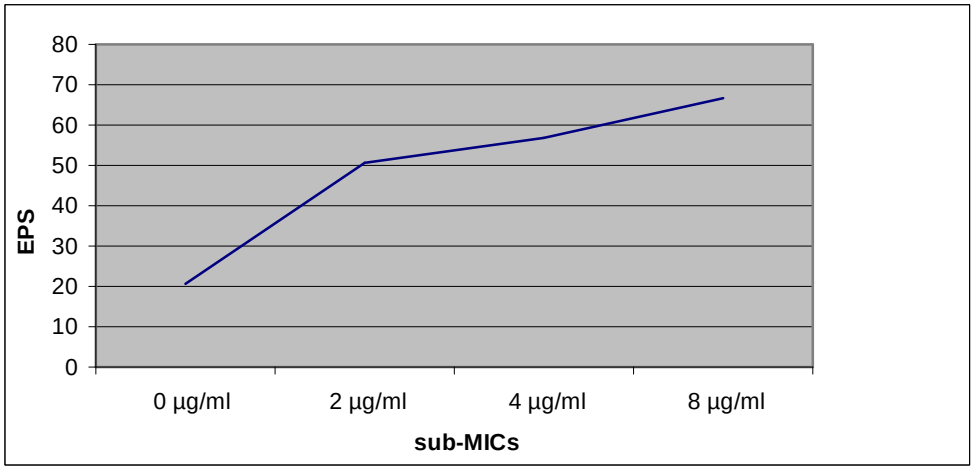

Fig.(4): Effect of sub-MICs of Lincomycine on crude EPS production

On the other hand growth on media containing sub-MICs of metronidazole remarkably reduce the amount of excreted crude exopolysaccharide.32 µg/ml of Metronidazole decreased the amount of crude EPS about 80% (Fig.5).

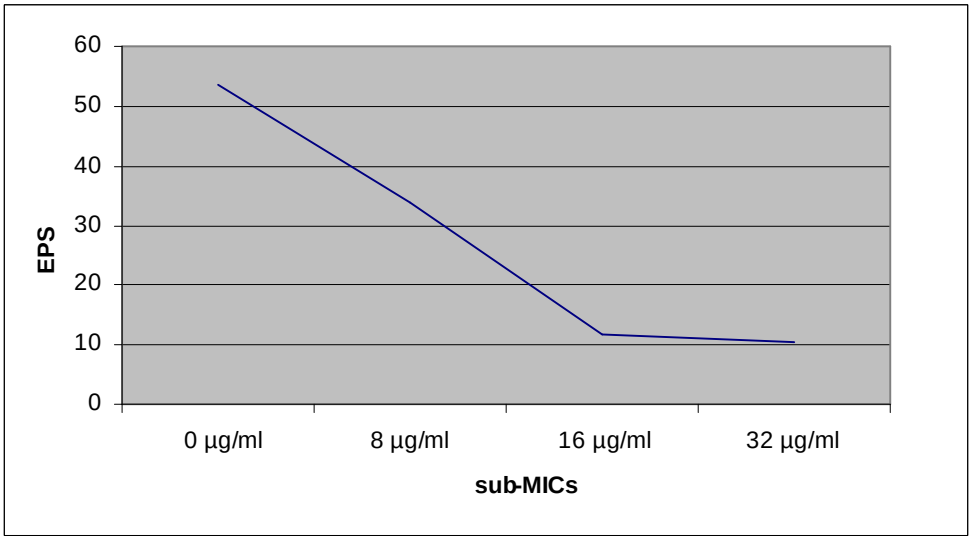

Fig.(5): Effect of sub-MICs of metronidazole on crude EPS production

Studying the effect of sub-MICs of Ciprofloxacine on crude EPS production showed that 2µg/ml of Ciprofloxacine remarkably increased crude EPS about 2 folds, but increasing Ciprofloxacine beyond this point had little effect on crude EPS production (Fig.6).

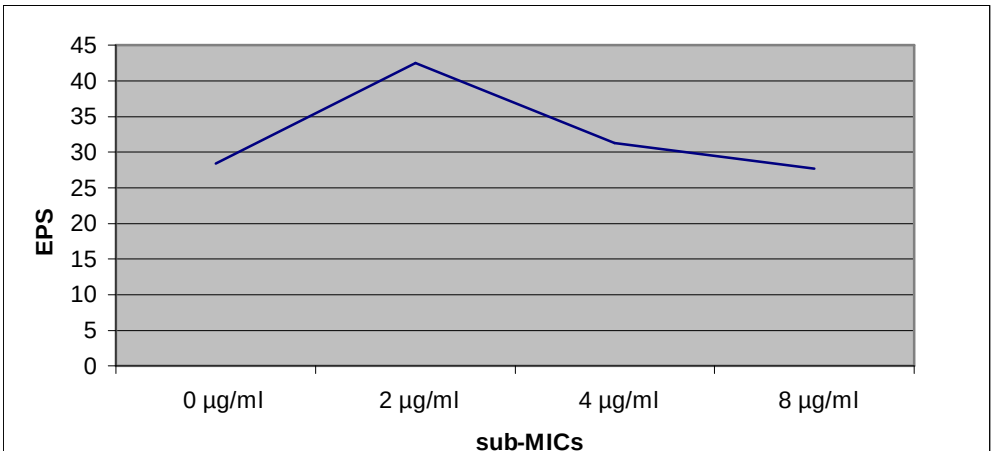

Fig.(6): Effect of sub-MICs of Ciprofloxacine on crude EPS production

There is no previous study referred to the effect of Lincomycine and metronidazole on slime formation and exopolysaccharide production in ***K.pneuomoniae***.

Other workers however have noted changes in the EPS production after growth in the presence of antibiotics like Cefuroxime and Ciprofloxacine [6] or in the presence of Cephalosporins [7]. Also there was studies on the effect of some chemicals like sodium salicylate [1] or iron and sulfur [10] on EPS production and slime formation respectively.

Since capsular polysaccharide have been proved to be the obvious vaccine candidate [2,4], based on this study, plating media with Lincomycine could be developed which would permit better production of capsular polysaccharide to be used for research purposes and vaccine production.

Referances

1. Domenico ,P.; Schwartz ,S.and Cuhan , B.A . "Reduction of capsular polysaccharide production in ***Klebsiella pneumoniae*** by sodium salicylate.Infect," *Immun.* Vol.57, pp. 3778-3782, 1989.
2. Podschun, R. and Ullmann,U. "***Klebsiella spp***.as nosocomial pathogens" *Epidemiology, Taxonomy, typing methods and pathogenicity factors*,1998.
3. Schembri,M.A.; Dalsgaard,D. and Klemm,P. "Capsule shields the function of short bacterial adhesion,"*J.Bacteriol*. Vol.186, pp.1249-1257,2004.
4. Zamze,S.;Martinez- pomres,L.;Jones,H.;Taylor,P.R.;Stillion ,R.J.;Gordon,s. and Wong,S.Y.C. "Recognition of bacterial capsular polysaccharide and lipopolysaccharides by the macrophage-Manoze Receptor," J.*Biol.Chem*. Vol. 277, pp. 41613-41623,2002.
5. Williams, P., "Sub-MICs of cofuroxime and ciprofloxacin influence interaction of complement and immunoglobulines with ***Klebsiella pneumoniae"*** .*Antimicrob. Agents. chemother*. Vol. 31 (5), pp.758-762,1987.
6. Kadurugamuwa, J.L.; Anwar, H.; Brown, M.R.W. and ZAK, O. "Protein antigens of encapsulated ***Klebsiella pneumoniae*** surface exposed after growth in the presence of Subinhibitory concentrations of cephalosporins,"*Antimicrob .Agents .Chemother* . Vol.28 (2),pp. 195-199,1985.
7. Kadurugamuwa J.L.; Anwar, H.; Brown, M.R.W. and ZAK, O. "Effect of Subinhibitory concentrations of cephalosporins on surface properties and siderophore production in Iron-depleted ***Klebsiella pneumoniae,"*** *Antimicrob.Agents .Chemother* . Vol.27(2),pp.220-223,1985.
8. Maniatis t. ;Fritch,E.F.and Sambrock, J. *Molecular cloning,a laboratory manual*, Cold spring harbor laboratory , cold spring harbor ,New York,1982.
9. National Committee for clinical laboratory standards (NCCLS). "Methods for dilution Antimicrobial susceptibility tests for bacteria that grow aerobically,"Approved standard M7-A3.*National Committee for Clinical Laboratory Standards*.Villanova,Pa,1993.
10. Palumbo S.A "Role of iron and sulfur in pigments and slime formation by ***P. aeruginosa"*** J.*Bacteriol*. Vol.111,pp.430-436,1972.