

Effect Of Gentamycin ,Tetracyclin And Ampiclox On Exopolysaccharide Production In Klebsiella Pneumonia

Muna Sabbar Jebar* MSc

Summary:

Background: *K. pneumonia* is surrounded by a polysaccharide layer referred to as capsule, which shields these organisms from host defenses and it is an important virulence factor.

Objectives: two isolates of *K. pneumonia* were used to study the effect of antibiotics on exopolysaccharide (EPS) production ,one of them have a high ability to produce (EPS) and another one have a low ability to produce (EPS).

Methods: agar dilution method was used to determine minimum inhibitory concentrations of antibiotic (gentamycin , tetracyclin ,ampiclox) , and (EPS) was extracted by precipitation with ethanol .

Results: The results showed that the gentamycin reduces the (EPS) production, while the tetracyclin and ampiclox slightly increase the production of (EPS) in high production isolate of *K. pneumonia*. However, the results of a low production isolate showed that there is no detectable effect of all three antibiotic on (EPS) .

Conclusions: there are deferent effects of antibiotics on (EPS) production.

Keywords: *K. pneumonia*, Exopolysaccharide , gentamycin , tetracyclin , ampiclox .

**J Fac Med Baghdad
2005; Vol. 47, No.4
Received June2005
Accepted Oct. 2005**

Introduction

Genus *Klebsiella* frequently cause human nosocomial infection, in particular, the medically most important *Klebsiella* species. *Klebsiella pneumonia* accounts for a significant proportion of hospital acquired urinary tract infection, pneumonia, septicemia and soft tissue infections (1). *K. pneumonia* is characteristically surrounded by a polysaccharide layer referred to as capsule . The capsule shields these organisms from host defenses and it is an important virulence factor (2) Typically, the larger capsule produced the more pathogenic strain is (3,10). The reduction or the loss of capsular polysaccharide by mutation results in increased susceptibility to phagocytosis and loss of virulence (4,17)

Brown (1977) showed that the composition of the surface components is greatly influenced by the growth environment (5), other workers have observed microscopically the change in capsule production in *K. pneumonia* after growth in the presence of antibiotic (6) .The reduction of exopolysaccharide (EPS) production may benefit in vivo because it renders the bacteria sensitive to opsonization and increases phagocytosis (7) .

Consequently, many studies dealt with the effect of antibiotic on EPS production in *K. pneumonia* . Gemmell (1984) showed that the sub minimuml inhibitory concentration (sub MIC) of penicillins and cephalosporins has a profound effect on material and consequently on the bacterial cell surface (8) .Kadurugamuwa et al. (1985) reported that the cephalosporins reduce capsule formation in encapsulated *K. pneumonia* (9).

Another study showed that cell growth in the presence of sub MIC of cephalixin ;ceftriaxone and cefotaxime reduces culture viscosity and capsule production (9).Domenico et al. (1989)proved that the addition of salicylic acid to culture media significantly reduce the quantity of EPS produced in *K. pneumonia* (11).

In this research the effect of three antibiotics on EPS production in *K. pneumonia*. is studied. Gentamycin, tetracycline, and ampiclox are chosen, which belong to different group of antibiotics.

Material and Methods

Bacterial isolate

Seven isolates of *K. pneumonia* are taken from urinary tract infection and cultured on tryptic soy agar to test their ability to produce EPS, and also two isolates selected, K1 which has the highest ability to produce EPS and K2 which has the lowest ability to produce EPS.

*Foundation of Technical Education, College of Health &Medical Technology, Medical analysis Department.

Determination of MIC

The MIC of Gentamycin (G),Tetracyclin (T) , Ampiclox (A) were determined by difold dilution method in agar using Macconkey Agar ,The lowest concentration of antibiotic resulting in complete inhibition of visible growth was taken as the MIC (12).

Viscosity measurement

The viscosity of EPS was determined as viscosity index on the bacterial growth at the concentrations of 0.5MIC, 0.25MIC, 0.125MIC, and a control to each antibiotic and each isolate by harvesting the growth with 10 ml of phosphate buffer saline (pH 7.2) for each plate . Then the viscosity was determined by measuring the length of time which the culture took to drain from 10 ml volumetric pipette divided by the length of time the distilled water took to drain from the same pipette (13).

Extraction & Measurement of EPS amount

The EPS was extracted by using adapted method according to (14,15, 16) as follows :

K. pneumonia isolates were grown on Macconkey agar containing 0.5MIC , 0.25MIC , 0.125MIC of G, T , A and on control plates . The bacterial growth was harvested with 10 ml of PBS (PH 7.2) and after that was well-shaken and then centrifuged then centrifuged (pH7.2) (6000g, for 20 min at 4 °C.)The clear supernatant fluid was heated for 30 min at 80°C to kill viable bacteria and to denature proteins . Denature material was then removed from supernatant by centrifugation (10000g ,for 20 min at 4 °C) .The EPS in supernatant was extracted by precipitation with three volumes of 95% ethanol . The precipitate was collected by centrifugation the sample at (10000g for 10 min at 4 °C) . The pellet was washed with 5 ml of 100% ethanol, then the ethanol was dried under a stream of air and the dry weight of the dried sample was determined.

Results

The results of MIC determination are shown in table (1)

Table (1) The MIC results

Isolate	MIC µg/ml		
	Gentamycin	Tetracyclin	Ampiclox
K1	16	64	32
K2	16	64	128

The effect of G, T, A on K1 and K2 viscosity are shown in figure Number (1)

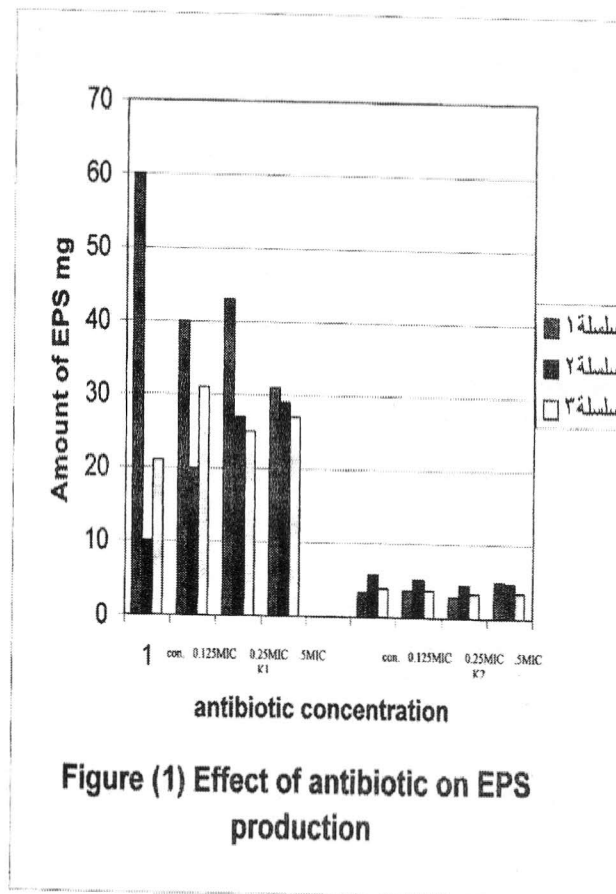


Figure (1) Effect of antibiotic on EPS production

The effect Antibiotic on K1 production EPS shown in figure number (2)

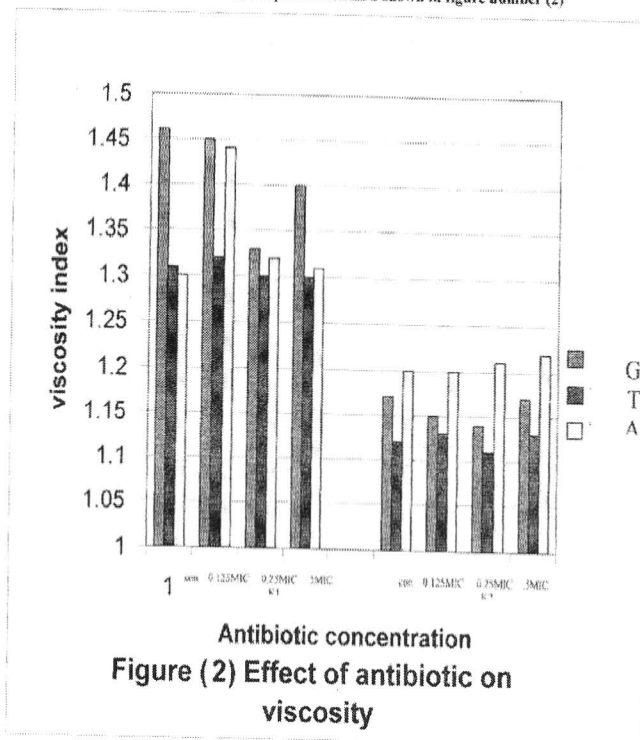


Figure (2) Effect of antibiotic on viscosity

Discussion

The results showed that the gentamycin reduces the viscosity and EPS production in K1. There is no previous studies dealt with the effect of gentamycin on viscosity and EPS of *K. pneumoniae*, but Gemmell (1984) showed that there is a reduction in the degree of encapsulation in *Bacteroides fragilis* after growth in the presence of clindamycin (aminoglycoside antibiotic) (8). Another study showed that the gentamycin reduces the production of EPS of *Pseudomonas aeruginosa* (19), and another study showed that the mucoid isolates from *P. aeruginosa* produce enzyme called polymannuronic, which has the ability to damage EPS (20). The place of this enzyme is periplasmic and the subMICs of gentamycin activates the release of this enzyme and reduce the EPS (21). May be there is a similar mechanism in *K. pneumoniae*.

The result showed that the ampiclox has little effect to on viscosity and EPS production. Leying et al. (1985) showed that the exposure of *E. coli* to subMIC of beta-lactam antibiotics has little effect on the qualitative composition of the outer membrane or cytoplasmic membrane (22). Another study showed that the ceftazidime increases capsular polysaccharide production in *K. pneumoniae* (18) and the bacteria may do that to protect them from the effect of antibiotics.

Also the results showed that tetracyclin has little effect to increase EPS production but has no effect on viscosity. No previous studies dealt with the effect of tetracycline on EPS in *K. pneumoniae*.

There is no detectable effect of antibiotics on viscosity and EPS production in K2, may be because it has already shown low potential to produce EPS.

References

- Podschun, R. & L'Umann, U. (1998) *Klebsiella* spp. As nosocomial pathogens: Epidemiology, Taxonomy, Typing and Antigenic factors. *Chn. Microbiol. Reviews*, 11(4):589-603.
- Robbins, J.B.; Schneerson, R.; Egan, B.; Vann, W. and Liu, D.T. (1980) Virulence properties of bacterium capsular polysaccharides - unanswered question. *P.I* 15-132. In: Smith, H.; Skehel, J. and Turner, M. (ed) *The molecular basis of microbial pathogenicity*. Dahlem Konferenzen, Federal Republic of Germany. Cited by: Domenico, P.; Schwartz, S. & Cunha, A. (1989) Production of capsular polysaccharide in *K. pneumoniae* by sodium salicylate. *Infect. Immun.* 57(12):3778-3782.
- Domenico, P.; Johanson, W. & Straus, D. (1982) Lobar pneumonia in rats produced by clinical isolated of *K. pneumoniae*. *Infect. Immun.* 37:327-335.
- Simoons-smit, A.; Verweij, J. & Maclaren, D. (1986) The role of *Klebsiella* antigens and virulence factors in *Klebsiella*. *J. Med. Microbiol.* 21:133-137.
- Brown, M. (1977). Nutrient depletion and antibiotic susceptibility. *J. Antimicrob. Chemother.* 3:198-201.
- Gemmell, C.; Spear, T. & Peterson, P. (1983) Morphological of various antibiotic. *Eur. J. Clin. Microbiol.* 2:217-218.
- Williams, P.; Lamber, P.; Brown, M. & Jones, J. (1983) The role of the O and K antigens in determining the resistance of *K. aerogenes* to serum killing and phagocytosis. *J. Gen. Microbiol.* 129:3075-3084.
- Gemmell, C. (1984) Potentiation of phagocytosis of pathogenic bacteria by exposure to low concentrations of antibiotic. *J. Antimicrob. Chemother.* 13:407-409.
- Kadumgamuwa, J.; Annnwar, H.; Brown, M. & Zak, O. (1985) Effect of subinhibitory concentrations of cephalosporins on surface properties and siderophore production in Iron depleted *K. pneumoniae*. *Antimicrob. Agents. Chemother.* 27:220-223.
- Domenico, P.; Straus, D. (1985) Extracellular polysaccharide production by *K. pneumoniae* and its relation to virulence. *Can. J. Microbiol.* 32:472-478.
- Doinenico, P.; Schwartz, S. & Cunha, A. (1989) Production of capsular polysaccharide in *K. pneumoniae* by sodium salicylate. *Infect. Immun.* 57(12):3778-3782.
- Miles, R. and Amyes, S. (1996) Laboratory control of antimicrobial therapy. In: Mackie & Macareney *Practical Medical Microbiology*, by Collee J. G.; Fraser, A.G.; Marmion, B.P. and Simmons, A. (14ed) Vol. I. Churchill Living Stone, New York, pp:151-179.
- Palumbo, A.S. (1972) Role of Iron and sulfur in pigment and slime formation of *Pseudomonas aeruginosa*. *J. Bacteriol.* 11:430-436.
- Pedersen, S.; Esperse, F.; Hoiby, N. and Shand, G. (1989) Purification, characterization and immunological cross reactivity of alginate produce by mucoid *Pseudomonas aeruginosa* from patient with cystic fibrosis. *J. Clin. Microbiol.* 27:691-699.
- Doig, Smith, N.R.; Todd, T. & Irvin, R.T. (1987) Characterization of the binding of *Pseudomonas aeruginosa* alginate to human epithelial cells. *Infect. Immun.* 55:1517-1522.
- Geers, T.; & Baker, N. (1987) The effect of sublethal concentrations of aminoglycoside on adherence of *Pseudomonas aeruginosa* to hamster tracheal epithelium. *J. Antimicrobiol. Chemother.* 19:561-568.
- Williams, P.; Lambert, P.; Haigh, C. (1986) The influence of O and K antigens of *K. aerogenes* on surface hydrophobicity and susceptibility to phagocytosis and antimicrobial agents. *J. Med. Microbiol.* 21:125-132.
- Held-TK; Adamczik, C.; Trautmann; M. Cross, A. (1995) Effect of MIC and subMIC of antibiotics on production of capsular polysaccharide of *K. pneumoniae*. *Antimicrobiol. Agents. Chemother.* 39(5):1093-1096.
- Majtan, V. & Hybenova, D. (1996) Inhibition of *Pseudomonas aeruginosa* alginate expression by sub inhibitor concentrations of antibiotics. *Folia. Microbio. Praha*, 41(1):61-64.
- Dunne, W. & Buckmine, F. (1985) Partial purification and characterization of a polymannuronic acid depolymerase produced by mucoid strain of *Pseudomonas aeruginosa* isolated from acystic fibrosis patient. *Appl. Environ. Microbiol.* 50:562-567.
- Dunne, W. (1985) Abstract number A79.85th Annual Meeting, American Society for microbiology, Las Vegas, Nevada. Cited by: Geers, T.; & Baker, N. (1987) The effect of sublethal concentrations of aminoglycoside on adherence of *Pseudomonas aeruginosa* to hamster tracheal epithelium. *J. Antimicrobiol. Chemother.* 19:561-568.
- Leying, H.; Karch, E.; Krolle, H. & Opferkuch, W. (1985) The influence of beta-lactam antibiotics, including monobactam on the outer and inner membrane of *E. coli* P 48-56. In: Adlam, D.; Hansand, H.; Opferkuch, W. (ed) *The influence of antibiotics on the host separate relationship II*. Springer Verlag, Barlin. Cited by: Williams, P. (1987) SubMICs of cefuroxime and ciprofloxacin influence interaction of complement and immunoglobulins with *K. pneumoniae*. *Antimicrob. Agents. Chemother.* 31(5): 758-762.