THE ROLE OF AGNO3 AND CLARYTHROMYCIN ON THE PRODUCTION OF EXOPOLYSACCHARIDE AND SLIME ,FORMATION BY *KLEBSIELLA* SP[+]

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Abstract :

In this study we have compared the effect of same materials like AgNo3 and clarythromycin as antibiotic in different concentration on the production of the slime and expolysaccharide which are produced from *klebsiella* sp . The results show that the concentration (4 mg/ml) of the AgNo3 has a positive effect , increasing the production of the slime and exopolysaccharide ,conversely the effect of clarythromycine was negative by decreasing the production of the slime and exopolysaccharide from *klebsiella* sp especially in the high concentration of this antibiotic .

The aim of this research is to show the effect of AgNo3 and clarythromycin on production slime and exopolysaccharide from *Klebsiella* sp.

المستخلص :

في هذه الدراسة تمت مقارنة تأثير بعض المواد مثل AgNo3 ومضاد clarythromycin على إنتاجية اللزوجة ومتعدد السكر يد لبكتريا *klebsiella* sp بتراكيز معينة وظهرت النتائج بـــان تركيـــز 4) (mg/ml من AgNo3 قد اثر تأثيراً إيجابياً ودنك بزيادة اللزوجة و إنتاج متعدد السكر يد وعلى العكـــس من ذلك فان تأثير clarythromycin كان سلبيا وخاصة في التراكيز العالية منه إذ قلل إنتاجهما من قبل بكتريا *kl.* Sp .

الهدف من هذا البحث هو لإظهار تأثير AgNo3 وclarythromycin على إنتاج اللزوجة ومتعدد السكر يد من بكتريا *Klebsiella* sp. .

Introduction :

Klebsiella usually develop prominent capsules composed of complex acidic polysaccharides [1]. The capsular repeating subunits ,consisting of four to six sugars and very often uronic acids (as negatively charged components) can be classified into 77 serological types [2] Capsules are essential to the virulence of *klebsiella* . The capsular material forms thick bundles of fibrillose structures covering the bacterial surface in massive layers [1].the surface structures of bacteria play a crucial role in the interaction with the host defenses during infection. Encapsulated *klebsiella* is

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known to be resistant to phagocytosis ,loss of hydrophilic capsular material by mutation renders the cells sensitive to opsonization and increased phagocytosis [3] .

Materials and methods :

Bacteria : Three laboratory strains of *klebsiella* sp (k1,k2, k3).

Media : Cells were grown on multer huntun agar(overnight culture) in different concentrations of AgNo3 and clarythromycin (1,2, 4,8,16,32,64)mg/ml for MIC estimation

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Viscosity measurements : The viscosity of the slime was expressed as the slime index and was determined before centrifugation of the culture by measuring the volumetric pipette divided by the length of time which distilled water took to drain from the same pipette [4).

Preparation of crude exopolysacchride (E.P.S.):

Bacteria cells were collected and harvested by centrifuge (6000 g)for (20) min at $4C^0$, the clear supernatant was heated for 30 min at $80C^0$ for denaturation of the proteins , centrifugation (12000g) for 20 min at $4C^0$,were added three volumes of 95% ethanol , centrifugation (10000)g for 10 min .the precipitate was collected and washed with 5ml of ethanol 100%, the ethanol was removed from the samples which were dried under warm air. Collect the crude E.P.S. was collected and balance it . [5, 6, 7]

Results:

One interesting observation is the slight influence of AgNo3 on the production of slime (viscosity) of *kl.* Sp. especially on the concentration (4 mg /ml) in (Fig1k1, Fig2k2, Fig3k3) ,but in another concentrations (8,16 mg/ml) (1,2mg/ml) (1,2mg/ml) the viscosity was increased compared with control. but not like the concentration of (4mg/ml).this result is the same with the production of crude exopolysacchride (Fig6k1, Fig7k2, Fig8k3).

The strain k1 is hypersensitive to the clarythromycin so the effect of clarythromycin on the viscosity and the production of the crude exopolusacchride will be studied on the two strains (k2, k3).

The viscosity and the production of crude exopolyusacchride slightly decrease especially in the high concentrations of clarythromycin (8,16,32mg/ml) (16, 32, 64mg/ml) seeFig4k2, Fig5k3, Fig9k2, Fig10k3).

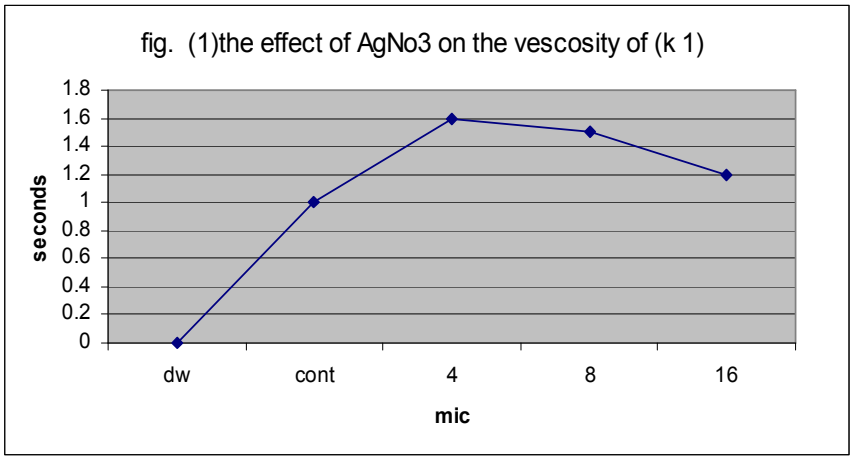


fig. (1)the effect of AgNo3 on the vescosity of (k 1)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
seconds
dw
cont
4
8
16
mic

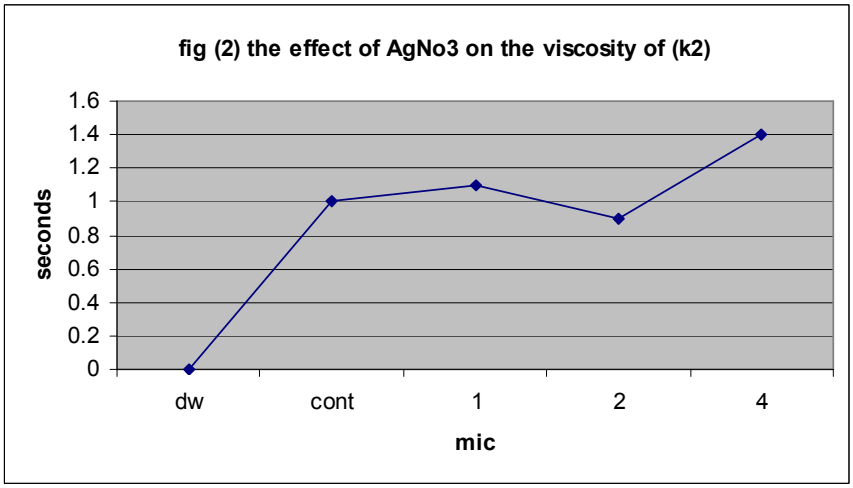


fig (2) the effect of AgNo3 on the viscosity of (k2)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
seconds
dw
cont
1
2
4
mic

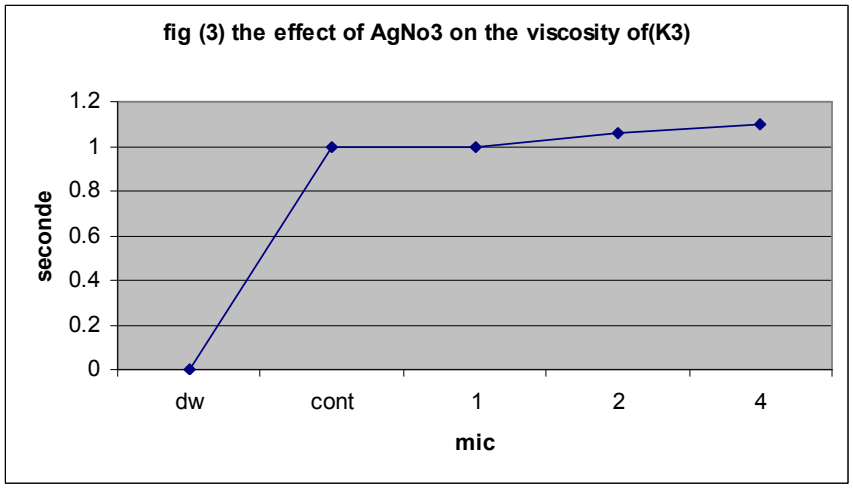


fig (3) the effect of AgNo3 on the viscosity of(K3)
1.2
1
0.8
0.6
0.4
0.2
0
seconde
dw
cont
1
2
4
mic

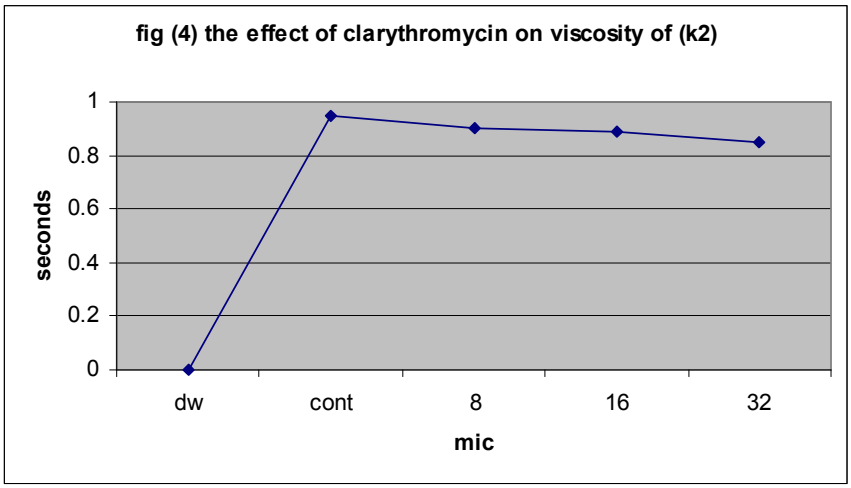


fig (4) the effect of clarythromycin on viscosity of (k2)
seconds
1
0.8
0.6
0.4
0.2
0
dw
cont
8
16
32
mic

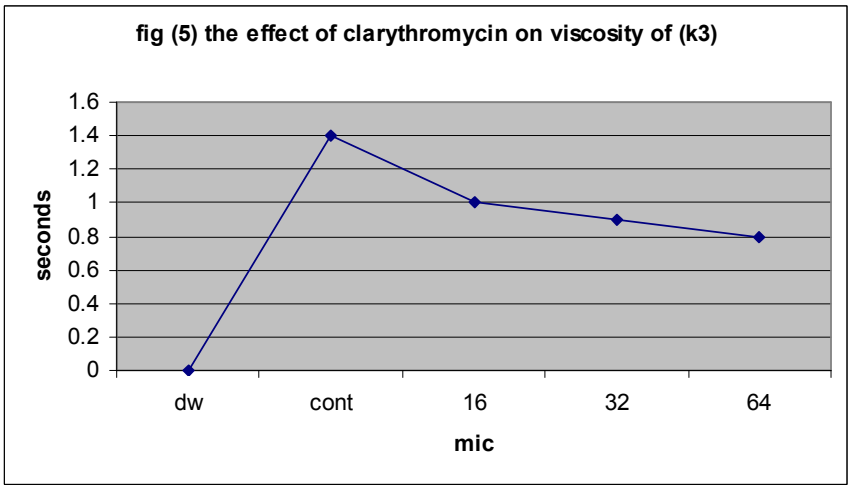


fig (5) the effect of clarythromycin on viscosity of (k3)
seconds
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
dw
cont
16
32
64
mic

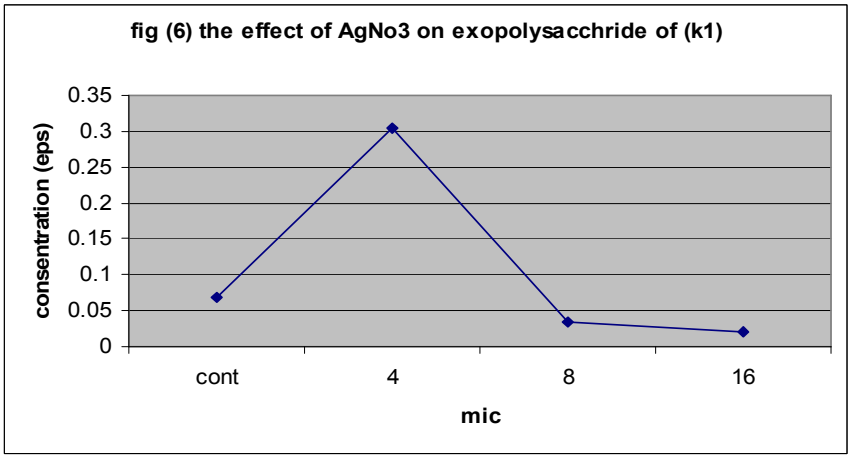


fig (6) the effect of AgNo3 on exopolysacchride of (k1)
consentration (eps)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
cont
4
8
16
mic

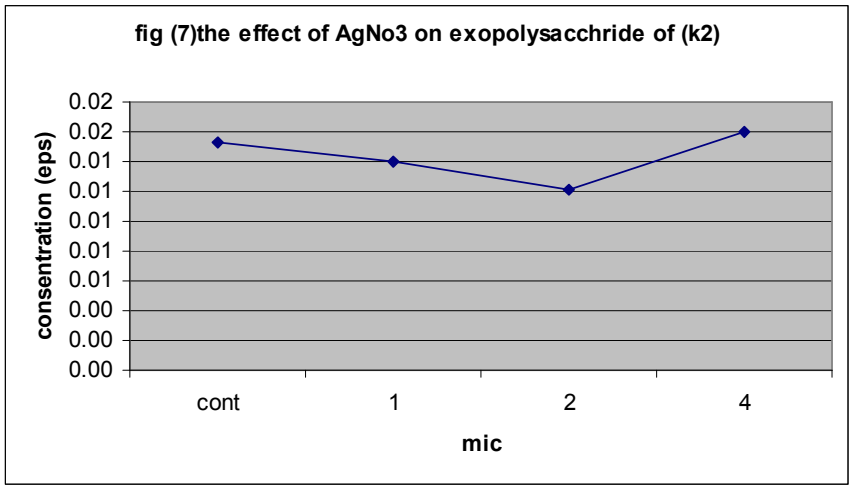


fig (7)the effect of AgNo3 on exopolysacchride of (k2)
consentration (eps)
0.02
0.02
0.01
0.01
0.01
0.01
0.01
0.00
0.00
0.00
cont
1
2
4
mic

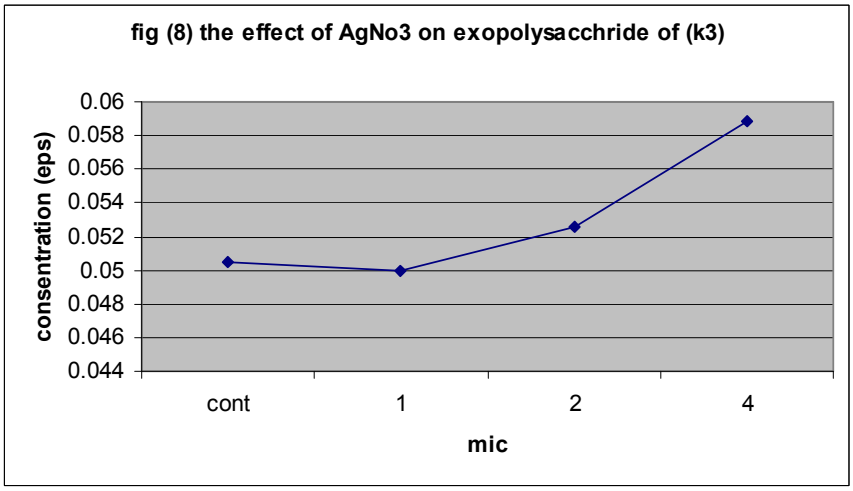


fig (8) the effect of AgNo3 on exopolysacchride of (k3)
consentration (eps)
0.06
0.058
0.056
0.054
0.052
0.05
0.048
0.046
0.044
cont
1
2
4
mic

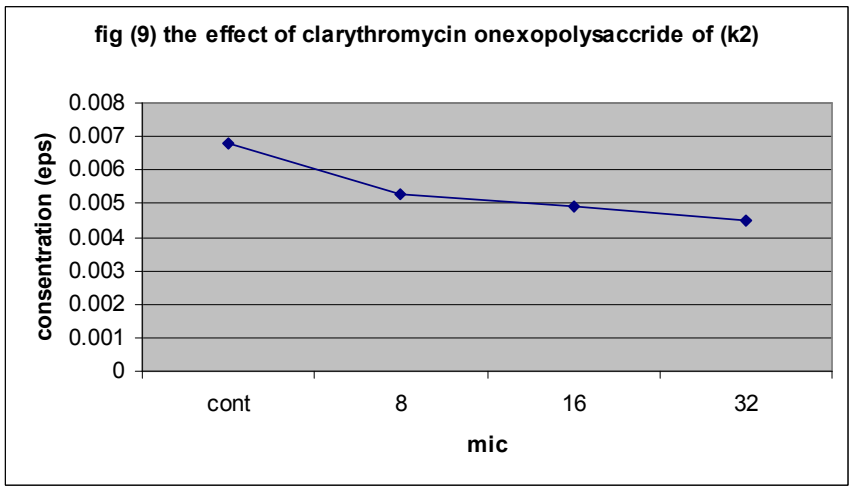
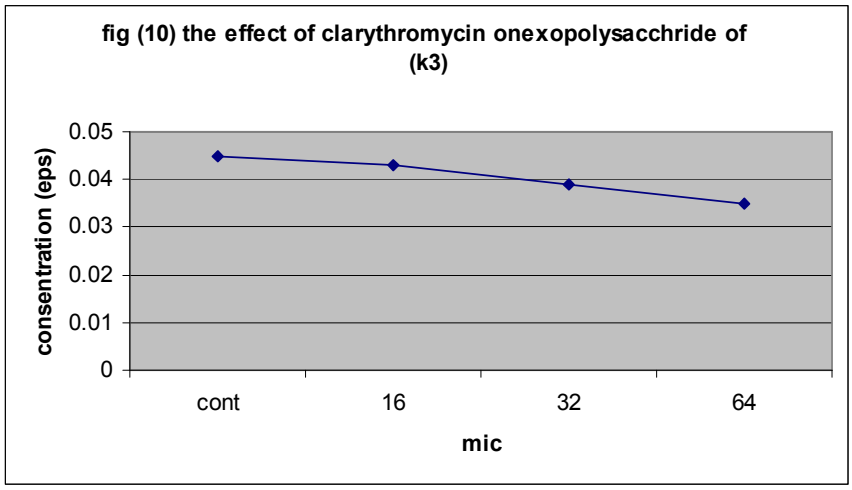


fig (9) the effect of clarythromycin onexopolysaccride of (k2)
consentration (eps)
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
cont
8
16
32
mic



Discussion :

In the present study we examined the sub MICs of agents belonging to two different classes,AgNo3 and clarythromycin which able to increase or decrease the viscosity and crude exopolusacchride production.

When the agent AgNo3 was added ,*kl* .sp. produced viscous slime .The AgNo3 in the concentration 4mg/ml supported the formation of slime and crude exopolysacchride ,this means that this concentration is the optimum concentration for producing a large amount of slime and crude exopolysacchride which can use this concentration in the future resurges.

This is like the effect of sulfur and iron on complex patterns of pigment formation and also increases the formation of slime from *ps.aeruginosa* ,[4].

Conversely the clarythromycin as antibiotic reduces the formation of slime and crude exopolysacchride in both low and high concentrations of it .The reasons are an unkown ; no previous study deals with this project. But there is a study on the effect of cephalosporin which can reduce the capsule formation from *kl. Pneumoniae* . [8].

Conclusions :

1- It appears that the best concentration of AgNo3 for slime and exopolysaccharide production is (4 mg /ml).
2- Clarythromycine has a negative effect in decreasing the slime and exopolysacchride production especially in high concentration of this antibiotic .

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