



## *Klebsiella pneumoniae*

\*

<i>Klebsiella pneumoniae</i>			
Acridin			
	(SDS) Sodium dodecyl sulphate	orange	
	<i>K.pneumoniae</i>		(%100)
			(%86–71)
	(%57–43)		
	(%29)		
	SDS		
-70)	SDS	orange	Acridine
	(%100–28)	Acridine	(%100)

# PLASMID CURING OF LOCAL ISOLATES OF KLEBSIELLA PNEUMONIAE ISOLATED FROM URINARY TRACT INFECTIONS AND ITS ROLE IN MULTIDRUG RESISTANCE

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**Abstract**

Curing of plasmid for 7 local isolates of *K. pneumoniae* have multi-drug resistance and isolated from urinary tract infections (UTI) by using acridin orange and sodium dodecyl sulphate (SDS) were investigated.

These results showed that the local isolates of *K. pneumoniae* were very resist (100%) to Ampicillin, Pepracillin, Amoxicillin, Penicillin G, Tetracyclin, Erythromycin and Gentamycin, high resist (71–80%) to Cephalexin, Cephalothin, Ceftazidine, Ciprofloxacin, Streptomycin and Tobramycin, moderate resist (43–57%) to Cefaclor, Cephadrin and Naldixic acid and weak resist (29%) to Cefotaxime while they were susceptible to Chloramphenicol and Amikacin.

It was found that SDS is more efficient in curing than Acridin orange, hence the losing of the resistance of antibiotics by using SDS was in the range (70–100%) while by using Acridin orange was in the range (28–100%). After curing of plasmid the Antibiotics resistance losed for Pipracillin, Amoxicillin, Penicillin G, Cephalexin, Cephalothin, Ceftazidin, Tetracyclin, Gentamycin and Tobramycin, this result confirm that the resistance of these antibiotics is born on plasmid, while the resistance of Ampicillin, Ciprofloxacin, Erythromycin and Streptomycin not losed, this result confirm that there resistance genes are born on the chromosome.

*Klebsiella pneumoniae*

*Klebsiella pneumoniae*

[ ]

[2] *Escherichia coli*

) 150  
( 50 -20

*Klebsiella*

2007

[ ]

)  
37° ( Himedia  
24

[ ]

.API-20E

[4]

DNA

( Himedia )  
[8] (Disc-diffusion method)

Ethidium bromide  
(SDS)

Acridine

*K.Pneumoniae*

[5] Sodium dodecyl sulphate

/ × .

(Pickand Patch) 24 37  
 ( )  
 24 °37

24 37

µg / ml

**Acridine**

**orange**

[9] Trevors

Acridine orange

*K.pneumoniae*

**Sodium dodecyl**

**(SDS) sulphate**

120 µg/ml

[10] Marcelo

SDS *K. pneumoniae*

5 (%1)

*K. pneumoniae*

5 24 37

% SDS

/ 10<sup>4</sup>

24 37

SDS

10<sup>-6</sup> 10<sup>-5</sup>

0.2 10<sup>-7</sup>

24 37

Acridine orange

150

110

23

%25.9

*Klebsiella*

*Klebsiella pneumoniae*

12

%10.9

10<sup>4</sup>

24 °37

/

[ ]

(API-20E)

10<sup>-7</sup> 10<sup>-6</sup> 10<sup>-5</sup>

0.2

19

24

°37

100

	µg/ml	code	
AL-Razi	10	AM	Ampicillin
AL-Razi	100	PRL	Pepracillin
AL-Razi	25	AX	Amoxicillin
AL-Razi	10	P	Penicillin G
Oxoid	30	CI	Cephalexin
Oxoid	30	CF	Cephalothin
Oxoid	30	CEC	Cefaclor
Oxoid	30	CE	Cephadrine
Bioanalyse	30	CTX	Cefotaxime
Bioanalyse	30	CTZ	Cephtazidine
Oxoid	10	CIP	Ciprofloxacin
Bioanalyse	30	TE	Tetracyclin
Bioanalyse	10	C	Chloramphenicol
Bioanalyse	15	E	Erythromycin
Bioanalyse	50	G	Gentamicin
Bioanalyse	26	S	Streptomycin
Bioanalyse	30	Tob	Tobramycin
Bioanalyse	30	NA	Nalidixic acid
Oxoid	10	AK	Amikacin

[13,12] Multi- (MDR)

Resistant drug

[12]

.2

2

(%100)

[14]

(%86-71)

[15]

(%57-43)

(%29)

[16]

ESBL

*K. pneumoniae*

جدول 2: اختبار الحساسية للمضادات الحيوية لعزلات

[11] . *K.pneumoniae*

[17]

[18] UTI

K.

SDS

Acridine orange

*pneumoniae*

3

.4

*K. pneumoniae*

:3

Acridine orange

Acridine orange								Antibiotic	No. of isolates
K. pneumoniae									
(%)	K7	K6	K5	K4	K3	K2	K1		
R	R	R	R	R	R	R	10	Ampicillin	1
66	45	79	80	42	85	50		Pepracillin	2
92	94	90	S	85	S	S		Amoxicillin	3
95	S	89	93	95	90	S		Penicillin G	4
I	67	81	60	I	78	80		Cephalexin	5
77	I	70	69	28	40	I		Cephalothin	6
I	75	90	71	80	81	S		Ceftazidine	7
I	R	R	R	R	I	R		Ciprofloxacin	8
85	66	88	83	87	90	S		Tetracylin	9
R	R	R	R	R	R	R		Erythromycin	10
86	84	69	79	72	91	70		Gentamycin	11

K. pneumoniae								Antibiotic	No. of isolates
K7	K6	K5	K4	K3	K2	K1			
100%	R	R	R	R	R	R	R	Ampicillin	1
100%	R	R	R	R	R	R	R	Pepracillin	2
100%	R	R	R	R	R	R	R	Amoxicillin	3
100%	R	R	R	R	R	R	R	Penicillin G	4
71%	I	R	R	R	I	R	R	Cephalexin	5
71%	R	I	R	R	R	R	I	Cephalothin	6
57%	I	R	I	R	R	R	S	Cefaclor	7
57%	S	I	R	R	R	R	S	Cephadrine	8
29%	S	I	I	R	R	I	S	Cefotaxime	9
71%	I	R	R	R	R	R	S	Ceftazidine	10
71%	I	R	R	R	R	I	R	Ciprofloxacin	11
100%	R	R	R	R	R	R	R	Tetracylin	12
-	S	I	S	S	S	S	S	Chloramphenicol	13
100%	R	R	R	R	R	R	R	Erythromycin	14
100%	R	R	R	R	R	R	R	Gentamycin	15
86%	R	R	R	I	R	R	R	Streptomycin	16
86%	R	R	R	R	R	R	I	Tobramycin	17
43%	I	S	S	R	R	S	R	Nalidixic acid	18
-	S	S	S	S	S	S	S	Amikacin	19

:I

:S

:R

*K. pneumoniae*

Extended Spectrum Beta –

Lactamase (ESBL)

*Klebsiella*

*K. pneumoniae*

R	R	R	I	R	R	R	Streptomycin	12
89	85	84	76	93	65	I	Tobramycin	13

[19] Ethidium bromide Novobiocin

. :R  
 .(%100 ) :S  
 .( ) :I

**K. pneumoniae** :4

R

SDS

[20]

K. pneumoniae							Antibiotic	Zone
K1-K7								
K7	K6	K5	K4	K3	K2	K1		
R	R	R	R	R	R	R	Ampicillin	1
87	88	85	S	89	82	98	Pepracillin	2
98	91	92	94	S	S	S	Amoxicillin	3
S	S	99	94	92	S	96	Penicillin G	4
I	87	89	83	I	80	91	Cephalexin	5
92	I	90	87	75	70	I	Cephalothin	6
I	90	86	89	92	S	S	Ceftazidine	7
I	R	R	15	R	I	R	Ciprofloxacin	8
S	92	86	85	82	S	S	Tetracylin	9
R	R	R	R	R	R	R	Erythromycin	10
95	S	86	91	90	S	85	Gentamycin	11
R	R	R	I	R	R	R	Streptomycin	12
S	92	90	86	S	91	I	Tobramycin	13

*K. pneumoniae*

. :R  
 .(% 100 ) :S  
 .( ) :I

Acridine

[23] orange

Acridine

4 3

SDS orange

*K. pneumoniae*

[24]

ESBL

[25] *K. pneumoniae*

Acridine

SDS

orange

.R

%100 -70 SDS

%100 - 28

SDS

12. Jabeen, K.; Zafar, A. and Hasan, R. **2005**. Frequency and Sensitivity pattern of extended spectrum beta – lastamsae producing isolates in atertiary care hospital Laboratory of Pakistan. *J. Pak. Med. Assoc.* **10**:436– 39.
13. Mathur, P., Kapil. A., Das. B. and Dhawan. B. **2002**. Prevalence of extended spectrum beta–lactamase producing gram negative bacteria in a tertiary car hospital. *Ind. J. Med. Res.* **115**: 153 – 157.
14. Yah, S.C.; Eghafona, N.O.; Enabulele, I.O. and Aluyil, H.S.A. **2006**. Ampicillin usage and Ampicillin resistant (Amer) plasmids mediated Escherichia coli isolated from diarrheagenic patients attending some teaching hospital in Nigeria. *Shiraz E-medical Journal.* **7**(4):4–18.
15. Yah, S.C.; Eghafona, N.O.; Oranusi, S. and Abouo, A.M. **2007**. Wide spread plasmid resistance genes among proteus species in diabetic wounds of patients in the Ahmadu Bello university teaching hospital (ABUTH) Zaria. *African Journal of Biotechnology.* **6**(15):1757 – 1762.
16. Inkhorn, A.F.; Neugausser, M.M.; Bearden, D.T.; Quinn, J. P. and penland, S.L. **2002**. Extended spectrum beta – lactamases: frequency, risk factors and outcomes. *Pharmacotherapy.* **22**: 14–20.
17. Araque, M.; Nieves, B.; Laurehi, L. and Rossolini, G.M. **2000**. Molecular basis of extended – spectrum beta – lactamase production in nosocomial isolates of K. pneumoniae. *from Meridan, Venzuella. Int. J. Antimicrob. Agents.* **15**(1): 37–42.
18. Jacobs, R.A; Guglielmo, B.J. **2005**. *Anti-infective Chemotherapeutic and Antibiotic Agents*. In: Tierney, L.M. McPhee, S.J, Papadakis M.A, eds. *Current Medical Diagnosis and Treatment*. 44<sup>th</sup> edition. New York, Lange Medical Books/ McGraw-Hill, pp.15-31.
19. El-Mansi, M.; Karen, J.A.; Craig, A.I.; Inche, L.K.; Linda, K. K. and David, J.P. **2000**. Isolation and curing of the Klebsiella species large indigenous plasmid using SDS. *Microbiology.* **151**(3):201–208.
20. Jacoby, G.A. **1994**. Properties of plasmids responsible for production of extended spectrum beta–lactamase. *Eur. J. Clin. Infect.* **13**(51): 2 – 11.
1. Neu, L. H. **1992**. *The Crisis in antibiotic resistance. Science.* **257**:1054 – 1073.
2. Hajarnis, S. **1996**. Suspected urinary tract infections: Identification of microorg-anisms and sensitivity to antibiotics in Seychelles. *Ind. Path. Microbiol.* **36**(2): 114 – 123.
3. Jacoby, G. A. and Sutton. L. **1991**. Properties of plasmids responsible for production of extended – spectrum beta – actamases. *Antimicrob. Agents Chemother* **35**: 164 – 189.
4. Steward, C.D.; Rasheed, J.K.; Hubert, S.K.; Biddle, J.W. and Raney, P.M. **2001**. Characterization of clinical isolates of Klebsiella pneumoniae from 19 laboratories using the National Committees for Clinical Laboratory Standards, extended spectrum beta–lactamase detection methods. *J. Clin. Microbial.* **39**: 2864 – 2872.
5. Mickelsen, P.A.; Plorde, J.J.; Gordon, K.P.; Hargisis, C. and Muclure, J. **1985**. Instability of antibiotics resistance in a strain of Staphylococcus epidermidis isolated from an outbreak of prosthetic valve endocarditis. *J. Infect. Dis.* **152**:50–58.
6. Holt, G.T.; Krie, R.N.; Sneath, P.H.A.; Staley, T.J. and Williams, T.S. **1994**. *Bergeys Manual of Determinative Bacteriology.* 9<sup>th</sup> edition. (Williams and Wilkins, Baltimore, Md., USA). pp.176- 211.
7. Collee, J.G. and Marr, W. **1996**. *Culture of bacteria. In: Mackie and Maccarthey. Practical medical microbiology.* (Eds. Collee, J.G., Fraser. A.G., Marmion. B.P. and Simmon. A.) 14<sup>th</sup> ed., Churchill Livingstone, New York, pp.113–129.
8. Yan, J.J.; Ko, W.C.; Tsai, S.H.; Wu, H.M. and Wu, J.J. **2001**. Outbreak of infection with multi-drug resistant Klebsiella pneumoniae carrying bla (IMP-8) in a university medical centre in Taiwan. *J. Clin. Microbial.* **39**: 4433-4439.
9. Trevors, J.T. **1986**. Plasmid curing in bacteria. *FEMS microbiology reviews.* **32**:109 – 199.
10. Marcelo, E.T.; Luis, A.A. and Jorge, H.C. **1993**. *Virulence plasmids. In: Plasmids: A partical approach* (Ed.): K.G. Hardy. Oxford university press, Oxford. pp. 5– 118.
11. National Committee for Clinical Laboratory Standards. **1999**. *Performances standards for antimicrobial susceptbility testing.* 9<sup>th</sup> ed., informational supplemented. Wayne, P.A.: National Committee for Clinical Laboratory Standards.

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- indigenous *Klebsiella*. *Pak J. Bot.* **35**(2):243–248.
24. Wang, M.; Sahm, M.F.; Jacoby, G.A. and Hooper, D.C. **2004**. Emerging plasmid-mediated quinolones resistance associated with the *qnr* gene in *Klebsiella pneumoniae* clinical isolates in the United States. *Antimicrob. Agents. Chemother.* **48**(4): 1295–1299.
  25. Paterson, D.L.; Mulazimoglu, L. and Casellas, J.M. **2000**. Epidemiology of ciprofloxacin resistance and its relationship to extended spectrum beta-lactamase production in *Klebsiella pneumoniae*. *Clin. Infect. Dis.* **30**:473–478.
  21. Nathisuwan, S.; Burgess, D.S. and Lewis, J.S. **2001**. Extended spectrum beta-lactamase: epidemiology, detection and treatment. *Pharmacotherapy.* **21**:920 – 926.
  22. Shahid, M.; Malik, A.; Akram, M.; Agrawal, L.M. and Khon, A.U. **2008**. Prevalent phenotypes and antibiotics resistance in *Escherichia coli* and *Klebsiella pneumoniae* at an Indian tertiary care hospital: plasmid-mediated Cefoxitin resistance. *Int. J. Infect. Dis.* **12**(3): 256 – 264.
  23. Sheikh, A.R.; Afsheen, A.; Sadia, K. and Abul Wanab, A. **2003**. Plasmid born antibiotic resistance factors among