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Mortality Pattern of *E.coli* PB-176 and its Transconjugants Harbouring Different Plasmids in Experimental Swiss Mice

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Abstract

The experiment conducted in Division of Microbiology CDRI, Lucknow All the seven haemolytic, drug resistant strains of PB-176, were tested for virulence in mice along with parent strain E.coliPB-176 (R^-hly^-). Four different dilutions of varying number of bacteria of each strain were inoculated intraperitoneally to 24 swiss mice in each batch. The mortality was observed upto ten days. The mortality at the $0.17x10^8$ cells/mouse R^+hly^+ strain was 91.6% and (88.8, 85.8, 88.8, 91.6, 88.8 and 84.5%) respectively. While at the dose $0.11x10^8$ cells/mouse the mean mortality for the same was 67.9% and (62.5, 59.6, 58.3, 56.6, 62.5 and 60.8%) respectively.

Keywords: *E.coli*, mortality, Plasmids.

Introduction

Escherichia coli usually considered to be an opportunistic pathogen which constitutes a large proportion of the normal intestinal flora of man and animals. This organism can, however contaminate, colonize and subsequently cause infection of extra intestinal sites and is a major cause of septicemia, peritonitis, abscesses, meningitis and UTI in men and animal. An impressive volume of epidemiological data has accumulated which demonstrates unequivocally that the wide spread and increasing occurrence of such R-plasmids or so-called R-factor present in bacteria progressively reduce or at least complicates effective treatment of human and animal bacterial infections. One of the important plasmids encoded property is the ability to produce haemolysin which is released extracellularly and lyse RBCs of several species of animals and human.

Material and Methods

The transfer studies of haemolytic factor were done according to Smith and Halls (1967) and LeMinor and LeCoueffic (1975).

All the haemolytic straisn were taken as donor. Three non haemolytic strains resistant to nalidixic acid (To which donor strains were sensitive) were taken as recipient i.e. *Salmonella typhimurium*, K-12 J62I and *E.coli* PB-176.

Conjugal transfer of antibiotic resistance factor

For conjugal transfer of R-factor, haemolytic colonies of *E.coli* PB-176 from blood agar were taken as recipient. Six non-haemolytic *E.coli* strain resistant to five antibiotics (streptomycin, penicillin, chloramphenicol, ampicillin, tetracycline) and sensitive to nalidixic acid were also used as donor. Conjugation was carried out in the same way as in haemolytic factor transfer studies. Conjugant mixtures were plated on blood agar plates containing double antibiotics (nalidixic acid 100 µg/ml. And respective antibiotics 50 µg/ml). Plates were incubated overnight at 37^{O}C. Then plates were observed for conjugant colonies of recipient and tested in the same way as in drug resistance transfer studies.

Measurement of virulence and estimation of LD_{50}

The virulence of bacteria for mice was assessed by a modification of the test described by Vanden Bosch *et al.* (1979).

Strains to be checked for virulence were grown overnight in nutrient broth and then centrifuged at 8000 rpm. For 10 minutes, supernatant was removed and pellets were washed in PBS twice. Pellets were resuspended in normal saline and a series of dilutions were made. Selected dilutions of known bacterial cells were injucted intraperitoneally/ intravenously in different batches of mice having 24 mice in each batch. Each mice was inoculated with 0.1 ml. Of each dilution and 0.1 ml. was spreaded over nutrient agar plate for counting the colonies. Mortality was recorded upto ten days.

A batch of 24 mice was also included as healthy control group, which was inoculated intraperitoneally with 0.1 ml. of normal saline.

Difference in virulence of two type of strains (plasmid bearing and non-plasmid bearind) was assessed by the mortality pattern of both strains in swiss mice.

Result and Discussion

To determine the cumulative effect of R-factor and haemolysin on the pathogenicity and virulence, a haemolytic conjugant of E.coliPB-176 was conjugated with six strains of E.coli resistant to different antibiotics. Hence six double conjugal strains of PB-176 with hly^{+} and R^{+} were obtained. At Ist day, the mortality was nil with all the strain, it was observed from 2^{nd} to 3^{rd} day, post-inoculation. Maximum mortality was observed upto ten days. At Ist day, the mortality was nil with all the strain, it was observed from 2^{nd} to 3^{rd} day, post-inoculation. Maximum mortality was observed from 2^{nd} to 6^{th} day in all the strains. Mortality on 8^{th}, 9^{th} and 10^{th} day and onwards was similar with each test strains. It is clear from the (Table 1 and Fig. 1) that PB-176 hly^{+} caused higher mortality than PB-176 hly^{-}. At the dose $0.17x10^{8}$ bacterial cells/mouse of PB-176 hly^{+} and PB-176 hly^{-}, the percentage mortality was 91.6% and 48.3%, respectively; likewise,

at the dose $0.11x10^8$ bacterial cells/mouse the mortality of both strains was 67.9% and 31.6% respectively. When each mouse of different batches was inoculated with $0.085x10^8$ bacterial cells/mouse and $0.056x10^8$ bacterial cells/mouse, the percent mortality of both hly^+ and hly^- strains was 42.9%, 20.8% and 17.9%, 8.3% respectively. There was no significant difference in mortality, caused by hly^+ strain and hly^+ R^+ strains. Likewise when each mouse was inoculated with $0.085x10^8$ cells/mouse and $0.056x10^8$ cells/mouse, the total percent mortality of PB-176 hly^+ and PB-176 R^+Hly^+ was observed 42.9% (38.7, 34.6, 41.6, 37.5, 42.9 and 26.3%) and 37.1% (19.5, 19.1, 20.8, 17.9, 17.9 and 19.1%) respectively. Mortality in each dose decreased slightly after introduction of R-factor to haemolytic strain, but this difference was statistically non-significant. The result found R. Bartoletti *et al.* (2016), F. Allerberger *et al.* (2009), I Vik *et al.* (2014), R. Funfustuck (2012), B.M. Kazemier *et al.* (2015), L.E. Nicolle *et al.* (2005), H.W. Baver*et al.* (2005), J. Wybran*et al.* (1989). M.A. Beerepoot *et al.* (2013), J.D. Sammon *et al.* (2014), P. Cassier *et al.* (2011), P.M. Hawkey (2018), H.S. Sader *et al.* (2015), M. Franz and W.H. Horal (1999), Y. Gao *et al.* (2017), D.D. Cardenas and T.M. Hooton (1995), C.E. Chenoweth *et al.* (2014) noticed.

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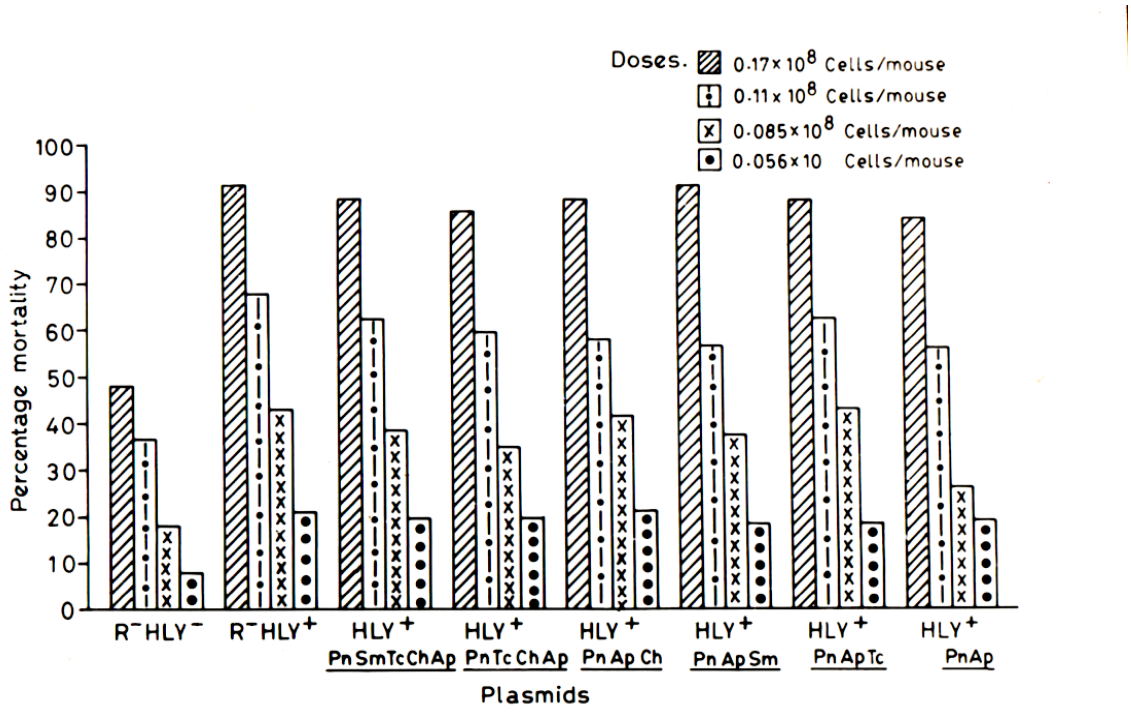

Fig.1 : Showing virulence of E.coli PB-176 strain before and after acquisition of different plasmids in experimental swiss mice.

References

A. Bosch, V.J. Benedi, R. Pares, and J. Jofre, (1988) "Enhancement of the humoral immune response and resistance to bacterial infection in mice by the oral administration of a bacterial immunomodulator (OM-89)," *Immunopharmacology and Immunotoxicology*, vol. 10, no. 3, pp. 333-343.

B. Kranjcec, D. Papes, and S. Altarac, (2014) "D-mannose powder for prophylaxis of recurrent urinary tract infections in women: A randomized clinical trial," *World Journal of Urology*, vol. 32, no. 1, pp. 79-84.

B.M. Kazemier, F.N. Koningstein, C. Schneeberger *et al*. (2015) "Maternal and neonatal consequences of treated and untreated asymptomatic bacteriuria in pregnancy: A prospective cohort study with an embedded randomized controlled trial," *The Lancet Infectious Diseases*, vol. 15, no. 11, pp. 1324-1333.

C.E. Chenoweth, C.V. Gould, and S. Saint, (2014) "Diagnosis, management and prevention of catheter-associated urinary tract infections," *Infectious Disease Clinics of North America*, vol.28, no. 1, pp. 105-119.

D.D. Cardenas and T.M. Hooton, (1995) "Urinary tract infection in persons with spinal cord injury," *Archives of Physical Medicine and Rehabilitation*, vol. 76, no. 3, pp. 272-280.

E. Allerberger, R. Gareis, V. Jindrik, and M.J. Struelens, (2009) "Antibiotic stewardship implementation in the EU: The way forward," *Expert Review of Anti-infective Therapy*, vol. 7, no. 10, pp. 1175-1183.

H.S. Sader, M. Castanheira, R.E. Mendes, R.K. Flamm, D.J. Farrell, and R.N. Jones, (2015) "Ceftazidime-avibactam activity against multidrug-resistant *Pseudomonas aeruginosa*isolated in U.S. Medical Centers in 2012 and 2013," *Antimicrobial Agents and Chemotherapy* vol. 59, no. 6, pp. 3656-3659.

H.W. Bauer, S. Alloussi, G. Egger, H. Blumlein, G. Cozma, and C.C. Schulman, (2005) "A Long-Term, multicenter, double-blind study of an *Escherichia Coli* extract (OM-89) in female patients with recurrent urinary tract infections," *European Urology*, vol. 47, no. 4, pp. 542-548.

I. Vik, M. Bollestad, N. Grude*et al.*, (2014) "TIbuprofen versus mecillinam for uncomplicated cystitis – a randomized controlled trial study protocol," *BMC Infectious Diseases*, vol. 14, p. 693.

I.E. Nicolle, S. Bradley, R. Colgan, J.C. Rice, A. Schaeffer, and T. M. Hooton, (2015) "Infectious diseases society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults," *Clinical Infectious Diseases*, vol. 40, no. 5, pp. 643-654.

J. Wybran, M. Libin, and L. Schandene, (1989) "Activation of natural killer cells and cytoline production in man by bacterial extracts," *Immunopharmacology and Immunotoxicology*, vol. II, no. 1, pp. 17-32.

J.D. Sammon, P. Sharma and H. Rahbar (2014) "Predictors of admission in patients presenting to the emergency department with urinary tract infection," *World Journal of Urology*, vol. 32, no. 3, pp. 813-819.

Le Minor, S. and Le Coueffic, E. (1975). Etude surles hemolysines des Enterobacteriaceae. *Ann. Micorbiol.* (Paris) 126: 313-332.

M. Franz and W.H. Horl, (1999) "Common errors in diagnosis and management of urinary tract infection. II: Clinical management," *Nephrology Dialysis Transplantation*, vol. 1`4, no. 11, pp. 2754-2762.

M.A. J. Beerepoot, S.E. Geerlings, E.P. Van Haarst, N. Mensing Van Charante, and G. TerRiet, (2013) "Nonantibiotic prophylaxis for recurrent urinary tract infections: A systematic review and meta-analysis of randomized controlled trials," *The Journal of Urolory*, vol. 190, no.6, pp. 1981-1989.

P. Cassier, S. Lallechere and S. Aho (2004) "Cephalosporin and fluoroquinolone combinations are highly associated with CTX-M β-lactamase producing *Escherichia coli:* A case-control study in a French teaching hospital," *Clinical Microbiology and Infection*, vol. 17, no. 11, pp. 1746-1751.

P.M. Hawkey, R.E. Warren and D.M. Livermore, (2018) "Treatment of infections caused by multidrug-resistant gram-negative bacteria: Report of the British society for antimicrobial chemother-apy/healthcare infection society/british infection association joint working partry," *Journal of Antimicrobial Chemotherapy*, vol. 73, no. 3, pp. iii2-iii78.

R. Bartoletti, T. Cai, E.M. Wagenlehner, K. Naber, and T.E. Bjerklund Johansen, (2016) "Treatment of Urinary Tract Infections and Antibiotic Stewardship," *European Urology Supplements*, vol. 15, no. 4, pp. 81-87.

R. Funfstuck, I.E. Nicolle, M. Hanefeld, and K.G. Naber, (2012) "Urinary tract infection in patients with diabetes mellitus," *Clinical Nephrology*, vol. 77, no. 1, pp. 40-48.

Smith, H.W. and Halls, S. (1967). The transmissible nature of the genetic factor in *E.coli* that controls haemolysin production. *J. Gen. Microbiol.* 47: 153-161.

Van Den Bosch, J.F.: Graaff, J.De. and Maclaren, D.M. (1979). Virulence of *E. coli* in experimental hematogenus pyelonephritis in mice. *Infect. Immun.* 25 (1): 68-74.

Y. Gao, T. Danforth, and D.A. Ginsberg, (2017) "Urologic Management and Complications in Spinal Cord Injury Patients: A 40 to 50 –year Follow-up Study," *Urology*, vol. 104, pp. 52-58.

Table 1.Mortality of E. coli PB 176 and its transconjugants harbouring different plasmids in experimental swiss mice

Strain	Dose: No. of cells/ mouse	Mortality* (Mean±SD) PB-176 and its transconjugats harbouring different plasmids in experimental swiss mice.									
		1	2	3	4	5	6	7	8	9	10
E. coli R^-Hly^-	$0.17x10^8$	0	3.6±0.57	6.6±3.2	8.6±3.2	9.6±2.5	10.3+1.5	11.6±1.2	11.6±1.2	11.6±1.2	11.6±1.2
	$0.11x10^8$	0	2.3±0.57	4±1	5±1	6.3±1.2	7±1	7.6±1.5	7.6±1.5	7.6±1.5	7.6±1.5
	$0.085x10^8$	0	1	2±1	3±1	3.3±1.5	4.3±1.3	4.3±1.3	4.3±1.3	4.3±1.3	4.3±1.3
	$0.056x10^8$	0	0	1±1	1.3±0.57	1.6±0.57	2±1	2±1	2±1	2±1	2±1
E. coli R^-Hly^+	$0.17x10^8$	0	7.6±1.5	10±1	11.6±0.57	14±1	16.3±1.5	20±2	22±2	22±2	22±2
	$0.11x10^8$	0	5.3±0.57	8±1	10.3±0.57	12±1	13.6±1.5	15.6±2.3	16.3±3.1	16.3±3.1	16.3±3.1
	$0.085x10^8$	0	2.6±1.5	6±1	7.6±2.3	8±1	9.3±1.2	10.3±1.2	10.3±1.2	10.3±1.2	10.3±1.2
	$0.056x10^8$	0	1.3±1.2	2.3±1.5	3.3±1.5	4.3±1.2	5±1	5±1	5±1	5±1	5±1
E. coli (PnSmTcChAp) Hly^+	$0.17x10^8$	0	7±2	9.7±1.5	12±1	14.7±1.5	17.7±1.5	19.7±1.5	21.3±1.5	21.3±1.5	21.3±1.5
	$0.11x10^8$	0	6.3±1.5	8.7±2.1	11.3±1.5	13±1	14.3±1.5	15±1	15±1	15±1	15±1
	$0.085x10^8$	0	3±1	6±1	7±1	8±1	8±1	9.3±0.57	9.3±0.5	9.3±0.5	9.3±0.5
	$0.056x10^8$	0	1.3±1.5	2±1	2.7±1.2	3.3±1.5	4.7±1.2	4.7±1.2	4.7±1.2	4.7±1.2	4.7±1.2
E. coli (PnTcChAp) Hly^+	$0.17x10^8$	0	7.3±0.57	9.6±0.57	11.3±0.57	14	17	18.6±0.57	20.6±0.57	20.6±0.57	20.6±0.57
	$0.11x10^8$	0	4.3±1.5	7±2	9.3±2.1	10.3±1.5	12.7±1.5	13.7±2.1	14.3±1.5	14.3±1.5	14.3±1.5
	$0.085x10^8$	0	2.6±0.57	4±2	5.3±2.5	6.3±1.5	7.3±1.5	8.3±1.5	8.3±1.5	8.3±1.5	8.3±1.5
	$0.056x10^8$	0	1	2±1	2.6±0.57	3±1	3.6±0.57	4.6±0.57	4.6±0.57	4.6±0.57	4.6±0.57
E. coli (PnApCh) Hly^+	$0.17x10^8$	0	6.6±0.57	9	12±1	14.3±2.5	16.7±1.5	19.3±0.57	21.3±1.2	21.3±1.2	21.3±1.2
	$0.11x10^8$	0	4.7±1.2	7±1	9.7±1.2	11.3±1.5	12.3±1.5	14±2	14±2	14±2	14±2
	$0.085x10^8$	0	3.3±1.2	5.8±0.57	6.6±0.57	7.6±0.57	9±1	9.6±0.57	10	10	10
	$0.056x10^8$	0	1.6±0.57	3.3±1.2	3.3±0.57	4±1	4.3±1.2	4.6±0.57	5±1	5±1	5±1
E. coli (PnApSm) Hly^+	$0.17x10^8$	0	6.3±1.2	9±1	12±1	14±1	14±1	19.6±1.2	22±1	22±1	22±1
	$0.11x10^8$	0	3.3±0.57	6.6±0.57	9	10.3±1.2	12±1	13±1	13.6±2.3	13.6±2.3	13.6±2.3
	$0.085x10^8$	0	1.3±0.57	2±2	4.3±1.5	6.3±1.5	7.3±1.5	8.6±0.57	9±1	9±1	9±1
	$0.056x10^8$	0	0.6±0.57	1.6±0.57	2	2.6±1.2	3.3±0.57	4±1	4.3±0.57	4.3±0.57	4.3±0.57

E. coli (PnApTc) Hly^+	$0.17x10^8$ $0.11x10^8$ $0.085x10^8$ $0.056x10^8$	0 0 0 0	6 3.3±0.57 2.3±0.57 1	8.6±0.57 6 5±1 2	10.6±0.57 8.3±0.57 6.6±1.2 2	12.6±1.2 11 6.6±1.2 3	16.6±2.1 12.6±0.57 8.3±0.57 3.6±0.57	19.3±2.9 14±1 9.6±1.5 4	21.3±1.2 15±1 10.3±0.57 4.3±0.57	21.3±1.2 15±1 10.3±0.57 4.3±0.57	21.3±1.2 15±1 10.3±0.57 4.3±0.57
E. coli (PnAp) Hly^+	$0.17x10^8$ $0.11x10^8$ $0.085x10^8$ $0.056x10^8$	0 0 0 0	6.3±2.1 4.3±1.2 2.3±1.2 1±1	8.6±1.5 6.6±0.57 3.6±0.57 2.3±1.2	11.3±2.1 9.3±1.2 5.6±0.57 2.6±0.57	13.6±1.5 11±1 6.3±1.2 3±1	16.3±2.1 12.3±1.2 6.3±1.2 3.6±0.57	18.3±0.57 14.3±1.2 6.3±1.2 4.3±0.57	20.3±0.57 14.6±0.57 6.3±1.2 4.6±0.57	20.3±0.57 14.6±0.57 6.3±1.2 4.6±0.57	20.3±0.57 14.6±0.57 6.3±1.2 4.6±0.57

* Mean mortality of three experiments: each comprising 24 mice.