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Effect of Drug Resistance on the Pathogenicity and Virulence of Salmonella Typhimurium

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Abstract: ***Various strains of E. coli. and Salmonella typhimurium were studied in vitro for the presence of plasmids controlling haemolysin, drug resistance, serum resistance and colonization factor etc. They were also tested in mice to determine the probable role of the plasmids on the host pathogenicity. To determine the effect of associated R-factor on the pathogenicity and virulence of S. typhimurium, antibiotic sensitive and resistant strains were tested for virulence in mice. The experiments showed that drug resistance plasmids enhance the lethality of their host. This causes a serious problem in effective treatment of patient suffering with Salmonella and E. coli infections.***

Keywords: ***S. Typhimurium, Plasmids, Drug Resistance, Mortality.***

I. INTRODUCTION

Multiple antibiotic resistance due to plasmids was discovered first in Japan. Since then an impressive volume of epidemiological data has been accumulated which demonstrates unequivocally that the wide spread and increasing occurrence of such R-plasmids or so called R factor present in bacteria complicates effective antibiotic treatment of human and animal bacterial infections. These drug resistance factor transfer themselves to other bacteria independently of bacterial chromosomes in other words even in the absence of chromosome transfer, by cell to cell contact (conjugation). This plasmid mediated resistance is quite frequent due to conjugal transfer of resistance from one organism to another (Infectious drug Resistance) and comparatively more serious problem than resistance controlled by chromosome.

II. MATERIAL AND METHODS

Drug resistance transfer studies were carried out by using the techniques of Watanabe and Fukasawa (1961a) with some modifications *E. coli* strains, resistant to one or more antibiotics and sensitive to nalidixic acid, were used as donors for transfer of antibiotic resistance.*S. typhimurium*was taken as recipient for *in vitro* antibiotic resistance transfer studies. The recipient strains was resistant to natidixic acid.

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A. Measurement of Virulence and Estimation of LD_{50}

The virulence of bacteria for mice was assessed by a modification of the test described by Van Den Bosch *et al.* (1979). Difference in virulence of two type of strain (plasmid bearing and non plasmid bearing) was assessed by the mortality pattern of both strains in swiss mice.

III. RESULT AND DISCUSSION

S. typhimurium(resistant to nalidixic acid and sensitive for all antibiotics) was conjugated with six resistant donor *E. coli*strains, resulting six conjugants of *S. typhimurium*with different patterns of antibiotic resistance were obtained. All the six R^+ strains of *S. typhimurium*and one R^- parent strain were tested in mice to determine the effect of R- factor on the pathogenecity and virulence of its *host*. For comparison of percentage mortality *S. typhimurium*R^- served as control. Four different dilutions with varying number of bacteria of each strain were inoculated intraperitonially to 24 mice in each batch, and mortality was observed upto ten days and onwards. Mortality was found to be nil on day 1, post inoculation. Mortality was constant from 9^{th} and 10^{th} day and onwards (Table-1). For *S. typhimurium*R^-, the percentage mortality was 98.6% at the dose 0.8 x 10^7 cells/mouse while for the six R^+ conjugants the mortality percentage was 85.5%, 83.3%, 87.5%, 83.3%, 87.5% and 83.3% respectively at the same dose.Total mortality percentage of*S. typhimurium*R^- and R^+ conjugants in swiss mice at the dose 0.4 x 10^7 cells/mouse was 77.4% for R^- strain and 66.6%, 58.3%, 66.6%, 62.5% and 47.11% for its R^+conjugants respectively. At the dose 0.26 x 10^7cells/mouse the mortality was 45.8% with R^+ strain and 41.6%, 41.6, 33.3%, 41.6%, 37.5% and 37.5% for R^- strain and 20.8%, 16.6%, 12.5%, 20.8%, 16.6% and with its R^+ strains. At the dose 0.16 x 10^7 cells/mouse total mortality percentage was 23.5% 17.9% for R^+ conjugants respectively. Total mortality percentage of R^+ strains in swiss mice was quite less than R^- strains. It seems that after introduction of R- factor to *S. typhimurium* the pathogencity decreases. A group of 24 mice which served as normal healthy control were inoculated with 0.2 ml normal saline/mouse and none of these control mice died during observation period. LD_{50} values of *S. typhimurium* R^- and its R^+ conjugants were calculated from 5^{th} to 10^{th} day. (Table 2) The molecular basis of understanding the plasmid induced pathogenecity of infectious disease is emerging as a new area of bacteriological research.

In this study the effect of the presence of antibiotic resistance plasmids on the pathogenecity and virulence of its host is being investigated. In the present work it was found that R- factor had no distinct effect on virulence of *E. coli*, **H.S. Sader et al, (2015)**. Some differences were detected but it appeared that in each case the strain initially (before genetic manipulation) was slightly more virulent. It confirms the results of **Michel *et al.*, (1980)** who founno difference between the mouse LD_{50} value of three antibiotic resistant variants of *E. coli* and their sensitive original type strains **Castro-Vargas RE *et al.*, (2020)** also studied the same in Salmonella sp. isolated from poultry. **Weill FX, 2010** reported the patients having typhoid fever and facing the challenges of resistant strains. In contradiction to our data **Qamar FN *et al.*,(2020)** studied the antimicrobial resistance in typhoidalSalmonella. It is not possible to conclude on the basis of these data whether R-plasmids lack a virulence enhancing determinants **J.D. Sammon*et al.*, (2014)**. In any event, the supposition that epidemic R-plasmids is responsible for the increased virulence of their host bacterium, lacks convincing supportive evidence.

Table: 1 Effect of R-factor on the Virulence and Pathogenicity of S. typhimuriumin experimental swiss mice.

Strain	Dose: No. of cells/ mouse	Mortality* (Mean±SD) in different days									
		1	2	3	4	5	6	7	8	9	10
*S. typhimurium*R^{-}	$0.8x10^7$	0	3	7	11	16±1	18+1	20±1	21.6±0.57	23.7±0.57	23.7±0.57
	$04x10^7$	0	2	4	7	11±1	13±1	16±1	16.6±0.57	18.6±0.57	18.6±0.57
	$0.26x10^7$	0	1	2	4	7±1	9±1	10±1	1104.6±0.57	11±1	11±1
	$0.16x10^7$	0	0	1	3	3.6±0.57	5±1	5.3±1.5	5.6±0.57	5.6±0.57	5.6±0.57
S. typhimurium (PnSmTcChAp)	$0.8x10^7$	0	2	6	10	13+1	15+1	16+1	18.6+0.57	20.6+0.57	20.6+0.57
	$04x10^7$	0	1	4	6	9±1	12±1	14±1	15±1	16±1	16±1
	$0.26x10^7$	0	0	2	4	6±1	7±1	8±1	9±1	10±1	10±1
	$0.16x10^7$	0	0	0.3±0.57	2±1	3.3±0.57	4.3±0.57	5±1	5±1	5±1	5±1
S. typhimurium (PnTcChAp)	$0.8x10^7$	0	1	5	8	11+1	16+1	18+1	19+1	20+1	20+1
	$04x10^7$	0	0	2	4	6.3±1.5	9±1	11±1	12±1	14±1	14±1
	$0.26x10^7$	0	0	1	3	5±1	7±1	9±1	10±1	10±1	10±1
	$0.16x10^7$	0	0	0	2	3±1	3.3±0.57	4±1	4±1	4±1	4±1
S. typhimurium (PnApTc)	$0.8x10^7$	0	2	5	8	11+1	15+1	17+1	19+1	21+1	21+1
	$04x10^7$	0	1	4	6	9±1	11±1	12±1	13±1	14±1	14±1
	$0.26x10^7$	0	1	3	5	5±1	6±1	8±1	8±1	8±1	8±1
	$0.16x10^7$	0	0	1	2	2.3±0.57	2.3±0.57	3±1	3±1	3±1	3±1

Table ...Continued .....

S. typhimurium (PnSmAp)	$0.8x10^7$	0	2	4	8	12+1	16+1	19+1	20+1	20+1	20+1
	$04x10^7$	0	1	3	6	7.3±0.57	8.3±0.57	9.3±0.57	10.3±0.57	10.3±0.57	10.3±0.57
	$0.26x10^7$	0	0	1	2	6±1	8±1	9±1	9±1	9±1	9±1
	$0.16x10^7$	0	0	0	1	1.6±0.57	3±1	3.3±0.57	4.3±0.57	4.3±0.57	4.3±0.57
S. typhimurium (PnSm)	$0.8x10^7$	0	3	5	7	11+1	15+1	17+1	19+1	21+1	21+1
	$04x10^7$	0	2	4	7	9±1	12±1	13±1	14±1	15±1	15±1
	$0.26x10^7$	0	0	1	3	5±1	7±1	8±1	8.6±0.57	9±1	9±1
	$0.16x10^7$	0	0	1	2	3±1	3.3±0.57	4±1	4±1	4±1	4±1
S. typhimurium (PnAp)	$0.8x10^7$	0	2	5	7	10+1	14+1	16+1	18+1	20+1	20+1
	$04x10^7$	0	1	4	7	9.6±0.57	12±1	14±1	15±1	16±1	16±1
	$0.26x10^7$	0	0	1	3	5±1	8±1	9±1	10±1	10±1	10±1
	$0.16x10^7$	0	0	1	3	4±1	4.3±0.57	5±1	5±1	5±1	5±1

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

Table 2 : Comparative LD_{50} values of drug sensitive *S. typhimurium* and its six drug resistant transconjugants in experimental swiss mice.

Strains	LD_{50}* (mean ± S.D.) x 10^7 Days					
	5^{th}	6^{th}	7^{th}	8^{th}	9^{th}	10^{th}
*S. typhimurium*R^{-}	0.45± 0.05	0.37± 0.03 NS	0.34± 0.04 NS	0.30± 0.01 P<0.05	0.28± 0.01 P<0.05	0.28± 0.01 P<0.05
S. typhimurium (PnSmTcChAp)	0.71± 0.09	0.47± 0.03 P<0.05	0.46± 0.02 P<0.01	0.43± 0.02 P<0.01	0.36± 0.02 P<0.01	0.36± 0.02 P<0.00
S. typhimurium (PnTcChAp)	0.71± 0.03	0.47± 0.03 P<0.005	0.46± 0.04 P<0.005	0.43± 0.03 P<0.005	0.36± 0.02 P<0.001	0.36± 0.02 P<0.001
S. typhimurium (PnApTc)	0.80± 0.02	0.43± 0.04 P<0.005	0.38± 0.03 P<0.005	0.37± 0.03 P<0.001	0.33± 0.04 P<0.001	0.33± 0.04 P<0.01

S. typhimurium (PnSmAp)	0.81± 0.01	0.56± 0.04 P<0.005	0.51± 0.02 P<0.001	0.44± 0.04 P<0.001	0.41± 0.02 P<0.001	0.41± 0.02 P<0.00
S. typhimurium (PnSm)	0.82± 0.02	0.42± 0.03 P<0.005	0.38± 0.02 P<0.001	0.36± 0.03 P<0.001	0.34± 0.03 P<0.001	0.34± 0.03 P<0.001
S. typhimurium (PnAp)	0.82± 0.02	0.41± 0.02 P<0.001	0.36± 0.02 P<0.001	0.33± 0.03 P<0.001	0.32 ± 0.02 P<0.001	0.32± 0.02 P<0.001

Note - All the 'p' values are expressed in comparison of 5th day's LD_{50} of each individual strain.

* D_{50} values are expressed as number of organism x 10^7 and mean of three experiments – involving 24 mice in each experiment.

IV. CONCLUSION

It was found that the only presence of R-factor is not sufficient to enhance the virulence of its host bacterium. Some additional factors such as homolysin, colicin and colonisation factors are necessary for the enhancement of pathogencity. Drug resistance provide only armor against the antibiotics.

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