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EC MICROBIOLOGY
Research Article

Loss of Antibiotic Resistance Transposons if Bacterial Pathogens Grown in Antibiotics Free Environment

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Received: April 13, 2026; **Published:** April 29, 2026

Abstract

Antibiotic resistance plasmid pCG86 was isolated from the pathogen ETEC present in the stool of dead piglet apparently responsible for the death of piglet after suffering from diarrhoeal disease. This ETEC carried plasmid pCG86 of 117Kb containing genes for the production of enterotoxin both heat stable and heat labile but the use of antibiotics at a low dose can't be effective because the piglet lacking body immunity but on the contrary produced transposons. What made me think there was an abuse of antibiotics because the piglets were lacking body immunity apparently essential for the efficacy of antibiotics. Dr. Carlton Gyles, (Professor of Veterinary College, Waterloo University, Canada) failed to save these piglets. An article was published in American Journal (Science) in the year 1977. It was collaborative project between Dr. Gyles (Canada) and Dr. Palchudhuri & Prof. Maas (USA). They showed this pathogen ETEC carries a single plasmid pCG86 carrying genes for the production of enterotoxin both heat stable and heat labile as well as antibiotic resistance transposons. Evidently antibiotics failed to save these piglets. Before this work Dr. Gyles sent the same E. coli ETEC isolate to his Ph.D. mentor Professor S. Falkow (University of California) with clinical history. Subsequently Dr. Falkow transferred pCG86 by mating with *E. coli* K-12 (711) and saved in a nutrient agar stab.Therefore, Professor Maas wanted me to characterize. After characterization we concluded that the antibiotic resistance plasmid pCG86 becomes Ent p307 but lost only antibiotic resistance characters (transposons) but not the toxin genes when transferred into *E. coli* K-12 (711).

Keywords: *Antibiotic Resistance Transposons; Bacterial Pathogens Growth; Antibiotics Free Environment*

Introduction

In 1961 cholera has affected greatly the Indian subcontinent along with almost the whole world. Nevertheless, cholera is an under-recognized problem in India; its endemicity in the country has been evidenced since the ancient times, India, where the disease is endemic, cholera outbreaks occur every year in between dry (March-April) and rainy (September-October) seasons [1]. The city Kolkata faced several outbreaks of cholera due to *V. cholerae* strains belonging to both the serogroups O1 and O139, and the biotypes, classical and El Tor of O1 serogroup [2,3]. Cholera toxin is heat-stable. Dr. Gyles sent the *E. coli* ETEC with the R plasmid pCG86, but he saved this strain in the presence of antibiotics and sent to his mentor Professor S. Falkow (university of California, USA), who stored this strain in an agar stab without adding any antibiotics [4]. In 1975 Professor W.K Maas received this strain from Dr. S Falkow for complete characterization. Therefore, we characterized and observed that antibiotic resistance pCG86 had become Ent p307 but lost only antibiotic resistance transposons [5].

We also received the original isolate of ETEC carrying an extra chromosome pCG86 from the stool of the piglet suffering from diarrhoeal disease caused by ETEC from Dr. Carlton Gyles and the same isolate from his mentor Professor Falkow who transferred it to *E. coli* K-12.

Then we characterized EntP307 (cryptic plasmid) of length 80 Kb and pCG86 (117 Kb). We now observed these two plasmids are homologous and the plasmids carry the same R replicon. In this article Santos., *et al*. have mentioned the Ent replicon instead of R replicon [5].

Result

Application of a new technique EM-heteroduplex analysis was applied by Palchaudhuri S to shows DNA sequence relationship between the two DNA bio-macromolecules F ins (100Kb) with a reference index at 58.8 Kb and the Ent P307(80 Kb) [6]. This heteroduplex Ent p307 does not have any homology with the F replicons (Rep F1A and RepF1B) but shows homology with incompatibility loci. In my recent article I have shown F plasmid was never a single replicon but precisely a co-integrate of two replicons F replicon and R replicon but the transposon Tn1000 never allowed these two replicons to function simultaneously [7]. But the antibiotic resistance Plasmid pCG86 and the Ent P307 has the same antibiotic resistance replicon (Rep F1C).

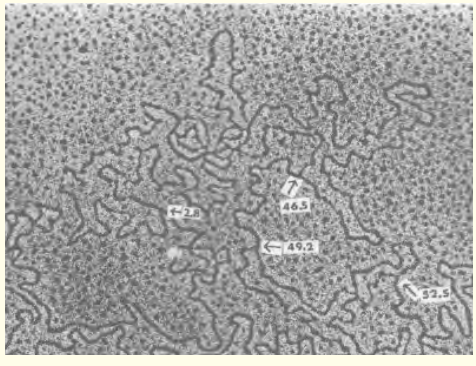

Figure 1: *Heteroduplex formed between E. coli K-12 sex factor F with a marker at 58.8 Kb and the non-transmissible cryptic plasmid Ent P307(enterotoxin positive).*

We also characterized the original isolate of ETEC carrying the antibiotic resistance plasmid pCG86, length 117 Kb and its deletion derivative Ent P307, length 80 Kb received from Dr. Falkow in *E. coli* K-12 (711). Interestingly the ETEC does not lose antibiotic resistance characters but in *E. coli* K-12 (711) the antibiotic resistance characters are lost. We now recognised that the antibiotic resistance characters are transposons or mobile DNA elements.

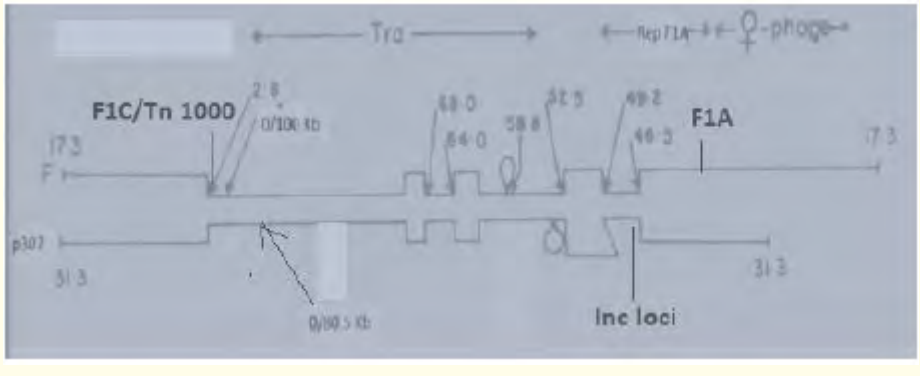

Figure 2: *Schematic diagram of the same heteroduplex bio-molecule (F ins,100 Kb/ Ent P307 80 Kb) to show the homology and non-homology between them.*

Discussion and Conclusion

Diseases caused by *E. coli* strains include diarrhoea, dysentery, haemolytic uremic syndrome (kidney failure), bladder infections, septicemia, pneumonia and meningitis [8].

Different strains are associated with different conditions. That is a strain of *E. coli* that causes diarrhoea will not cause urinary tract infections or meningitis and vice versa. The versatility of *E. coli* strains is due to the fact that different strains have acquired different sets of virulence genes. Joshua Lederberg joined the laboratory of Dr Tatum as a summer student. His project was to draw a kill curve using laboratory bacterium *E. coli* K-12 after exposure to different doses of X-irradiation, they failed to think about the genesis of maleness but showed the gene transfer by the intimate contact between the radiation exposed *E. coli* K-12 and the population not yet exposed. However, the word "Serendipity" was used for their ignorance (1946) [9]. After some years a British doctor W. Hayes claimed that he also discovered the maleness of *E. coli* K-12 but they were in a bitter conflict for several years but Lederberg applied this knowledge in developing *E. coli* K-12 genetics. Such conflict between these two groups of investigators continued for several years until Molecular Biology was born. F factor carries four mobile DNA elements IS1, IS2, IS3 and Tn1000. Antibiotic resistance transposons formed a ring of r-det and associated with the r replicon by using the IS1 in a direct order. What is more we have formed several different types of Hfr males by using homologous recombination between F (the IS2 and IS3 located at different sites of bacterial chromosome forming stable Hfr donors. However, *E. coli* chromosome has a single origin of replication at 84.5 min of the chromosome (100 min or 4736 KB). What helps the *E. coli* K-12 or *E. coli* C to prevail as monocopy? My answer is the transposon Tn1000. Are *E. coli* C and *E. coli* K-12 males? My answer is "No". *E. coli* C is the female but functions as surrogate mother of the bacteriophage phiX174 [10].

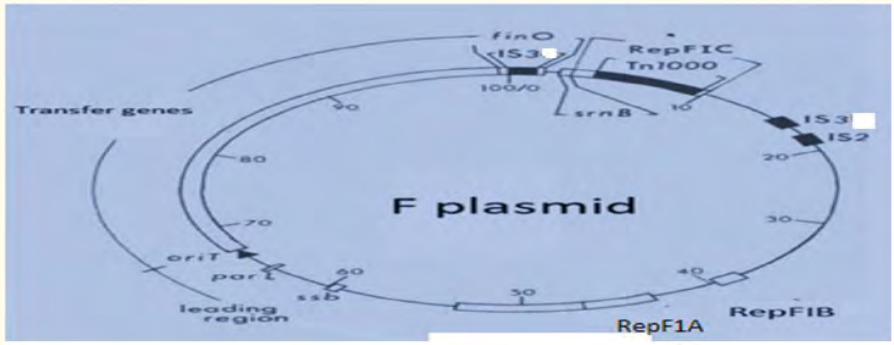

Figure 3: *Map of F plasmid as co-integrate of two replicons F1A functional replicon and the F1C R replicon remains silent by the insertion of transposon Tn1000 (5.7 Kb)..*

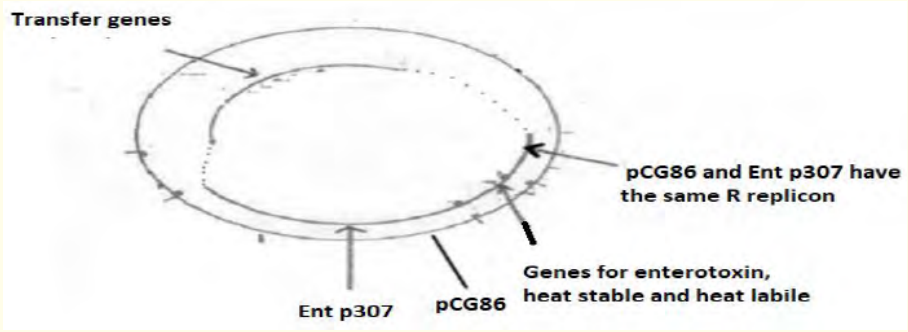

Figure 4: *pCG86 and Ent P307 contain the antibiotic resistance replicon (the F1C replicon prevails silently as a co-integrate of F plasmid but nonfunctional by the insertion of transposon Tn1000).*

Why we started *in vitro* gene cloning using a multicopy cloning vector pBR322 but carrying antibiotic resistance transposons. Such a technique has contaminated our planet with antibiotic resistance transposons!

Unlike antibiotic resistance transposons (Tn1, Tn2, Tn3 etc) Tn1000 does not carry any genetic marker but prevails silently in F plasmid and keeps the F in a monocopy state [11]. This Transposon Tn1000 stops the R replicon by its physical insertion into the R replicon and stops from functioning but in cis. Does it function in trans? The Tn1000 also controls the copy number of F or R replicons and their functional states; but never allowed the two replicons to function simultaneously. How does the Tn1000 (5.7 Kb) function if we introduce the multicopy cloning vector pBR322 in the *E. coli* F plasmid? The mini-F is lacking the Tn1000 and therefore the cloning vector pBR322 uses the *E. coli* (C600) but not the *E. coli* K-12 (12). Question remains how the F factor was born in *E. coli* K-12 as an extra-chromosome? Now I want to mention that the male specific bacteriophage M13 touches the F pili (Type IV) of F+ male (bio-signal) to be drawn into the cytoplasm for the genetic continuity [13]. M13 progeny then come outside by puncturing the cell membranes (adhesion zone?). The host *E. coli* F+ mother may survive as an Hfr male to avoid sharing the same adhesion zone.

Antibiotic plasmid pCG86 carries antibiotic resistance characters and genes for production of enterotoxin (LT and ST). When pCG86 is transferred into *E. coli* K-12 derivative (711), it becomes Ent P307, Ent P307 produces both toxins heat stable and heat labile but lost antibiotic resistance transposons. My conclusion is antibiotic resistance transposons are lost when it prevails in the growth environment free of antibiotics.

That shows Tn1000 does not allow to function both F and R replicons simultaneously. At the same time if *E. coli* K-12 F+ does not allow the multicopy cloning vector pBR322 to act as cloning vector. Ent P307 and pCG86 have the same R replicon but the *E. coli* sex factor F carries two replicons without allowing the both to function simultaneously [7].

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Volume 22 Issue 5 May 2026