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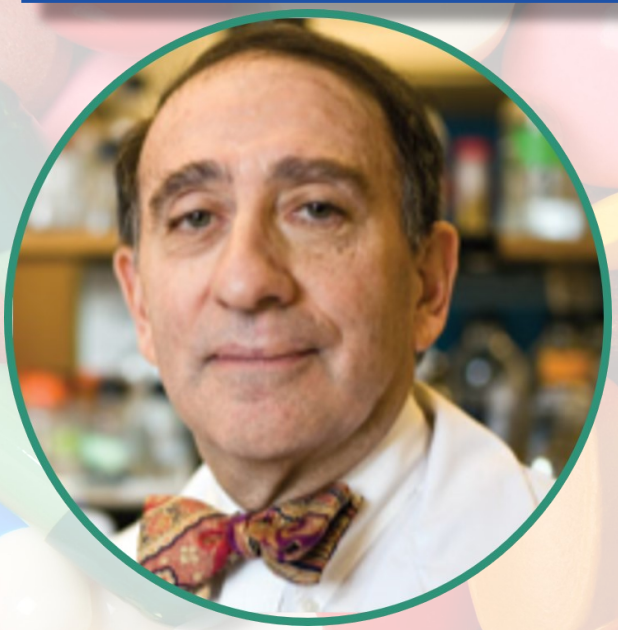
NEWSLETTER

PUBLISHED SINCE 1983 BY THE ALLIANCE FOR THE PRUDENT USE OF ANTIBIOTICS

Professor Stuart B. Levy
(21 November 1938 - 4 September 2019)



The scientific and medical communities mourn the passing of Professor Stuart B. Levy. He was a visionary who foresaw the consequences of the inappropriate use of antibiotics and who coined the now much-used phrase “antibiotic stewardship”. He co-founded APUA to bring together an international network to advocate for the prudent use of antibiotics in both the professional and public arenas.

This special APUA tribute newsletter features just a few of Professor Levy’s key publications and highlights their continued relevance today. It also pays tribute to the gentleman who was Stuart Levy.

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ALLIANCE FOR THE PRUDENT USE OF ANTIBIOTICS

The Alliance for the Prudent Use of Antibiotics (APUA) and the International Society of Antimicrobial Chemotherapy (ISAC) were very sad to hear the news that Professor Stuart Levy passed away on Wednesday 4 September 2019 after an extended illness. He would have turned 81 in November.

Stuart B. Levy M.D. was both a physician and researcher. He was Professor of Molecular Biology and Microbiology and of Medicine and Director of the Center for Adaptation Genetics and Drug Resistance at Tufts University School of Medicine in Boston. Amongst the many offices he held, he was a past President of the American Society of Microbiology (ASM) and founder of APUA which he served as President until very recently.

Stuart Levy was best known for his work on antibiotic resistance. His 1976 prospective farm study showing that antibiotic resistance elements can be transferred from intestinal flora of farm animals to workers was a breakthrough, demonstrating that the use of antibiotics as livestock growth-enhancers was a dangerous practice with significant risks to clinical care. In 1978 he discovered that the mechanism of tetracycline resistance was an energy-dependent antibiotic efflux pump. His research into multiple drug resistance revealed a regulatory locus, mar, for intrinsic antibiotic resistance and virulence among Enterobacteriaceae and other bacteria. He published over 300 papers and edited four books devoted to antibiotic use and resistance.

Stuart Levy championed the prudent use of antibiotics and wrote the landmark book, *"The Antibiotic Paradox: 'How Miracle Drugs Are Destroying the Miracle'"* now in its second edition and translated into four languages. The ASM book "*Frontiers in antimicrobial resistance : a tribute to Stuart B. Levy"* was published in 2005 and is based on his work.

Stuart Levy was Chairperson of the U.S. Fogarty Center study of "Antibiotic use and resistance worldwide" and helped write the U.S. Office of Technology Assessment report on antibiotic resistant bacteria. He consulted for international and national organisations including WHO, the U.S. National Academy of Sciences and Institute of Medicine and the FDA. In 2011 he was presented with ISAC's highest award, the Hamao Umezawa Memorial Award. Amongst many other accolades, he received the Hoechst Roussel Award for esteemed research in antimicrobial chemotherapy from ASM and the 2012 Abbott-ASM Lifetime Achievement Award.

APUA is in mourning for its founder and would like to convey heartfelt sympathy to Stuart Levy's family and friends. He will always be remembered as the father of "antibiotic stewardship" and his legacy will live on through the activities of APUA which ISAC is honoured to support.

Professor Pierre Tattevin, APUA Chair
On behalf of The APUA Board

The Antibiotic Paradox: a short review

Robert Gaynes

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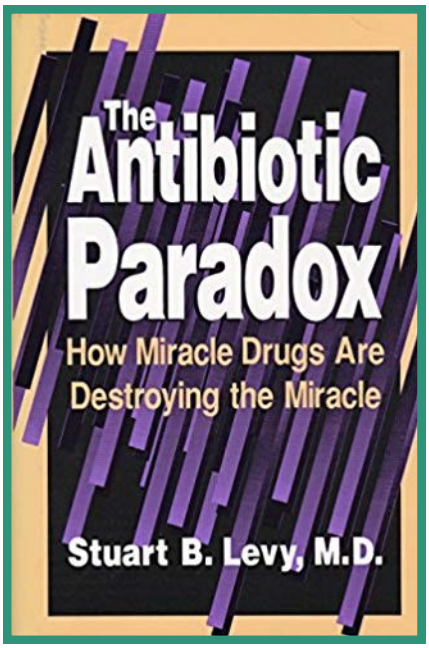
In 1992, Stuart Levy published *The Antibiotic Paradox: How Miracle Drugs are Destroying the Miracle*. (New York: Plenum Press). I am privileged to write this review using my copy, signed by Prof. Levy himself in 1997 when we first worked together. In his preface he wrote, "Antibiotics have been called the single most important therapeutic discovery in the history of medicine… While to some extent antibiotics have merited this appellation, it paradoxically has caused some dent in their armor… leading to their misuse and overuse. Bacteria have responded to the widespread applications of antibiotics finding ways to become resistant, insensitive to the killing effects of these powerful drugs."

Prof. Levy spent most of his career studying, educating and sounding the alarm on the misuse and overuse of antibiotics and the calamitous effects that this misuse/overuse brings, namely antibiotic resistance. Through numerous scientific papers, lectures, consultations and the founding of a visionary professional organisation, the Alliance for Prudent Use of Antibiotics (APUA) in 1981, Prof. Levy devoted his life to this cause. Nowhere in his writing, however, can his voice be heard more clearly than in his book, *The Antibiotic Paradox.*



In the book, one can read his discussion of the history of the development of antibiotics, the proliferation of antibiotic resistance and discussion of overuse of antibiotics, which remain as valid today as when they were first published. In 2002, he published *The Antibiotic Paradox: How the Misuse of Antibiotics Destroys Their Curative Powers.* In its second edition, he updated information that was originally presented in the first edition. The updated edition also included steps that the public, the pharmaceutical industry and various health care organisations can take to control the problem of antibiotic misuse and resistance.

Prof. Levy was deliberate in his writing approach in the book to educate a wide audience, not just healthcare professionals. In readable, straightforward prose, he described the development of antibiotic resistance, the genetic components in its proliferation, and the role antibiotic pressure plays in the selection of resistant bacteria in plants, animals and humans. He spent several chapters explaining how the millions of pounds of antibiotics used in veterinary medicine, agriculture and aquaculture, which are the bulk of antibiotics produced in the United States, facilitate the selection of resistant microorganisms. Many people believed the animal and human ecospheres were separate and the development of antibiotic resistance in one sphere would not affect the other. Prof. Levy provided many examples of how animal-associated resistant strains eventually infected human beings, some from Levy's own work. The morbidity associated with these infections is significant, and the costs of treatment are staggering.

Through the educational efforts of many including Prof. Levy, in part from *The Antibiotic Paradox* publication, the use of antibiotics in feed has slowed, due to developing alternative techniques to promote growth in animal husbandry. This has lessened the development of resistant microbial strains, though much work remains worldwide.

Prof. Levy's legacy may be difficult to characterise but his book, *The Antibiotic Paradox*, certainly should be included in that legacy. He described the founding of APUA in the book as "an internationally-based group whose membership extends to more than 80 countries of the world, communicates basic tenets of proper antibiotic usage and the problems of antibiotic resistance." He thought it essential that APUA act "outside of political and economic pressures. Its members are individuals, doctors, dentists, pharmacists, veterinarians, biologists, microbiologists, public health officials and others whose professions include handling antibiotics directly or confronting resistance in the home, hospital or laboratory—making people all over the world cognisant of the resistance problem." Prof. Levy has helped the world achieve that awareness so that, in his words, "We could then control the rise of antibiotic resistance and assure the success of antibiotics now and for generations to come." Now is the time for all of us to carry on Prof. Levy's work.

Landmark papers on the consequences of antibiotic use in farm animals for humans

Pierre Tattevin

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In 1976, almost 40 years before the emergence of the 'One Health' concept, Stuart Levy and colleagues published pioneering works on the connection between increased use of antimicrobials in farm animals, and the emergence of antibacterial resistance in humans[1, 2]

The first landmark paper was published in the prestigious Journal *Nature* in March 1976[2]: The authors transferred a mutant plasmid *pSL222-6* expressing resistance to chloramphenicol, tetracycline, sulphonamides and streptomycin into *Escherichia coli*, and introduced them directly into the intestines of four chickens. Then, chickens were fed with tetracycline-supplemented food, to enhance colonisation with tetracycline-resistant *E. coli*. The authors could verify that all chickens were indeed colonised. Two chickens each were then placed in two cages (A and C), each containing 50 chickens. One cage (A) was placed on tetracycline-supplemented feed, the other (C) was not. Two other cages of 50 chickens with (B), and without (D) tetracycline-supplemented feed, were placed approximately 50 feet from the experimental cages.

Table 1 Number of chickens excreting *E. coli* containing *pSL222-6

Day	Cage			
	A	B	C	D
0	0	0	0	0
7	1	4	0	0
14	1	6	0	0
49	5	8	0	0
56	6	8	0	0
63	7	12	0	0
% Chickens excreting mutant *R* plasmid	14	24	0	0

* Does not include inoculated chickens placed in cages A and C: chickens in cages A and B were fed tet-feed; C and D received normal feed.

Stuart Levy and colleagues could demonstrate (Table):
i) the spread of tetracycline resistance among chickens in the cage where food was supplemented by tetracycline (A), but not in the other cage (C), a demonstrative illustration of the selective pressure;
ii) transmission of resistance even in a distant cage, if chickens receive tetracycline-supplemented food (cage B), but not in the absence of this selective pressure (cage D).

During these experiments, they also screened the faeces from 11 family members living on the farm, and three laboratory workers, for the presence of the plasmid. On two occasions, the plasmid was detected but only temporarily. The absence of antibiotic use in these two humans probably explains why the plasmid was only temporarily detected. The authors could not determine if the transmission of tetracycline resistance between two distant cages (50 feet) occurred by air or on the clothes of the feed handler[2].

That same year, a few months later (September 1976), Stuart Levy published in the *New England Journal of Medicine* a clear demonstration that antibiotic-supplemented feed is a factor contributing to the selection of antibiotic-resistant bacteria in humans[1]. They showed, at a larger scale, that:
i) intestinal flora of chickens fed with tetracycline-supplemented food contained almost entirely tetracycline-resistant organisms, and that this emergence occurred early (within one week after tetracycline-supplemented food was started);
ii) the farm workers became progressively colonised by tetracycline-resistant organisms, although much more slowly, and at lower rates: within six months, 31.3% of weekly faecal samples from farm dwellers contained >80% tetracycline-resistant bacteria in farms where chickens were fed by tetracycline-supplemented food, as compared to 6.8% of samples from the neighbours' farmers who fed their chickens with antibiotic-free food ($P<0.01$)[2]

The concluding sentence was prophetical:
"*These data speak strongly against the unqualified and unlimited use of drug feeds in animals husbandry and speak for re-evaluation of this form of widespread treatment of animals.*"
Unfortunately, it took several decades before the world realised how true, and how important, these pioneering works were!

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Remembering Stuart Levy

Kimberly Thurler
Excerpted from an article for Tufts University (now.tufts.edu)

Colleagues, friends and family remember Stuart Levy as a gifted researcher and compassionate, humble human being.

"Stuart was a rare combination," said John Leong. "He had a far-reaching vision of the consequences of inappropriate use of antibiotics, a keen understanding of the need for public education and policy change, an ability to communicate effectively, and a cutting-edge lab that studied the mechanisms of bacterial resistance."

The son of a doctor, Levy grew up steeped in medicine and science. He, his identical twin brother Jay and sister Ellen all ended up pursuing careers in academic medicine.

The twin brothers enjoyed switching identities, pulling off a notable prank as undergraduates. They successfully executed a week-long switch with Jay attending classes at Williams while Stuart hung out at Wesleyan, his twin's university. More recently, at the 100th anniversary celebration of the American Society for Microbiology, of which Stuart was President, the brothers marched in identical attire. "Some people," Jay recalled, "wondered how Stuart could be in two places at once."

Levy enrolled in the University of Pennsylvania School of Medicine in 1960. While on leave as a visiting research fellow at the Institut Pasteur, he met noted Japanese scientist Tsutomu Watanabe, who introduced him to a breakthrough discovery: that resistance to antibiotics can transfer from one bacterium to another, even across species. "This was unheard of previously. It was the beginning of studies on transferrable drug-resistance genes and infectious drug resistance," Levy told *The Scientist*.

Levy joined the Tufts School of Medicine in 1971. He published a study in the *New England Journal of Medicine* showing chickens raised on feed containing low-doses of antibiotics developed intestinal bacteria that were highly resistant to antibiotics which could be transferred to farm workers. The agricultural industry was sceptical. Prevailing wisdom was that low-dose antibiotics, routinely fed to promote livestock growth rather than treat disease, would result in low-level resistance—and only in the animals themselves. Levy's work has been credited with prompting the FDA to shift its guidelines on the use of antimicrobial drugs in food-producing animals.

In 1978, Levy's lab showed that *E. coli* resistance to tetracycline is due to the bacteria actively pumping the antibiotic out of the cell. Controversial at the time, this "active efflux" mechanism is now an accepted paradigm for a critical class of antibiotic resistance and is also a mechanism for resistance to drugs that treat cancer.

Levy believed that professional and public education was essential to preventing a looming health crisis. He became a quotable expert sought out by leading news media.

"Bacteria have seen dinosaurs come and they've seen them go," he told Dan Rather. "So we aren't going to destroy the bacterial world. We live in the bacterial world." He repeatedly called for "prudent use" of antibiotics, which he termed "societal drugs" because use by one person affects others.

He lobbied for incentives to make development of new antibiotics economically feasible. Along with Nobel Prize winner Walter Gilbert, he founded Paratek Pharmaceuticals, which developed a new tetracycline derivative, omadacycline, to which target bacteria were not resistant. He also called for rigorous management of antibiotics at hospitals and for advanced diagnostics to identify diseases earlier and more accurately to better target appropriate antibiotic use.

In 1981, he co-founded APUA, now part of the International Society of Antimicrobial Chemotherapy, which brought together infectious disease specialists from more than 100 counties. "On a shoestring, he put together this worldwide network to call attention to the problem and document it. And finally, the world woke up," Berman said.

Levy never shied away from controversy. When he advocated prohibiting antimicrobials like triclosan from common products such as soap and hand sanitiser because they left behind a dangerous residue associated with antibiotic resistance, product manufacturers protested loudly. Changes in FDA regulations vindicated him and he had the satisfaction of seeing the US launch a National Action Plan for Combating Antibiotic-Resistant Bacteria and the World Health Organization name antimicrobial resistance as one of the top threats to global health.

Levy's work also inspired the new Tufts Center for Integrated Management of Antimicrobial Resistance which will tap researchers from across the university to work alongside colleagues at the medical centre.

"Stuart Levy was a towering figure," Ralph Isberg said, "not because of his physical stature but because of the force of his ideas."

Matching those ideas were his kindness, humility, integrity and love of life. Describing himself as "an optimist to my toes," he made friends all over the world, and his fluency in seven languages enabled him to support them through good times and bad, as well as meet his wife of 35 years, Cecile Pastel Levy, a native of France.

His children—Arthur, Suzanne, and Walter—recall a father who regularly tucked them into bed when they were small; read their schoolwork; taught them how to tie the perfect bow tie that was among his trademarks; shared his love of music, painting, and singing; and talked about his work without condescension whether he was invited into their elementary school classroom or college lecture hall.

Leong described Levy as "confident, as he needed to be." But, he continued, "he was always extremely gracious, never dismissive. When I arrived in 2012, I asked him what role he'd like, what he needed. Stuart had only one request. He wanted to lecture first-year medical students and educate them on antimicrobial resistance. He didn't ask for more space or money. I thought that was remarkable."

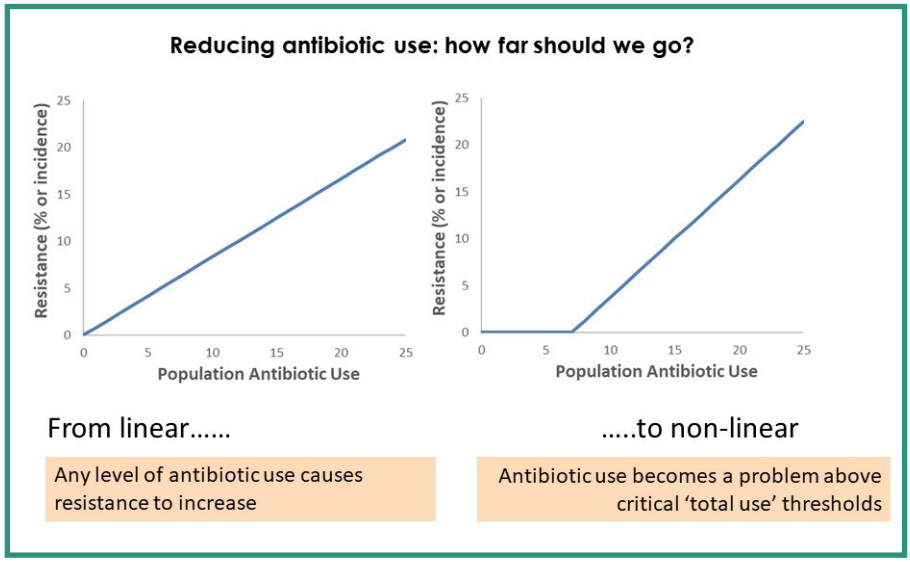
Antibiotic use versus resistance has a non-linear relationship

Ian Gould[1], Cesar A. Nebot[2], Mamoon Al Deyab[3], José-María López Lozano[4]

[1]Medical Microbiology, Aberdeen Royal Infirmary, Scotland, UK; [2]University Defence Centre, Polytechnic University of Cartagena, Cartagena, Spain; [3]Department of Pharmacy, University of Huddersfield, England, UK; [4]Research Group on Health Sciences Data Analysis, University of Murcia, Murcia, Spain

With current problems in antibiotic resistance and new antibiotic development it is important to understand how best we can optimise antibiotic use to delay the development of resistance

Time Series Analysis (TSA) techniques have been used to study the relationship between antibiotic use and resistance for 20 years now[1]. The theory was that resistance, measured over time and from an ecological perspective, is a stochastic phenomenon resulting from the dynamic interaction of several factors, (e.g. antibiotic use, changes in the microbiome, infection control measures). Previously, analysis was based on a linear concept of the relationship between the trigger, e.g. antibiotic use and the outcome, resistance: i.e., the more antibiotic use, the more resistance, regardless of the level or intensity of use.



Not withstanding this, an extremely important observation using linear ecological analysis, was the dynamic character of the relationship between antimicrobial use and antimicrobial resistance such that any specific antimicrobial use precedes specific resistance with unique lags. Ultimately though, for every resistance problem and its causative antibiotic use, there is a specific impact (how much resistance rises when antibiotic use increases).

Balancing the drug-resistance equation[2]

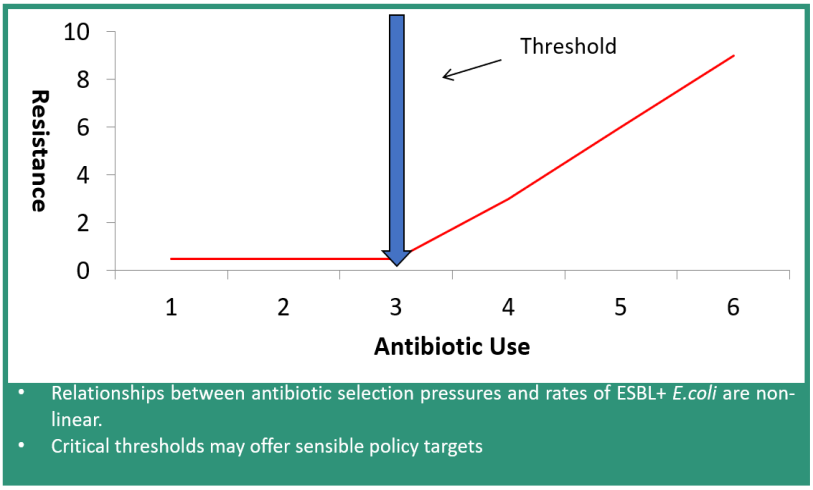
In a ground-breaking editorial published in 1994, Stuart Levy hypothesised that the relationship between intensity of antibiotic use and resistance might not be linear. Non-linear relationships are common in other biological systems but hadn't been considered in the context of antibiotic resistance up until then. Levy suggested that there might be a threshold of antibiotic use beyond which resistance would be triggered. Correspondingly, below that given threshold or level of antibiotic use, resistance would remain below epidemic levels, only as a sporadic phenomenon, because the cost to the microbe of carrying the resistance genes would outweigh any possible survival values.

Thresholds

In the last few years, on the basis of Levy's 1994 hypothesis, we introduced statistical methodology from the field of econometrics, suitable for the identification and estimation of non-linear models[3-6]. This methodology is known as Multivariate Adaptive Regression Splines (MARS), based on the separation of the data into sections or "regions" in which the ratio of the explanatory variables to the dependent variable changes and allows the identification of the nodes in which that change occurs. This statistical approach has allowed us to detect multiple antibiotic use / resistance combinations in which, up to a certain threshold, no relationship is detected between the use of antibiotics and resistance, but beyond that threshold the relationship is positive.

A threshold is an estimate of the maximum use of any antibiotic in a population that can be used over a specific period without generating resistance to that antibiotic. This can be converted into a maximum number of patients to be treated with that antibiotic in the population (e.g. a community or hospital, ward or unit). Each antibiotic has a threshold for each resistance although this is likely to be variable depending on the microbe, use of other antibiotics, and other factors still

to be researched such as the patient population, infection prevention and control (IPC) measures and the adaptability of the bacteria.



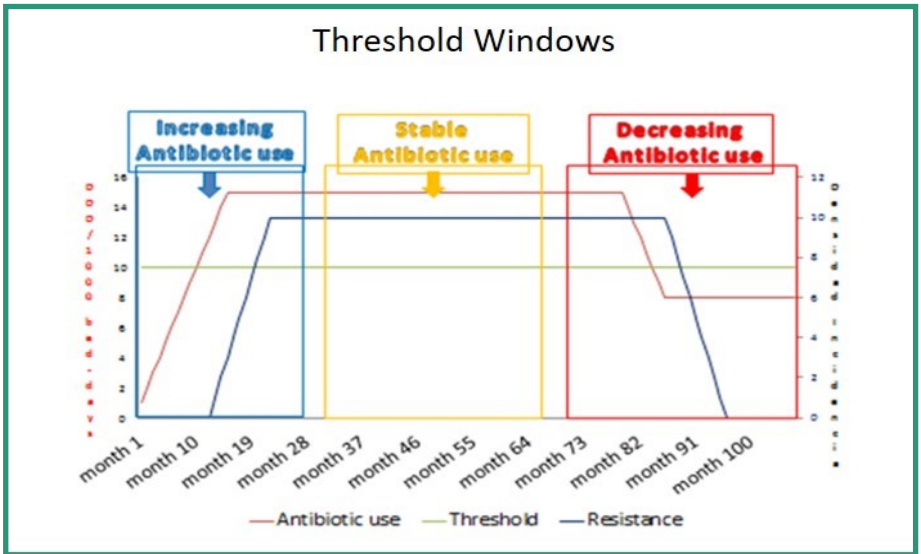
- Relationships between antibiotic selection pressures and rates of ESBL+ *E.coli* are non-linear.
- Critical thresholds may offer sensible policy targets

Thresholds too have been found for other factors, e.g. pertaining to MRSA, alcohol hand rub use, admission screening, number of positive admissions for MRSA, bed numbers and length of stay all displayed non-linear associations with MRSA prevalence. Ceiling effects too have been described, where above a certain level of use more resistance does not arise. Similarly, for MRSA, econometrically we have not found a threshold for 3rd generation cephalosporins, as even low levels of use increase MRSA prevalence.

A recent advance by our group has been provision of a confidence interval around each threshold estimation[3]. This is one of our econometric contributions; MARS does not provide this measure of uncertainty of the threshold estimation. This is relevant for establishing policies.

Further Questions

If we were able to detect thresholds for all antibiotics used in a particular hospital, unit or community, we could establish a policy of use aimed at not exceeding those thresholds for each antibiotic in the hope that problematic resistances would remain at acceptable levels. This would be similar to establishing quotas (max number of treatable patients) in order to remain under the threshold, something akin to carbon credits to reduce CO_2 production. Detection of thresholds requires long time series data sets, often of several years use and resistance and usually measured in monthly periods. Further research is needed on likely factors to influence specific thresholds, such as population vulnerability (maybe lower in geriatric inpatients), molecular epidemiology (e.g. MRSA strain), intensity of IPC measures (contributes in multivariate analyses) or the epidemic phase of an outbreak strain (compensatory mutations may occur to lighten the cost of resistance).



Time-series length and epidemic phase may affect ability to detect threshold

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Tetracycline resistance determinants

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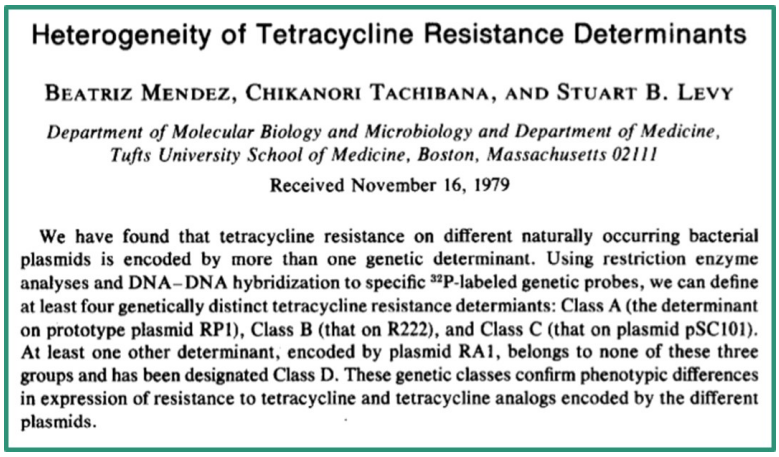
Prof. Levy had a great interest in tetracycline resistance genes in Gram negative bacteria, the various tetracyclines developed, and the history of the field. He has published >75 papers that discuss the active efflux *tet* genes and chromosomal *mar* gene, various tetracycline analogs, as well as, the history of the tetracyclines. One of his first papers in the field was published in *Nature* in 1970 where he studied the segregation of transferable plasmids in *E. coli* minicells. This was 39 years after the first tetracycline compound Aureomycin™ was discovered in the early 1940s by Lederle Laboratories Division of American Cyanamid. This first tetracycline had a wide range of activity. It was a broad-spectrum antibiotic which had activity against Gram positive and Gram negative bacteria and was the first antibiotic to be given this label. Other pharmaceutical companies discovered other tetracycline compounds. Tetracycline was first introduced for clinical therapy in 1948. These antibiotics inhibit protein synthesis by binding to the 30S ribosomal subunit. There are now other broad spectrum antibiotics in different classes of antibiotics.



Heterogeneity of Tetracycline Resistance Determinants

BEATRIZ MENDEZ, CHIKANORI TACHIBANA, AND STUART B. LEVY

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Received November 16, 1979

We have found that tetracycline resistance on different naturally occurring bacterial plasmids is encoded by more than one genetic determinant. Using restriction enzyme analyses and DNA–DNA hybridization to specific ^{32}P-labeled genetic probes, we can define at least four genetically distinct tetracycline resistance determiants: Class A (the determinant on prototype plasmid RP1), Class B (that on R222), and Class C (that on plasmid pSC101). At least one other determinant, encoded by plasmid RA1, belongs to none of these three groups and has been designated Class D. These genetic classes confirm phenotypic differences in expression of resistance to tetracycline and tetracycline analogs encoded by the different plasmids.

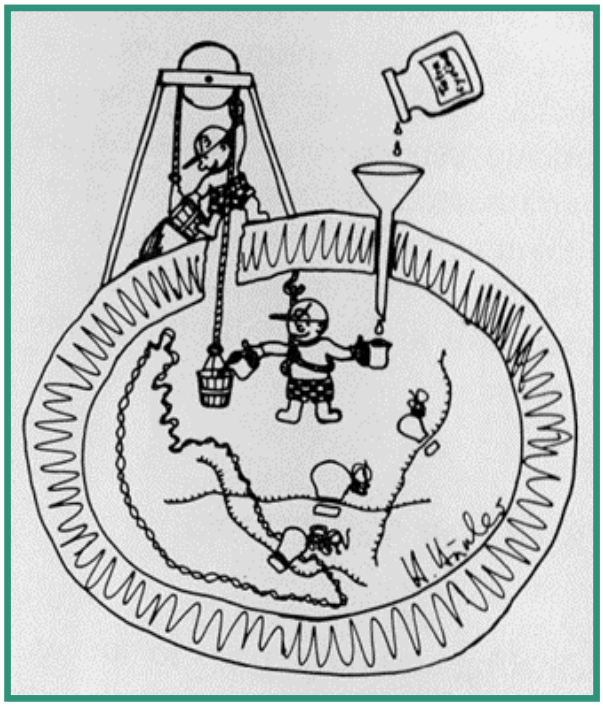
I have chosen two of these tetracycline papers of Prof. Levy to provide a mini-review because they have had a great impact on the tetracycline resistance gene nomenclature as well as other antibiotic resistant nomenclatures as having direct impact on my research career.

Prof. Levy's 1980 paper describes the identification of four genetically distinct tetracycline resistance determinants which were labeled Class A, B, C & D. These four genes were confirmed to have phenotypic differences in expression of resistance to tetracycline, minocycline and chelocardin and were encoded by different plasmids from members of the Enterobacteriaceae and Pseudomonadaceae. These were the first four characterised *tet* genes which conferred resistance by an active efflux mechanism which decreased the accumulation of tetracycline in the host bacterial cell. Previously in Enterobacteriaceae, *Staphylococcus* and some anaerobic species were shown that they were inducibly resistant to higher levels of tetracycline if the hosts had previously been exposed to subinhibitory concentrations of the drug. An inducible negatively regulated protein was identified in the inner membrane of the bacteria carrying one of these genes.

The initial discovery that there are different types of tetracycline resistance genes was a major breakthrough in the field of antibiotic resistance. The four genes were shown to be unrelated using DNA-DNA hybridisation, which was the state of the art for the day. The paper then went on to show the distribution of these different genes using ^{32}P-labeled fragments to determine carriage of the different *tet* genes against 25 different strains representing 12 different species carrying plasmids from different incompatibility groups and different resistant patterns using filter hybridisation. The four genes described in the 1980 paper are the standard for the active efflux *tet* class of genes and are exclusively found in Gram negative genera.

Prof. Levy demonstrated in the 1980 paper that a *tet* gene could be associated with a transposon, in this case the highly studied Tn*10*. The *tet*(B) gene encoded conferred resistance to minocycline while the other three efflux genes did not.

Today we know that these efflux genes are regulated by a specific repressor gene which is upstream of the structural gene and is read in the opposite direction from the structural gene. Today there are 33 genetically distinct *tet* efflux genes in this class of *tet* resistance genes — many of great clinical significance[3.] Today we know that these *tet* genes are α-helices that are divided into two halves, α and β, by a large putative cytoplasmic loop designated the interdomain region. The α and β domains of the protein (N-terminal and C-terminal halves, respectively) have presumably evolved from a duplication of a single domain. A number of the *tet* genes including the first four described in the 1980 papers have 45–75% identity. Hybrid interclass Tet protein constructions, complementation studies and second-site suppressor studies showed that interactions between both domains are required for function.



The molecular methods described in Levy's 1980 paper went on to become the standard method for surveillance of the distribution of various tetracycline resistance genes. Similar methods were used to identify heterogeneity in *tet* resistance genes in *Streptococcus* spp. The methods changed with the introduction of polymerase-chain reaction (PCR) assays in the 1990s for the detection of different tetracycline resistance genes.

In the second paper, a short publication, Prof. Levy worked with others in the field to define a nomenclature system for *tet* genes which still works today some 30 year later. From this work the tetracycline resistance gene nomenclature center was borne[3.] Prof. Levy's laboratory developed a form for authors to fill out so that they could request new names for newly identified genes that were <80% related by amino acid identity with other *tet* genes previously described and given names. The aim of this paper and the centre was to make sure that highly related *tet* genes were given the same name. At this time, <80% identity was the best discrimination that could be done. Names were then provided and a website was developed to provide the information free to all who were interested. At the same time, I modeled the nomenclature for macrolide -lincosamide-streptogramin genes after the *tet* system and provided a website for these genes as well. Today there is a total of 60 different *tet* genes with other mechanisms including ribosomal protection [n=13], enzymatic inactivation of the antibiotic [n=13] and one with unknown mechanism of action.

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Hospital and societal costs of antimicrobial-resistant infections in a Chicago teaching hospital: implications for antibiotic stewardship

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Ten years ago, Prof. Levy co-authored a notable paper applying economics to analyse antimicrobial resistance (AMR)[1]. It describes economic analysis of the Chicago Antimicrobial Resistant Project dataset, with the aim of measuring the cost attributable to antimicrobial resistant infections (ARI) in hospitalised patients.

A random sample of high-risk patients hospitalised in 2000 in Chicago was selected. To increase the number of patients for the subgroup analysis, additional high risk patients with antibiotic resistant organisms were selected. A sensitivity analysis including three study designs was conducted. Regression was used to adjust for potential confounding in the random sample and in the sample expanded with additional patients with ARI. Propensity scores were used to select matched control subjects for each patient with ARI for a comparison of mean cost for patients with and without ARI. All patient resource use was abstracted from electronic and paper medical records. Service costs included all support costs related to administration, employees, buildings, etc.

In 2009, drug-resistant organisms were classified in four subgroups: (1) methicillin-resistant *Staphylococcus aureus*, (2) vancomycin-resistant enterococci, (3) *Escherichia coli* resistant to fluoroquinolones / 3rd generation cephalosporins or *Klebsiella* species resistant to 3rd generation cephalosporins and (4) amikacin- or imipenem-resistant *Enterobacter*, *Pseudomonas* or *Acinetobacter* species.

23,904 patients were hospitalised and 4,944 (20.7%) met the eligibility criteria. The random sample of 1,253 patients was expanded by 138 patients with ARI, resulting in a total of 1,391 patients, of whom 188 (13.5%) had ARI. Patients with ARI had significantly different APACHE III scores, HAI rates and death rates compared to those without ARI. Among those with ARI, 34 (18.1%) died compared with 36 (3.0%) without ARI (P< 01). The mortality odds ratio, adjusted for APACHE III, ICU care, and HAI, was 2.16 with an attributable mortality rate of 6.5% or 12 excess deaths for episodes caused by ARI alone. Hospital stay was prolonged by 6.4 – 12.7 days. The medical costs attributable to ARI ranged from $18,588 to $29,069 per patient. Using the lowest estimates from the sensitivity analysis resulted in a total cost for this single hospital of $13.35 million in 2008 dollars in this patient cohort. These figures raised to $18.75 million using the highest estimates.

The authors concluded that this detailed analysis of the cost of antibiotic resistance in a single large teaching hospital express the magnitude of the problem in the United States that should lead to increased efforts to control AMR. Additionally, they suggest that this data could form the basis for a more comprehensive evaluation of the cost of resistance and the potential economic benefits of prevention programmes.

Since then, many attempts to estimate the burden of this serious public health issue have been made. It was recently suggested that ARI treatment costs have doubled since 2002[2] in the US. Excess costs were estimated to be $1,383; for the year 2014, the national cost approximates to $2.2 billion annually. A recent systematic review[3] of 214 studies found that the excess costs ranged from non-significance to $1 billion per year whilst economic burden ranged from $21,832 per case to over $3 trillion in GDP loss. These variations show that methodological assumptions and biases can occur dependent on chosen outcome and perspective. Another report[4] estimated that the total economic cost of resistance for five main pathogens (*S. aureus, E. coli, K. pneumoniae, A. baumanii* and *P. aeruginosa)* was $0.5 billion and $2.9 billion in Thailand and the US respectively. Finally, the overall AMR cost for year 2015 reached EUR 109.3 million in France with a mean of EUR 1103 per stay[5]; extrapolation to the entire National database estimated that this figure could potentially reach EUR 287.1 million if all cases would be identified. The mean excess length of hospital stay attributable to AMR was estimated at 1.6 days.

Calculating the economic costs of ARI will always be difficult due to many confounding factors and biases, but there is no doubt that the complexity and seriousness of the whole problem is a huge issue (e.g. mortality, increased hospital stay, the increase need of broad spectrum antibiotics, the lack of new treatment options for MDR bugs and costs). The concept of attributing costs to ARI was introduced by Roberts and Levy a decade ago and is one which will remain highly significant for a long time to come.

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Tribute from the AUPA Bulgaria Chapter

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Selected Works of Stuart Levy

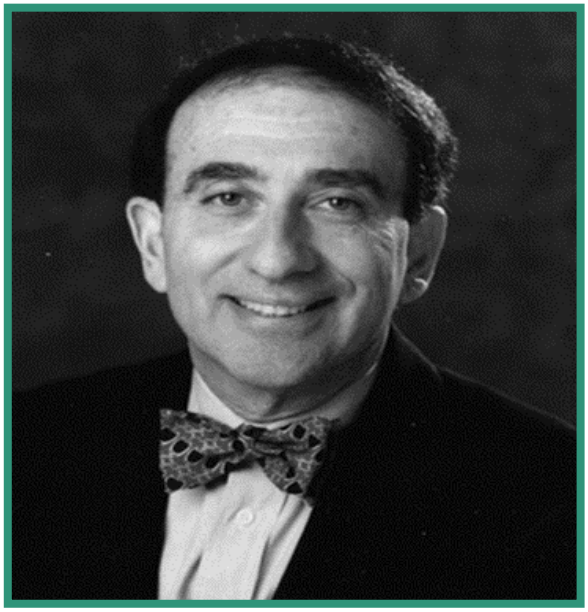
The Antibiotic Paradox. *How Miracle Drugs Are Destroying the Miracle. Plenum Press. NewYork, London. 1992.*

"Antibiotics have been called the single most important therapeutic discovery in the history of medicine. The seemingly endless miracles attributed to these drugs have led to their misuse and overuse. Bacteria responded by finding ways to become resistant. Antibiotics sow the seeds of their own potential downfall by selecting rare strains of bacteria… These resistant traits can be transferred or spread from one kind of resistant bacteria to other bacteria… Our goal is to encourage making them (antibiotics) even *more* effective by curbing the emergence and spread of resistant forms… The goal of improving antibiotics must concern all members of society using these drugs, namely: consumers, whether they be humans, animals, or the agriculture industry; prescribers, whether they be physicians, veterinarians, or plant pathologists; and the producers and companies that are making and marketing these drugs".

Antibiotic Resistance: Consequences of Inaction

Clin Infect Dis. 2001; 33 (Suppl 3) S124-9

"Bacterial resistance presents therapeutic dilemmas to clinicians worldwide. The warnings were there long ago, but too few people heeded them. Thus an emerging problem has grown to a crisis. Resistance is an ecological phenomenon stemming from the response of bacteria to the widespread use of antibiotics and their presence in the environment. We must work to remedy the lack of action in the past. By improving antibiotic use and decreasing resistance gene frequency at the local level, we can move towards reversing the resistance problem globally".



Factors impacting the problem of antibiotic resistance

The 2000 Garrod Lecture. *J Antimicrob Chemother.* 2002, 49, 25-30

"While it is clear that antibiotics are pivotal in the selection of bacterial resistance, the spread of resistance genes and of resistant bacteria also contributes to the problem. We currently face multiresistant infectious disease organisms that are difficult and, sometimes, impossible to treat. In order to curb the resistant problem, we must encourage the return of the susceptible commensal flora."

Legacies

Stuart Levy was the founder of the Alliance for the Prudent Use of Antibiotics (APUA). APUA is recognised all over the world for supporting awareness about antimicrobial resistance and ways to control it as well as the need for appropriate use of antibiotics (antimicrobial stewardship) and infection control activities. The APUA country Chapters were given the opportunity to apply for small grant for research activities. The APUA Newsletter provides

information on the latest scientific news and gives a platform to famous researchers, young investigators or country experts. APUA has led several key projects including The ROAR project (Reservoirs of Antimicrobial Resistance). The Global Advisory for Antibiotic Resistance Data (GAARD) involved several of the world's largest multinational enterprises tracking global trends in resistance as well as the Centers for Disease Control and Prevention, WHO, and the WHO Collaborating Centre for Surveillance of Antimicrobial Resistance, which serve in advisory roles.

In 2001, a comprehensive document was published in collaboration with WHO: *Antibiotic resistance: synthesis of recommendations by expert policy groups*. Alliance for the Prudent Use of Antibiotics: JL Avorn, JF Barrett PG Davey, SA McEwen, TF O'Brien and SB Levy. *WHO/CDS/CSR/DRS/2001.10*.

Prof. Stuart B. Levy pioneered work in drug influx mechanisms. He workedat the Center for Adaptation Genetics and Drug Resistance, Tufts University School of Medicine and was a physician at Tufts Medical Center. He worked tirelessly to campaign on the rational use of antibiotics amongst both his peers and the public at large.

He was President of the American Society for Microbiology in 1999. As Vice-President of the National Science Advisory Board for Biosecurity (NSABB) he worked hard to highlight the fact that antibiotic resistant / pan-resistant bacteria can pose unique biosecurity problems. The NSABB produced important documents, including "Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the potential Misuse of Research Information". *A Report of the National Science Advisory Board for Biosecurity (NSABB),* June 2007.

He was also a valued Vice-President of the International Round Tables on Dual Use Life Sciences Research, Bethesda (2007—2008) which was co-sponsored by WHO and the U.S. Government.

Conclusions

Prof. Stuart Levy died on 4 September 2019 but he will always be with us. His work will live on in future generations who will develop his legacy to promote the prudent use of antibiotics. Prof Stuart Levy was indeed, a great scientist and an even greater human being!

Photo of the participants in the International Round Table on Dual Use Life Sciences Research, Bethesda, 2007 – Prof. St. Levy – in the middle of the first row