Article ID: WMC004872 ISSN 2046-1690

Pathogen Traffickers: Disease causing pathogens do not have legs

Peer review status:
Yes

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Previous Article Reference: http://www.webmedcentral.com/article_view/4668

Article ID: WMC004872

Article Type: My opinion

Submitted on:08-Apr-2015, 01:54:34 PM GMT **Published on:** 08-Apr-2015, 02:02:22 PM GMT

Article URL: http://www.webmedcentral.com/article_view/4872

Subject Categories:VIROLOGY

Keywords: Iron, Human immunodeficiency virus, Engineered avirulent Pseudomonas aeruginosa strains, competition

How to cite the article: Osayande JO. Pathogen Traffickers: Disease causing pathogens do not have legs. WebmedCentral VIROLOGY 2015;6(4):WMC004872

Source(s) of Funding:

None

Competing Interests:

None

Pathogen Traffickers: Disease causing pathogens do not have legs

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Abstract

The Ebolavirus is introduced into the human population through close contact with the sweat, blood, secretions, organs or other bodily fluids of infected animals such as chimpanzees, gorillas, fruitbats, monkeys, forest antelope and porcupines. It first surfaced in1976, and today thousands ofdeaths have been recorded. Does this mean that humans should not come in contact with each other? This question would be answered when a form of vaccine or treatment or solution is proffered against the Ebola virus and other disease causing organisms in times of deadly disease outbreak. This short communication is proposing the reinvention of the Vitek Machine and the engineering of avirulent bacteria strains against deadly pathogens. The engineered strains will be constructed in such a way that they are able to compete with pathogenic organisms for essential nutrients needed to survive and establish themselves within a host, "The era of the survival of the fittest".

Introduction

The Ebola virus first appeared in 1976 in two simultaneous outbreaks - in Nzara, Sudan; and in Yambuku, in the Democratic Republic of Congo. Since its outbreak, more than 4000 deaths has been recorded and a spread to more than four African countries and other locations in the world. One is left to wonder what happened to Quarantine simply defined as the strict isolation imposed to prevent the spread of diseases.

When Ebola was first discovered, was quarantine in existence?

I ask about quarantine, maybe I simply should ask about education; proper education of people especially during times of disease outbreak.

It is possible that quarantine existed; proper education too, but what about 'Good heart'?

A lot of people have contagious diseases, even when they know how dangerous these diseases are and the simple need for them to get isolated; they rather choose to become very heartless or empty hearted to travel with these diseases so that others can also become contaminated. Imagine if only Ebola remained in the place where it first surfaced, a lot of deaths would not have been recorded now.

The current idea is written because of the outbreak of the Ebola virus and also because of the fact that, over the years, no remedy is coming forth as regards the issues of antibiotic resistance and viral infections (in particular the Human immunodeficiency virus).

Phage therapy is being praised, even to the extent of being regarded as a last resort, but we as scientists should remember that what we are talking about here are viruses, being introduced to kill bacteria.

When disease-causing organisms successfully find themselves into the body of a host, they need a lot of materials (including essential nutrients) to enable them feel comfortable and get established within the host environment .

A number of these disease-causing organisms need essential nutrients to be able to survive and knowledge of such nutrients can be an area that can be targeted.

In addition, using other strains better equipped in fighting for these nutrients can be an exploitable avenue to ameliorate the pain and suffering of sick people.

I am proposing the Engineering of avirulent bacteria strains against pathogenic organisms. These engineered avirulent strains should be constructed to be better equipped in fighting for essential nutrients needed by pathogenic organisms (which may be virus or even deadly bacteria) to survive or thrive within a host, it will simply be a case of the survival of the fittest.

It has been scientifically documented that iron for instance is required for particular steps of the HIV replication life cycle in cells (Pradeep *et al.* 2010), and this same iron is important in a number of processes in *Pseudomonas aeruginosa*. Can avirulent strains of *Pseudomonas aeruginosa* be designed to compete with the virus (for example the HIV) for iron such that those particular steps of the HIV replication life cycles requiring iron is blocked? If the virus is unable to replicate, then it cannot survive. Could this be a remedy?

My proposition to us scientists is to carry out

experimental findings able to demonstrate which essential nutrients disease- causing pathogens need to survive, and how avirulent bacteria strains can be engineered to compete with these deadly pathogens for these essential nutrients within a host. The best competitor survives, while the other dies, "The era of the survival of the fittest".

The Ebola Virus issue

The Ebola virus is introduced into the human population through close contact with the sweat, blood, secretions, organs or other bodily fluids of infected animals such as chimpanzees, gorillas, fruit bats, monkeys, forest antelope and porcupines.

Early symptoms are similar to those of flu but are followed by severe vomiting and diarrhoea, and eventually internal and external bleeding.

It started in Guinea in February and spread to neighbouring Liberia and Sierra Leone.

The virus then spreads in the community through human-to-human transmission.

Symptoms begin with fever, muscle pain and a sore throat, then rapidly escalate to vomiting, diarrhoea and internal and external bleeding. The incubation period can

be up to 21 days. Since March, there have been 1,201 cases of Ebola and 672 deaths in Guinea, Liberia and Sierra Leone, according to the World Health Organisation (WHO).

Patrick Sawyer, an American, travelled from his home of Minnesota to attend the funeral of his sister who died from Ebola. He was able to board multiple flights despite vomiting and suffering from diarrhoea, arriving in Nigeria where he died. The scientist who helped discover the Ebola virus said the outbreak in West Africa was unlikely to trigger a major epidemic outside the region, adding he would happily sit next to an infected person on a train. But Professor Peter Piot said that a "really bad" sense of panic and lack of trust in the authorities in West Africa had contributed to the world's largest-ever outbreak. The Belgian scientist, now based in Britain, urged officials to test experimental vaccines on people with the virus so that when it inevitably returns, the world is prepared; News culled from the Telegraph (July, 2014).

During holiday and often times, a lot of people travel with children and family. With the spread of some deadly diseases like the Ebola virus, one would wonder if there is anywhere safe. Human traffickers lure their victims who end up being forced into some kind of slavery they never intended in their lives, pathogen traffickers may do so without knowing it. We talk about disease spread, but do these pathogens causing diseases have legs?

Pathogens are transported by human beings who travel from one place to the other. People travel on holidays with their personal effects. They use sponge, soap and towels, and in addition, make use of toilets and bathrooms, without actually knowing the person or persons who had previously used these bathrooms and toilets. These same personal effects are never disinfected, they are brought back and their usage is continued after the holidays. While some persons simply leave these toiletries behind or throw them away, others do not. One would be left to imagine what would happen if a sick or an infected person previously lodged in a hotel room before a healthy person‘s arrival. Majority of these hotel rooms are not properly cleaned up nor disinfected.

Proper attention is not paid to the cleaning of rooms in hotels or hospitals. In some countries, no diploma is required to do a cleaning job, yet it is one of the most sensitive job ever. Majority of the cleaning staff are either illiterates or not highly educated. Some clean with protective or disposable gloves on, while it is on the contrary for others. In addition, the hotel or hospital management worldwide leave the issues of cleaning to these classes of people. One would wonder how diseases are transmitted from one place to the other.

I was listening to news sometime ago of a patient who went to hospital with a simple wound, she ended up

with *P. aeruginosa* infection, these group of bacteria cause a number of hospital related infections. But how did she get infected? Next to her ward where she was hospitalized was a soldier earlier diagnosed with this bacterial infection. However, the cleaning team succeeded in transporting it to her. Using the same cleaning materials, the whole hospital is regularly cleaned. Attention is not paid to the regular cleaning activities. The illiterate or not so highly informed cleaner goes from one hospital room to the other using the same mop, the same hand gloves etc.

A healthy person visits the hospital one day and the next day comes back sick with a new infection, most probably acquired from the hospital. It is assumed that these infections are hospital related, but nothing is done to address the reason the healthy person got infected.

Who is to blame in the area of pathogen transmission, the pathogen or the person who traffics the pathogen without knowing it? Can something be done to

enlighten 'Cleaners' all over the world?

We hear of Scientific and Medical Conferences, Scientists and Medical Doctors coming together to discuss and in addition, among other things, how the latest vaccine in Science and Medicine can be developed. These conferences sometimes focus on disease spread but the actual causes of the problems are not addressed, the actual persons responsible for disease spread are not invited to the conference.

It would be extremely exciting also to see an organized scientific conference having as its attendees : Cleaners (hotels and hospitals) and environmental sanitation workers all over the world, where , 'The harm caused by not disposing off gloves used in cleaning for instance one hospital room before proceeding to clean another' can be demonstrated or shown. Can there be a way to help cleaners, the cleaning industries and human beings all over the world on how to minimize disease spread? Disease causing pathogens do not have legs, yet they are everywhere.

THE ERA OF THE SURVIVAL OF THE FITTEST: Virus Kill Bacteria

Bacteriophages are viruses that infect and replicates within a bacterium and phage therapy is the therapeutic use of bacteriophages to treat pathogenic bacterial infections. This technique actually involves injecting viruses into humans in order to get them cured or treated of bacterial infections; a treatment some consider as "Last Resort" and because of fear, its use have not been widespread. Please read the underlying article.

Phage therapy gets revitalized By Sara Reardon

Nature 510:15-16 (05 June 2014) DOI: 10.1038/510015a

The rise of antibiotic resistance rekindles interest in a century-old virus treatment.

For decades, patients behind the Iron Curtain were denied access to some of the best antibiotics developed in the West. To make do, the Soviet Union invested heavily in the use of bacteriophages 'viruses that kill bacteria' to treat infections. Phage therapy is still widely used in Russia, Georgia and Poland, but never took off elsewhere. This is a virus, and people are afraid of viruses says Mzia Kutateladze, who is the head of the scientific council at the Eliava Institute in Tbilisi, which has been studying phages and using them to treat patients for nearly a century.

Now, faced with the looming spectre of antibiotic resistance, Western researchers and governments are giving phages a serious look. In March, the US National Institute of Allergy and Infectious Diseases listed phage

therapy as one of seven prongs in its plan to combat antibiotic resistance. And at the American Society for Microbiology (ASM) meeting in Boston last month, Grégory Resch of the University of Lausanne in Switzerland presented plans for Phagoburn: the first large, multi-centre clinical trial of phage therapy for human infections, funded by the European Commission.

Ryland Young, a virologist at Texas A and M University in College Station, attributes the previous lack of Western interest to clinicians' preference for treating unknown infections with broad-spectrum antibiotics that kill many types of bacterium. Phages, by contrast, kill just one species or strain. But researchers now realize that they need more precise ways to target pathogenic bacteria, says microbiologist Michael Schmidt of the Medical University of South Carolina in Charleston. Along with the rising tide of strains resistant to last-resort antibiotics, there is growing appreciation that wiping out the human body's beneficial microbes along with disease-causing ones can create a niche in which antibiotic-resistant bacteria can thrive. Antibiotics are a big hammer, Schmidt says. 'You want a guided missile'.

Finding a phage for a bacterial target is relatively easy, Young says. Nature provides an almost inexhaustible supply: no two identical phages have ever been found. As a bacterium becomes resistant to one phage by shedding the receptor on the cell surface that the virus uses to enter the Eliava Institute researchers simply add more phages to the viral cocktails that patients receive. Kutateladze says that they update their products every eight months or so, and do not always know the exact combination of phages that make up the cocktail.

Resch, who is one of the leaders of the Phagoburn study, says that regulatory agencies would need to figure out how to oversee such a rapidly evolving product before the therapy could progress beyond clinical trials. He hopes that phage therapy will be treated in a similar way to the seasonal influenza vaccine, for instance, which is updated every year as new flu strains emerge.

The fact that the European Union (EU) is contributing €3.8 million (US$5.2 million) to the Phagoburn study shows that it is willing to consider the approach, Resch says. Beginning in September, researchers in France, Belgium and the Netherlands plan to recruit 220 burn victims whose wounds have become infected with the common bacteria *Escherichia coli* or *Pseudomonas*

aeruginosa. The patients will be given phage preparations from a company in Romainville, France, called Pherecydes Pharma, which has isolated more than 1,000 viruses from sources such as sewage or river water and screened them for the ability to kill pathogenic bacteria. To lower the chance that resistance will develop, the patients will receive a cocktail of more than a dozen phages that enter bacterial cells in different ways. If the phage treatment fails, patients will then receive standard antibiotics.

Although governments are starting to pay attention to phage therapy, pharmaceutical companies remain reluctant to get on board, Young says. Because phage therapy is nearly a century old, it would be difficult for a company to claim a treatment as intellectual property, and therefore recoup its costs. Young says it is likely that a 2013 ruling by the US Supreme Court against the patenting of natural genes would also apply to phages isolated from nature. Jérôme Gabard, chief executive of Pherecydes, says that the company is banking on hopes that developing and characterizing precise combinations of natural phages to target particular bacteria will be patentable.

An engineered phage could, in theory, be patented. At the ASM meeting last month, researchers led by synthetic biologist Timothy Lu at the Massachusetts Institute of Technology in Cambridge presented work on a phage engineered to use a DNA-editing system called CRISPR to kill only antibiotic-resistant bacteria. The phage injects the bacterium with DNA, which the microbe transcribes into RNA. If part of the bacterium‘s antibiotic-resistance gene matches that RNA sequence, an enzyme called Cas9 cuts up the cell‘s DNA, killing it.

In initial trials, the researchers found that their phage could kill more than 99% of the *E. coli* cells that contained specific antibiotic-resistance gene sequences, whereas it left susceptible cells alone. Giving the phage to waxworm larvae infected with resistant *E. coli* increased the worms‘ chance of survival. The researchers are now starting to test the system in mice (human trials are a long way off).

Gabard does not expect that phage therapy will ever replace antibiotics. But he says that he can envisage regulatory agencies approving it for patients in whom drug treatments have failed. And some people with antibiotic-resistant infections are taking matters into their own hands. Kutateladze says that an increasing number of EU patients are travelling to Georgia for phage treatment. She adds that doctors in some EU countries send patients‘ samples to the Eliava Institute, which then sends back a phage cocktail specific to the bacterium causing the infection. When there‘s no hope, you‘ll do anything,Ability to carrySchmidt says.

Meanwhile, researchers are watching the Phagoburn study with interest, hoping that it will lay the groundwork for moving the technology into the clinic. We just need one really great success for the field to really open up? says Lu; Culled from Nature News

The article above made me remember the days when I was working (during my PhD thesis) at the Queen Astrid Military Hospital (Burn Unit, Nederoverhembeek), located in Belgium. My former Supervisor, Dr Jean-Paul Pirnay was actually working very hard on this topic (using viruses to kill bacteria), my question then was, ?Could this be the era of the survival of the fittest?? Can bacteria also be engineered to kill viruses? Bacteria strains will not be introduced into a sick host just like that. The strains will first of all be made "avirulent" and avirulent bacteria strains are non-pathogenic.

My proposition today to scientists (including me) all over the world is to see how things can also go the other way round. In effect, trying, if possible, to "Engineer avirulent bacteria strains against viruses to reduce or treat viral infections". I propose that we re-strategize the way the virus has been dealt with. Please read my little write up below.

***Pseudomonas aeruginosa*, the human immunodeficiency virus and iron: What Role Does Iron Play in the HIV Infection process?**

Pseudomonas aeruginosa

Pseudomonas aeruginosa, a gram negative bacterium and a member of the rRNA homology group I *Pseudomonas* family, have several characteristics which includes

- Ability to carry out strict respiratory type of metabolism using oxygen as terminal electron acceptor (they can also grow anaerobically using nitrate as final electron acceptor, a process called denitrification)
- Growth at an optimum temperature of 37°C, and some may even grow at temperatures as high as 42°C and as low as 4°C (Iglewski, 1991). Having very simple nutritional needs, the simplest medium for growth of these species of gram negative bacteria consists of acetate as sole carbon source and ammonium sulphate as nitrogen source. Ability to withstand a variety of physical conditions like temperature, high salt concentrations, weak antiseptics and resist many antibiotics attributed to their being in possession of multidrug efflux (Mex) systems, outer membrane permeability barrier, and chromosomal β-lactamase activities such that in cases of infection with these bacteria, majority of administered antibiotics face extrusion out of the cell of an infected host (Masuda *et al*. 1999; Schweizer, 2003).

Infection by *P. aeruginosa* causes serious problem in patients hospitalized with cystic fibrosis, burns and cancer; mortality rates arising from these diseases are as a result of infection with this pathogen. *P. aeruginosa* is associated with many hospital acquired infections and has become popular because of the frequency with which it is involved in human diseases such as urinary tract infections, colonization of the lungs of cystic fibrosis patients, severe corneal infection, and diarrhoea (Van Balen *et al*., 2003, Todar's online book of Bacteriology).

The production of water-soluble, fluorescent pigments (pyoverdine) makes them characteristically referred to as true *Pseudomonas* (*sensu stricto*) and members of this group of bacteria are found within the gamma-subclass of the proteobacteria (De Vos *et al*., 1989).

P. aeruginosa is able to thrive in various ecological niches such as soil, river water, and plants. It has also been isolated from burn wound and found to inhabit the lungs of cystic fibrosis patients and once within these environments, in the cystic fibrosis lungs for instance, this organism displays long-term persistence due to decreased efficacy of antibiotics and formation of biofilms which makes the penetration of antibiotic difficult.

Siderophore

Iron, which is an essential but insoluble element, is required in soluble quantities by bacteria, however, the predominant form of iron is its ferric form which is insoluble, the relative amount of bioavailable iron is therefore low resulting in its scarcity (Poole and Mckay, 2003; Braun and Braun, 2002).

To narrow down iron limitation in microbial environment, bacteria secrete compounds called siderophores which serve as iron chelators in iron limiting environment. In addition, bacteria uses alternative means such as surface reduction of ferric iron to the more bioavailable ferrous forms, lowering the pH of their environment and stripping off iron bound to protein complexes as avenues to increase the amount of usable iron in their environment (Bagg and Neiland, 1987; Neilands, 1995; Abdul-Tehrani *et al.* 1999; Ratledge and Dover, 2000; Ankenbauer *et al*. 1985; Crosa, 1989). Siderophores are low molecular weight compounds which bind iron with high affinity, and not only do they bind iron, they also solubilise them, mediating their transport into the bacterial cell. Once synthesised and secreted, siderophores mediate iron exchange between the bacterial cell and the external environment (Guerinot, 1994; Matzanke, 1991; Winkelmann, 2002).

SIDEROPHORES OF P. aeruginosa

P. aeruginosa produces two siderophores; Pyoverdine and pyochelin.

Siderophore Receptors

In*Pseudomonas aeruginosa*, iron bound to siderophore (ferri-siderophore) is successfully internalized following the recognition at the outer membrane level, by a specific iron repressed outer membrane receptor (IROMP).

Several siderophores produced by other bacteria or fungi like cepabactin, coprogen, desferriferrichrysin, desferriferricrocin, desferriferrioxamine B and desferriferrioxamine E has been observed to promote iron uptake under iron limiting conditions into *P. aeruginosa* in addition to being growth stimulated by its own siderophores (Meyer, 1992; Llamas and Bitter, 2006).

More than thirty TonB- dependent receptors are present in the genome of *P. aeruginosa*, with two endogenous and multiple heterologous siderophores to choose from, *P. aeruginosa* is highly equipped in its need to sequester iron making it a strong competitor for iron in relation to other florescent species.

Role of Iron in the HIV Infection process

The progression of HIV infection towards its more advance stages is accompanied by increasing body iron stores, there is urgent need for careful clinical studies to clarify the role of iron status on the course of HIV infection.

Increased bone marrow iron is associated with shortened survival and increased opportunistic infections. Iron could be playing an important role in the interaction between host and virus. Knowledge of these interactions is necessary to predict morbility response to disturbance in host iron homeostasis (Pradeep *et al.,* 2010).

Iron is important to*Pseudomonas aeruginosa*, (Meyer *et al*. 1996) where it serves as a signal for biofilm development in this organism (Banin *et al.* 2005).

Various studies in the past have elucidated how iron dependence by microorganisms can be utilized as an avenue of drug discovery. Looking at the *P. aeruginosa* strains I worked with for example, following PCR amplification , variants of *P. aeruginosa* were presented based on their ability to utilize iron (Strains Br678 and Mi162, both are clinical *P. aeruginosa* isolates from burn wound, Br678, a pyoverdine positive *P. aeruginosa* strain able to produce pyoverdine under iron limiting conditions (CAA medium), Mi162 a pyoverdine negative *P. aeruginosa*

strain unable to produce pyoverdine but yet was growth stimulated by iron under iron limiting conditions (CAA medium, please see reference 18) .

The outbreak of the Ebola Virus in Africa is of great worry, and the above news (culled from Nature) speaks about viral cocktails, can 'Avirulent bacteria' (*Pseudomonas aeruginosa*) cocktails be engineered into HIV or other deadly viral situations to see which organism survives best in a sick host? The fight will then be between the engineered avirulent bacteria strains and the virus competing probably for a singular nutrient for survival within the host.

Pradeep *et al.,* in 2010 further documented that iron is central to physiology in addition to being required for particular steps of the HIV replication life cycle in cells. If iron is important, then can bacteria be engineered to compete with virus for iron so that the virus is unable to replicate?

If bacteria is able to compete for iron with virus, then those particular steps of the HIV replication life cycle may be affected in cells. Can this be a remedy?

In addition, even in areas of antibiotic resistance, tougher avirulent bacteria strains (which I may like to call "beneficial bacteria") better equipped for survival can be engineered against disease causing bacteria, the only thing we as scientist should concentrate on now is to see;

- First and foremost, what compounds or nutrients are essential for the survival of a pathogenic organism in a host such that in the absence of these essential nutrients (for example) the pathogenic organism is

unable to thrive. For instance, iron serves as a signal for biofilm development in *P.aeruginosa* (Banin *et al.,* 2005), and the biofilm serves as a protective covering (some kind of protective shield) against administered antibiotics, so if avirulent strains of the same *P. aeruginosa* or other bacteria species which are better equipped to compete for iron could be engineered and subsequently introduced as a form of treatment into a sick host, the biofilm would not be developed and administered antibiotics will find their way directly to the targeted pathogenic bacteria. I think a great difference will be made, also in the situations of viral infections. It will only boil down to the survival of the fittest organism within a host and the pathogen which is unable to survive, dies.

It may sound strange or may be considered as nonsense, perhaps, it may be worth trying out!

REINVENTION OF THE VITEK MACHINE

The Vitek System (bioMerieux,Inc. Hazelwood, MO), is an automated antibiotic susceptibility testing and microbiology identification system that evaluates an optical signal generated by individual biochemical reactions contained within a variety of microbe identification cards. The VITEK 2 technology with the Advanced Expert System™ offers:

- Knowledge base developed from >100,000 references
- >2,000 described phenotypes
- >20,000 MIC distributions
- >100 resistance mechanisms detected
- >99 organisms
- On average, provides a resulting range of five to seven MIC doubling dilutions per antibiotic
- Extended MIC range to enable low-level resistance detection
- Resistance-oriented results that highlight unusual phenotypes
- Deduced antibiotic results to meet formulary requirements
- Checks every result every time

Vitek® 2 Automated Systems

VITEK® 2 is a fully automated system that performs bacterial identification and antibiotic susceptibility testing.

VITEK 2 offers

- Intuitive software
- User interface screen for immediate notification of system status to increase productivity.
- Unique vacuum filler provides both safety and the highest level of automation.
- Elimination of many manual steps:
- Designed for simple temperature verification.
- Completed tests are automatically ejected into an ergonomic trash container.

****But there is no where it is stated that it can be used for mutant identification.

Following my work at the laboratory (Osayande J.O., 2013; Ferripyoverdine receptors and General metabolism in *Pseudomonas aeruginosa*, Preliminary results, WebmedCentral Microbiology,

4(6):WMC004302, I observed that this machine could identify constructed mutants (while the wild type *P. aeruginosa* used in the experimental study were scored 98% , mutants were scored 97%, see table 1) and in addition could also detect changes (please see reference 20) introduced into the culture‘s media.

I am proposing a reinvention of the vitek machine to help reduce scientific work load based on my observations.

The vitek machine is a highly automated machine that cannot only identify organisms, in addition, can detect a change in the culture‘s media and subsequently presents the effect on the result automatically generated.

In my work at the laboratory, introduction of Iron (Fe) and Gentamicin into the culture media could lead to differential substrate utilization for the wildtype and ferripyoverdine mutants of *Pseudomonas aeruginosa* used; this kept me thinking so many things can be done.

- There are worldwide scientific publications explaining how scientists are trying to develop vaccines, these publications contain information about constructed mutants where the authors try to show the importance of one or more genes in several pathogenic organisms (Alvarez-Ortega *et al.* 2010)

- The vitek machine can serve as a Small Laboratory Workstation (SLW) by helping to minimize time spent on carrying out experimental procedures , this kind of SLW will be used for the identification of mutants, and in addition used to see how these different mutants will thrive in the presence or absence of various antibiotics, enabling scientists to score genes in relation to antibiotics, that is, in the presence or absence of some genes, some organisms can either resist or become more susceptible to some antibiotics impregnated in the vitek identification cards

- Customization of the Vitek cards. In addition, there are compounds that acts as antidotes against pathogenic organisms, some are already known, while others are yet to be tried, NEW cards impregnated with special Compounds or Synthetic eCaps (synthesized compounds known to minimize or reduce symptoms of disease conditions; see reference 18) can be reinvented and utilized in the vitek machine.

It is going to be a whole lot of work but I promise a fruitful outcome whereby following a few hours of running the vitek experiment, the adverse effect a compound or compounds can have on an unknown pathogenic organism or a mutant (as the case may be) will be automatically shown by the machine, such that no time is wasted.

MY PROPOSITION

- I propose, if possible, a construction of a database of such mutants (Alvarez-Ortega *et al.* , 2010) showing the relevant genes that have been mutated in the past from several pathogenic organisms and such database incorporated into the vitek machine

- Development of new vitek cards (as stated earlier above) to be biochemically tested against these mutants or other pathogenic organisms

- Control experiments should be run in parallel. The vitek machine has this potential. It contains a lot of wells where different experimental conditions can be carried out in different tubes

- Presently, there is Vitek 2 technology, the reinvented machine might be called something else and will be used for my probable proposition. However, it will also have the ideal vitek machine cards (substrate and antibiotic identification cards), in addition to the new (customized) cards impregnated with synthetic compound or ecaps known to be effective against disease-causing organisms. Such a mechanical construct might be able to show the effect synthetic compounds or ecaps impregnated into the new cards have on pathogenic organisms or mutants and such results are then ultimately automatically generated by the machine thereby simplifying laboratory experiments.

- There are compounds with prohibited usage in laboratories because of the damaging effects they have on humans, but however, these compounds are also potential poisons to disease -causing bacteria. Such compounds can be impregnated into these cards to generate preliminary results in such a case that information is provided on what best can be used as antidotes for pathogenic organisms. The only little work left for scientists then is to see how to detoxify these compounds and incorporate them into vaccines. This version of the vitek machine I am proposing will further reduce laboratory work load.

The reinvention of the Vitek Machine is proposed in this issue. If this is done, it would be in agreement with the discoverer of the Ebola virus who urged officials to test experimental vaccines on people with the virus so that when it inevitably returns, the world is prepared.

A machine that can test viruses, bacteria (known or yet to be identified), vaccines etc. is needed at this point in time and also in the future in severe cases of disease outbreak.

CONCLUSION

With the little knowledge I have, I tried to address issues related to health and propose some few ideas on how best we can help each other as scientists, (no

offence to cleaners). I do not know where these ideas may be put to use. But wherever laboratory experiments are run and trials are carried out based on these ideas, please credit should never be taken for using these ideas.

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Illustrations

Illustration 1

Cathecolate-Fe complex

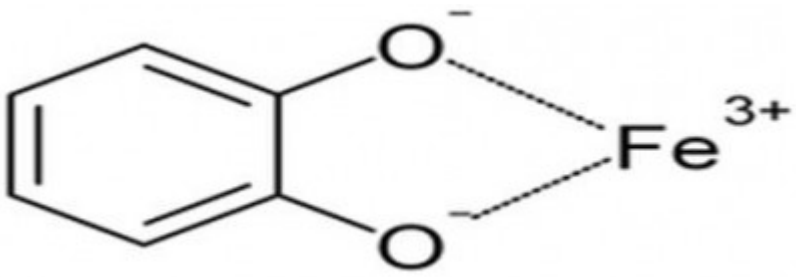

Illustration 2

Table 1: VITEK gram negative identification of P. aeruginosa wildtype and ferripyoverdine receptor mutants strains used.

Strain	Reference	Identification	Confidence
PAO1	Stover *et al.* 2000	98% probability *Pseudomonas aeruginosa*	Excellent identification
PAO1-*pvdD pchEF FpvA*	Ghysels *et al.* 2004	97% probability *Pseudomonas aeruginosa*	Excellent identification
PAO1-*pvdD pchEF FpvB*	Ghysels *et al.* 2004	97% probability *Pseudomonas aeruginosa*	Excellent identification
PAO1-*pvdD pchEF FpvAFpvB*	Ghysels *et al.* 2004	97% probability *Pseudomonas aeruginosa*	Excellent identification