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A study on mutagen induced development of antibiotic resistance in some pathogenic bacteria

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ABSTRACT

The evolution of antibiotic resistance by pathogenic bacteria poses a major task for human health. Whereas it is unambiguous that natural selection promotes resistance-conferring mutations, our understanding of the response of the mutation rate to antibiotics is limited. Ever since effective antibiotic treatment was first introduced almost seventy years ago, it has been a notable success story and antibiotic treatment is without a doubt the single most important medical procedure or treatment ever invented as measured by the reduction in human morbidity and mortality. However, the present studies based on the industrial area like pharmaceutical, where chemical mutagen release as an effluent. Continue contact with chemical mutagen some microbes got changes and show different behavior when antibiotic used. The intensive contact of microbes with chemical mutagen resulted in antibiotic resistance among almost all major human pathogens, and the resulting loss of therapeutic options might generate a post-antibiotic era where present and future medical advances are negated. As resistant bacteria significantly reduce the possibilities of treating infections efficiently and boost the risk of complications and fatal outcome for patients with severe infections, it represents a major public health concern and economic problem both within the globally worldwide.

Key words: Antibiotic resistance, Chemical mutagen, Infection, Microbes.

INTRODUCTION

Antibiotics have been truly called miracle medicine but sixty years of use and misuse of antibiotics has resulted in increased frequencies of resistance for most combinations of antibiotics and bacteria. In fact, this bacterial adaptive evolution has been so successful that certain bacterial infections are almost untreatable with antibiotics. For any resistance mechanism the bacterium is not only altered in its ability to withstand the drug but potentially also in its interaction with the host and environment (Andersson, 2003).

Now these days due to speedy development of industry and production of many toxic compounds which contain heavy metals, toxic product and certain other mutagenic substance that can cause mutation. Mutation has two impacts on environment that can be beneficial or harmful. Mutation has not always bad impact on environment but also has some good impact. Mutation can be advantageous and lead to evolutionary changes in genotype that`s why it is also called ultimate fuel for evolution and it has been widely accepted that these deleterious causing disease, developmental delays, structural abnormalities and other effect. Like the other living organisms, bacteria are continuously exposed to environ-mental stresses and are able to adapt themselves to severe fluctuations of the environment. There are many factors causing stresses in bacteria. Among them, the presence of antibiotics produces different types of vital stresses. Studying the evolution of the bacterial cell structure under such stressful conditions is an important research topic because it provides us with an insight of how bacteria become antibiotic resistant (Do, 2013).

As we know that many microbial floras present in eco-system but aquatic ecosystem are the final destination of domestic, municipal, and industrial effluent or liquid, whose effect can affect the quality of water, aquatic animals, and microbes. Industrial waste containing broad range of pollutant that is depending on the industry for example tennary, chemical and fertilizer industry. There waste contains dyes, many toxic chemicals like, EtBr, Benzene, Acryl amide etc. All the chemicals which is mentioned above are carcinogen and it has bad impact on ecosystem and microbial flora and that can change in their genetic level which has very bad and dangerous impact on ecosystem, due to the chemical mutagen some microbes are get mutated for particular antibiotic and some are leads to the produc-tion of secondary metabolites In our environment there have many microbial flora (like *Staphylococcus aureus, Streptococcus pyogenes, Clostridium perfrigens, E. coli* etc) which are cosmopolitan and mostly found in soil, also has ability to cause disease on human being. Most of the microbes are resistance against antibiotic only few are sensitive but by EtBr and several others chemical mutagen they all are get mutated and it leads to changes in their genetic level but some are become resistance against particular antibiotic. Although it is not necessary that mutation has always bad impact it also can be good. We are using four microbes for detection of effect of chemical mutagen and their antibiotic test. *S. aureus* is the gram-positive bacteria cocci or spheroidal, non-motile, arranged in group of clusters and they grow on nutrient agar media at 37^0, *S. pyogenes* the gram positive bacteria and they are spherical or ovoid cells; non motile and nonsporing. *E. coli are* gram negative rod shape, they do not form spores. *C. perfrigens* is an anaerobic, gram positive, rod shaped and spore forming bacteria that can be readily found in soil, dust, feces, feed, poultry litter and in gastrointestinal tract of healthy bird *C. perfrigens* is the third most common causes of food poisoning in United Kingdom (Das *et al*., 2008). The increase of antibiotic resistance is broadly seen by organisations like the European Food Safety Authority, the WHO and the Lancet Infectious Diseases Commission as a consequence of the use and overuse of antibiotics in both human and veterinary medicine (Andreoletti *et al*., 2008, Laxminarayan, 2013, WHO, 2011, WHO, 2014). In this study for detection of mutagen induced development of antibiotic resistance we are used following six different chemicals as mutagen Sodium nitrite ($NaNO_2$), Sodium nitrate ($NaNO_3$), Congo red (CR), Acryle amide (AA), Ethidium bromide (EtBr), and Benzen (BZ).

METHODOLOGY

Bacterial strains

Following Bacterial strains are selected for the study of impact of chemical mutagen -*Staphylococcus aureus (*MTCC3160*), Streptococcus pyogenes (*MTCC4122*), Escherichia coli (*MTCC443*), and Clostridium perfrienges (*MTCC450*).*

Antibiotic

We are using some antibiotic to know the sensitivity against particular microbes. And these are Tetracycline, Ampicillin, Penicillin, Streptomycin, Chloramphenicol and Gentamicin (liquid)

Media

Here in entire work NAM (Nutrient Agar media) and Nutrient Broth is used as growth medium. It is used for establishment of culture, sub culturing, and also for Antibiotic test.

Chemicals

There have many chemicals used in entire work for exa-mple Sodium nitrite ($NaNO_2$), Sodium nitrate ($NaNO_3$), Congo red (CR), Acryle amide (AA), Ethidium bromide (EtBr), and Benzen (BZ). All these chemicals used here

as a mutagen as we know that all these chemicals have mutagenic impact on microbes as well as in animals.

Establishment of culture in NAM media

Lyophilized glass vials containing powder form of bacterial culture has been broken by using autoclaved forceps and bacterial culture has inoculated into nutrient broth and also spreaded in NAM plates.than bacterial culture has incubated for 24-48 hrs at 37°C in incubator and after 48 hrs culture is used for the test.

Preparation of antibiotic disc

Antibiotic disc has been prepared by using antibiotic powder and whatman filter paper no.42. First of all 1mg/ml concentration of stock solution has been prepared for the entire six antibiotic i.e. Tetracycline, Streptomycin, Ampicillin, Penicillin, Cloramphrnicol, and Gentamicin. Here by using 1mg/ml stock solution, 10ug/ml of antibiotic solution has been prepared which is used for the preparation of antibiotic disc, and 10ug/ml is a standard for antibiotic disc. After preparation of 10ug/ml antibiotic dilution, punched chromatography paper had dipped and left for 24 hrs. So for the preparation of 10ug/ml antibiotic solution 0.1 ml of 1mg/ml antibiotic solution mixed in 0.9 ml distilled water and after preparation of above antibiotic solution, disc (Whatman filter paper no 42, prepared by punching machine) has dipped in 10ug/ml concentration of antibiotic solution.

Antibiotic test of microbes

Antibiotic test of all the microbes (*E. coli, S. aureus, S. pyogens,* and *C. perfringes*) has been done. In this process first of all 1ml of 48 hrs old culture has spreaded in NAM plates than 10ug/ml antibiotic disc has been placed on Petri plates and incubated for 24-48 hrs and zone of inhibition has been observed. Antibiotic test has been observed. Antibiotic test help for the determination of effect of antibiotic for particular microbes rather that is sensitive or resistance for that microbes (Figure-2).

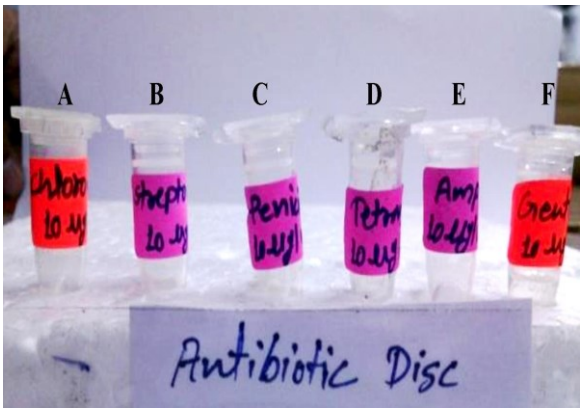

Figure 1

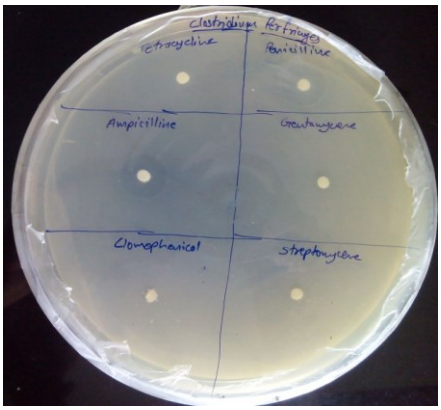

Figure 2

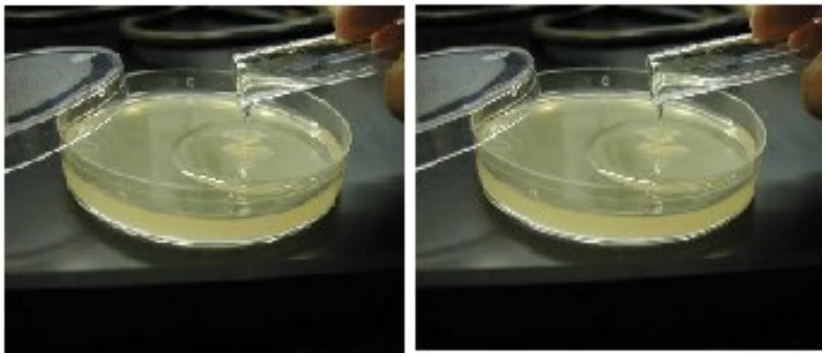

Figure 3

Figure 1 – A to F, Showing Different Antibiotic Disc
Figure 2 - Antibiotic test of microbe (*Clostridium*)
Figure 3 - Pour plate method

Preparation of stock solution of chemical mutagen

There have so many chemicals which have mutagenic property. For the detection of effect of chemical mutagen on microbes, stock solution of chemical mutagen has been prepared. Here 1mg/ml stock solution of chemical mutagen has been prepared, chemicals which is used for mutation is Benzene, acryl amide, $NaNo_2$, $NaNo_3$, Congo red etc. These all are that chemical which is mostly used in textiles, agricultural, fertilizers, and in many other industries. 20ul of stock solution of chemical mutagen is used for 20 ml media, and its concentration is 10ug/ml and here this concentration is used for the mutation.

Pour plate method

In this method first of all, 100ul of 24 hrs old culture has been inoculate in empty petriplate and simultaneously 20ul of 1mg/ml antibiotic and 200ul of 1mg/ml chemical mutagen has been added in 20ml of NAM media at 38-42^{0}C and mixed properly than poured into petriplate having 100ul of bacterial culture and incubate for 24-48hrs (Figure -3).

RESULT AND DISCUSSION

Table 1 show the mutagenic impact of various chemical on microbes, our present study is based on the evolution of antibiotic resistance by different chemical on pathogenic bacteria which cause severe disease on human being. Here we worked on four different bacterial strain *Staphylococcus aureus, Streptococcus pyogenes*, *Escherichia coli*, *and Clostridium perfrienges* as a tester strain and optical density of all the culture was 0.7 at 520 nm. After incubation of all the tester strain with mutagenic agent cultures are inoculated on Nutrient Agar Medium and antibiotic disc (Figure-1) placed, after 24-48 hrs.

Table 1: Showing mutagenic impact of various chemical on microbes.

S. No.	Bacterial strain	Chemical mutagen	No.of colonies (Showing sensitivity against particular antibiotic)					
			Peni	Amp	Genta	Tetra	strept	Chlo
1.	*S.aureus*	$NaNO_2$	-ve	-ve	-	-	-ve	-
		$NaNO_3$	-ve	-ve	-	-	-ve	-
		CR	-ve	-ve	-	-	-ve	-
		AA	-ve	-ve	-	-	-ve	-
		EtBr	-ve	-ve	-	-	-ve	-
		BZ	-	-	-	-	+ve(175)	-
2.	*S. pyogens*	$NaNO_2$	-ve	-ve	-ve	-ve	-	-
		$NaNO_3$	-ve	-ve	-	-	-ve	-
		CR	-ve	-ve	-	-	-ve	-
		AA	-ve	-ve	-	-	-ve	-
		EtBr	-ve	-ve	-	-	-ve	-
		BZ	-ve	-ve	-	-	-ve	-
3.	*E. coli*	$NaNO_2$	-ve	-ve	-ve	-	-	-
		$NaNO_3$	-ve	-ve	-ve	-	-	-
		CR	-ve	-ve	-ve	-	-	-
		AA	-ve	-ve	-ve	-	-	-
		EtBr	-ve	-ve	-ve	-	-	-
		BZ	-ve	-ve	-ve	-	-	-
4.	*C. perfrigens*	$NaNO_2$	-ve	-ve	-	-	-ve	-
		$NaNO_3$	-ve	-ve	-	-	-ve	-
		CR	-ve	-ve	-	-	-ve	-
		AA	-ve	-ve	-	-	-ve	-
		EtBr	-ve	-ve	-	-	-ve	-
		BZ	-ve	-ve	-	-	-ve	-

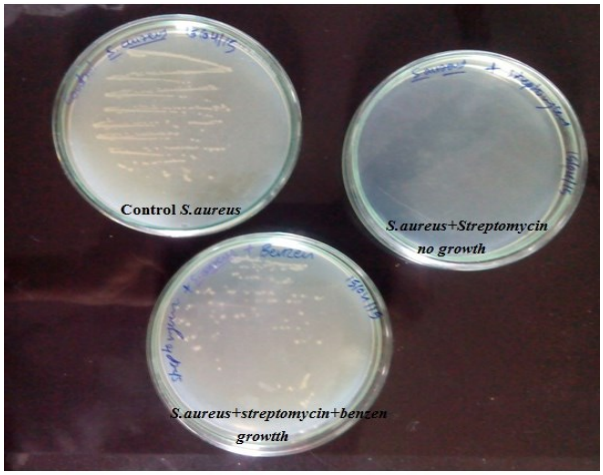

Figure 4 - Showing mutation in *S. aureus* by chemical mutagen benzene against streptomycin antibiotic.

It has been observed that *S. aureus* treated with Benzene shows the resistance against streptomycin but normally it is sensitive (Figure-4) and other sample *S. Pyoggenes, C. perfringes, and E. coli* were showing negative result but *S. aureus* got mutated in Benzene against streptomycin. *S. aureus* sensitive in streptomycin but after treatment with benzene it gets resistance, which shows the confirmation of mutation.

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