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Regular Article

Study on Efficacy of Expired and Active Forms of Various Antibiotics on *Saccharomyces cerevisiae*

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Antibiotics are among the most frequently prescribed medications in modern medicines. The cell protection strategies in the organisms, development of resistance in previously susceptible microbes, the inevitable progression of microbes exposed to antibiotics to develop resistance, were the nesisities that ensures the need for continual cycles of discovery and development of new antibiotics. A large variety of antibiotics are available in the drug market today, several others being added regularly in combat with various pathogens that cause disease in humans as well as in animals. Our present study focused to investigate the change in efficacy of commonly used antibiotics such as amoxicillin, ampicillin, sparfloxacin, cefixime. We have collected antibiotics with before and after their expiry dates. A simple eukaryotic model organism *Saccharomyces cerevisiae* is used to study the comparative understanding of this microbe with these different antibiotics. In our investigation we found that response of *Sacchromyces cerevisiae* towards different antibiotics varied in its intricacies. Fresh forms of antibiotics have significantly inhibiting the growth of *Saccharomyces cerevisiae* as compared to expired forms. The observations revealed that expired forms of antibiotics loose their efficacy drastically.

Keywords: Antibiotic resistance, Drug efficacy, Microbes, *Saccharomyces cerevisiae.*

Antibiotics are the substances that can be produced or derived from certain fungi or bacteria and other micro organisms that can destroy or inhibit growth of other microbes. Their mechanisms of action include (1) interaction with the cell membrane, leading to leakage of cytoplasmic components; (2) interference with the synthesis of membrane components; (3) interference with nucleic acid synthesis; and (4) interference with microtubule assembly. The origins of natural antibiotics are noted along with cell protection strategies in the producer organism and the development of resistance in previously susceptible microbes. Some bacteria are naturally resistant to certain antibiotics (inherent resistance). Clinical resistance is commonly due to the emergence of resistant organisms following antibiotic treatment (acquired resistance). This emergence, in turn is due to selection of resistant mutants of the infective species (endogenous resistance) or, usually to transfer of resistance genes from other naturally resistant species (exogenous resistance). The inevitable progression of microbes exposed to antibiotics to develop resistance ensures the need for continual cycles to discover and development of new antibiotics. So, new antibiotics have to be designed and produced almost at a regular

basis to overcome this problem. Choosing the right drug at the appropriate concentration and the required time is a way to combat drug resistance.

In addition to this, all the antibiotics available in the market today lose their efficacy after their expiry period. The danger of taking expired antibiotics is not only loosing their chemical integrity, but they could play a role in creating antibiotic resistance. Many antibiotics are coated with a smooth film that ensures the medication gets released at the right point in the digestive tract. If that coating is compromised, the antibiotic may be released into blood stream too soon, and cause intestinal complications such as diarrhoea. It could also irritate stomach lining. There is always a risk to benefit ratio considered while developing a drug. The risk to benefit ratio should in general be less risk and more beneficial to the patient where as in the case of the expired drugs the risk factor is increasing drastically. In our present study we investigated the changes in efficacy of some commonly used antibiotics: Amoxicillin, Ampicillin, and Sparfloxacin, Cefixime before and after their expiry period. We have used a simple eukaryotic model organism, *Saccharomyces cerevisiae*.

MATERIALS AND METHODS:

Strain: *Saccharomyces cerevisiae* is used as a test organism for this study.

Medium: The strain was grown in a liquid broth of YPD medium, prepared using 2g of dextrose, 2g of peptone and 1 g of yeast extract dissolved in 100 ml of double distilled water, and the pH was adjusted to 6.8 and then sterilized. To this 2 % of gelling agent, agar is used.

Drugs: Both active (non-expired) and expired drugs of: Amoxicillin, Ampicillin, Sparfloxacin, Cefixime.

Method: 5 ml of the media are taken in each of seven test tubes. 1% seed culture is added to these tubes containing 5ml medium and drugs with varrious concentrations were added. The inoculated experimental cultures were grown at room temperature for 48 h. The harvested cultures were measured for its turbidity at 600 nm using UV-VIS spectrophotometer (Ultraspec-1100 of Amersham Biosciences). The optical density (OD) values were tabulated for microbial growth obtained from non-expired and expired drug of different concentrations. Similarly repeated for the other drugs also, 3 such experiments were conducted for each drug (Table 1-4).These data were represented in graphs (Fig 1-4)

Cell count: Liquid culture was taken and diluted in ratio of 1:10. The liquid sample containing immobilized cells is placed on the haemocytometer chamber; it is covered with a cover glass, and leave for capillary action to completely fill the chamber with the sample. Looking at the chamber through a microscope, the number of cells in the chamber was determined by counting. The number of cells of *Saccharomyces* grown both in expired as well as in non-expired antibiotics containing YPD medium was given in the tables 1-4 is obtained from the average of the three experiments.

RESULTS AND DISCUSSIONS:

A reduction in the microbial population was observed in all of the four active drugs and expired drug at higher concentration which was taken under study. Expired drug at lower concentration showed increase in the growth of microorganism

As observed from the OD values (figure 1,2,3&4) and viable cell count from haemocytometer (figure 5,6,7&8). The drug concentration (both active & expired) and microbial growth were inversely proportional. There was significant reduction in microbial population with the increased concentration of active & expired drug.

A flaggy curve of inhibition was observed in microbial population when treated with expired drug of Amoxicillin indicating no change in microbial population. But, there was increase in population at higher concentration. A bell shaped curve was observed when treated with active Amoxcilin under low concentration of Amoxicillin the microbial population and at higher concentration the population was decreased (figure 1)

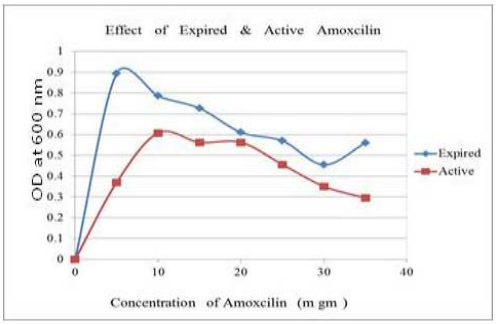

Figure 1: Graph shows the OD values of microbial growth treated with active & expired Amoxicillin. The concentration of amoxicillin taken is given in x axis and the OD values of microbial growth is taken in y axis.

Similar inhibitory curve was observed when treated with expired drug of Ampicillin at very high concentration of expired drug there was decrease in population showing slightly linear line. At high concentration of 35mg both active and expired drug showed same effect (figure 2). Flagy shaped inhibitory curve was observed when treated with both active and expired Sparfloxacin, indicating no population fluctuation. As the expired drug taken under study was of 2 months expiry. So, close O.D. values were obtained between active and expired drugs (Figure 3)

When treated with expired and active Cefixime similar curves were obtained at high concentration showing decrease in microbial population. At certain points there was coincidence in OD values due to expired duration was less. But yet low concentration of 0 to 10 mg range the bell shaped curve was observed with expired drug and declined line was observed with active drug (Figure 4).

Table 1: Data shows the OD values of microbial growth under various concentration of Amoxillin. 5ml of YPD medium was taken in each of 15 boiling test tubes were inoculated with 50µl of 48h grown seed culture and 50, 100, 150, 200, 250, 300, 350µl of expired and non-expired Amoxillin stock (10 mg/ml) solution along with control (0.0µl). The inoculated cultures were allowed to grow at room temperature in a shaker at 150rpm for 48 h. Harvested cultures were measured for its growth based on its turbidity using UV-VIS spectrophotometer at 600nm. The OD values for the individual tubes were tabulated, and are the average of 3 such experiments.Counting was done using haemocytometer .The number of cells mentioned here are the averages of 3 such experiments.

Concentration of Amoxillin in mg	OD of non-expired Amoxillin at 600 nm	OD of expired Amoxillin at 600nm	Number of cells from non- expired Amoxillin	Number of cells from expired Amoxillin
0	0	0	0	0
5	0.895	0.37	73.666	138.666
10	0.786	0.608	104.66	125
15	0.727	0.563	86	102.333
20	0.611	0.563	92.333	90.666
25	0.57	0.456	86.333	109.666
30	0.455	0.351	69.333	84.333
35	0.56	0.295	46.666	111.666

Table 2: Data shows the OD values of microbial growth under various concentration of Ampicillin. 5 ml of YPD medium was taken in each of 15 boiling test tubes were inoculated with 50 µl of 48hr grown seed culture and 50,100,150,200,250,300,350µl of expired and non-expired Ampicillin stock (10 mg/ml) solution along with control (0.0µl). The inoculated cultures were allowed to grow at room temperature in a shaker at 150 rpm for 48hr.Harvested cultures were measured for its growth based on its turbidity using UV-VIS spectrophotometer at 600nm.The OD values for the individual tubes were tabulated, and are the averages of 3 such experiments. Pippetted out 10µl of harvested culture from all 14 tubes and control into an e tube and diuted them with 990µl of distilled water and cell counting was done using haemocytometer.

Concentration of Ampicillin in mg	O.D of non-expiredAmpicillin at 600nm	O.D of expired Ampicillin at 600nm	Number of cells from non-expired Ampicillin	Number of cells from expired Ampicillin
0	0	0	0	0
5	0.754	0.613	111	122.333
10	0.683	0.562	98	113.666
15	0.663	0.554	101	115.333
20	0.76	0.49	76	97.333
25	0.438	0.411	69.666	80.333
30	0.364	0.308	55	62.666
35	0.285	0.228	40.666	47.666

Table 3: Data shows the OD values of microbial growth under various concentration of Sparfloxacin. 5ml of YPD medium was taken in each of 15 boiling test tubes were inoculated with 50µl of 48hrs grown seed culture and 50,100,150,200,250,300,350µl of expired and non-expired Sparfloxacin stock (10mg/ml) solution along with control (0.0µl).The inoculated cultures were allowed to grow at room temperature in a shaker at 150rpm for 48hr. Harvested cultures were measured for its growth based on its turbidity using UV-VIS spectrophotometer at 600nm.The OD values for the individual tubes were tabulated below are the averages of 3 such experiments. Pippetted out 10µl of harvested culture from all 14 tubes and control into an e tube and diluted them with 990µl of distilled water and cell counting was done using haemocytometer.

Concentration of Sparfloxacin	O.D of non-expired Sparfloxacin at 600nm	O.D. of expired Sparfloxacin at 600nm	Number of cells from non-expired Sparfloxacin	Number of cells from expired Sparfloxacin
0	0	0	0	0
5	0.782	0.694	128	102
10	0.705	0.656	110	89
15	0.591	0.544	77	69
20	0.511	0.463	65	57
25	0.433	0.381	51	42
30	0.358	0.308	35	31
35	0.267	0.245	26	20

With expired Amoxcilin flagy shaped curve was shown at low concentration indicating no fluctuation in cell count. But at high concentration a wavy shaped curve was observed indicating high rate of fluctuation in viable cell counts. With low concentration of active Amoxicillin, a bell shaped curve was observed with increase and decrease in cell count and at high concentration. A declined curve of slightly bell shaped was observed showing decreased cell count. At 20 mg concentration, the cell count was same for both active & expired drug (Figure 5)

Table 4: Data shows the OD values of microbial growth under various concentration of Cefixime. 5ml of YPD medium was taken in each of 15 boiling test tubes were inoculated with 50µl of 48hr grown seed culture and 50, 100, 150, 200,250,300,350µl of expired and non-expired Cefixime stock (10mg/ml) solution along with control (0.0µl).The inoculated cultures were allowed to grow in room temperature in a shaker at 150rpm for 48hs. Harvested cultures were measured for its growth based on its turbidity using UV-VIS spectrophotometer at 600nm.The OD values for the individual tubes were tabulated below are the averages of 3 such experiments. Pippetted out 10µl of harvested culture from all 14 tubes and control into an e tube and diluted them with 990µl of distilled water and cell counting was done using haemocytometer.

Concentration of Cefixime	O.D. of non-expired Cefixime at 600nm	O.D. of expired Cefixime at 600nm	Number of cells from non-expired Cefixime	Number of cells from expired Cefixime
0	0	0	0	0
5	0.74	0.782	120	132
10	0.723	0.827	101	145
15	0.673	0.716	83	92
20	0.627	0.69	72	86
25	0.386	0.514	46	65
30	0.326	0.368	35	41
35	0.256	0.286	20	31

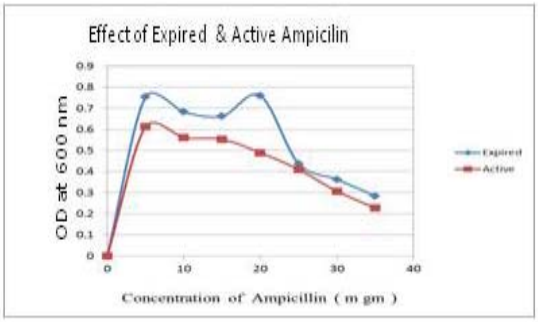

Figure 2: Graph shows the OD values of microbial growth treated with active & expired ampicillin. The concentration of ampicillin taken is given in x axis and the OD values of microbial growth is taken in y axis.

Similar systematic flaggy shaped inhibitory curve was observed with only change in height of their peaksat at low concentration, when treated with active & expired Ampicillin. At higher concentration of expired Ampicillin, continuous declined linear line was observed. But with active drug, it was slight flagy shaped indicating the fluctuation in cell count (Figure 6).

A flagy shaped curved was observed with both active & expired drugs of sparfloxacin with only change in height of the curve (Figure 7). With expired Cfixime, on cells a constant increase in the cell count was observed at low concentration and a constant decrease in cell count was observed at high concentration. With active cefixime, at low concentration a flag shaped curve depicting a high cell count was observed. Cell counts were similar for high concentration of both active and expired cefixime. (Figure 8)

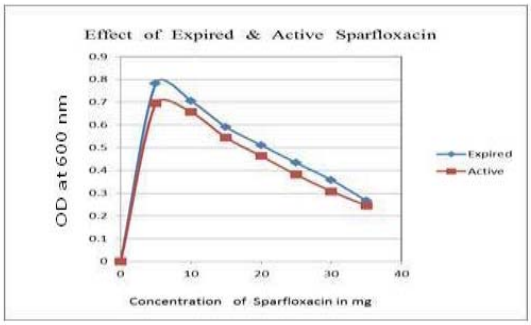

Figure 3: Graph shows the OD values of microbial growth treated with active & expired sparfloxacin. The concentration of sparfloxacin taken is given in x axis and the OD values of microbial growth is taken in y axis.

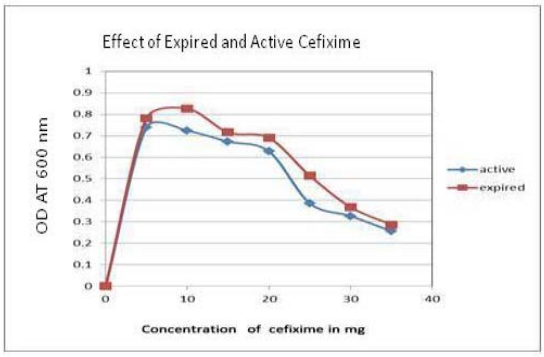

Figure 4: Graph shows the OD values of microbial growth treated with active & expired cefixime. The concentration of sparfloxacin taken is given in x axis and the OD values of microbial growth is taken in y axis.

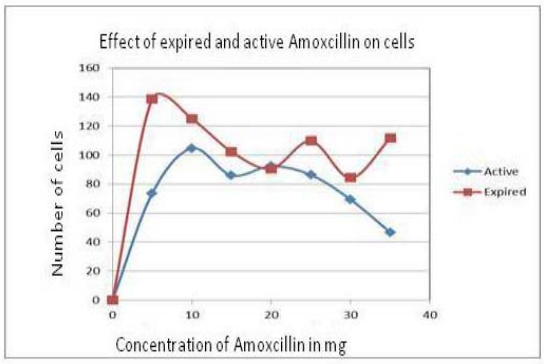

Figure 5: Graph shows the number of cells observed from microbial growth treated with active and expired amoxicillin. The concentration of amoxicillin taken is given in x axis & the number of cells observed in microbial growth is taken in y axis.

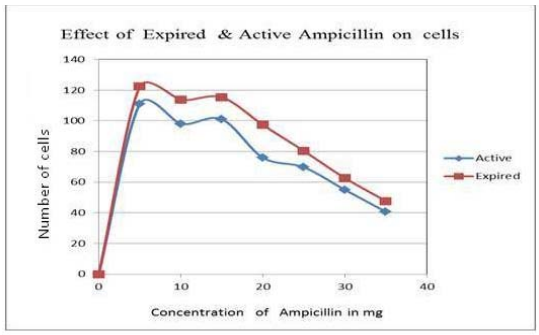

Figure 6: Graph shows the number of cells observed from microbial growth treated with active and expired ampicillin. The concentration of ampicillin taken is given in x axis & the number of cells observed in microbial growth is taken in y axis.

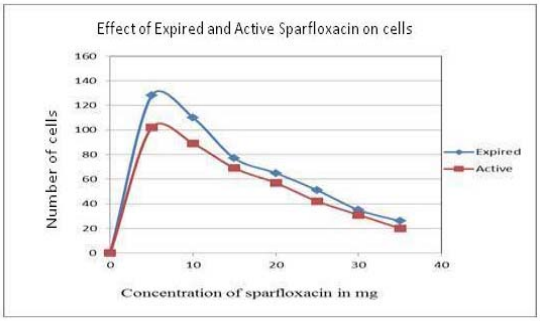

Figure 7: Graph shows the number of cells observed from microbial growth treated with active and expired Sparfloxacin. The concentration of sparfloxacin taken is given in x axis & the number of cells observed in microbial growth is taken in y axis.

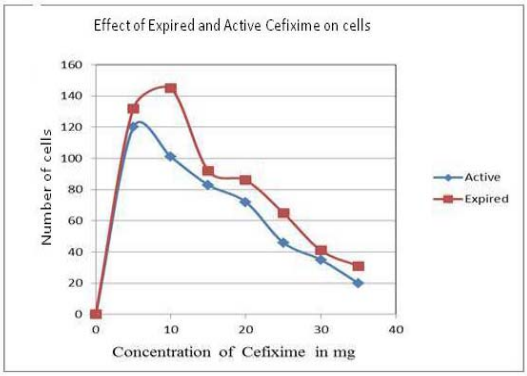

Figure 8: Graph shows the number of cells observed from microbial growth treated with active and expired Cefixime. The concentration of cefixime taken is given in x axis & the number of cells observed in microbial growth is taken in y axis.

CONCLUSION: The cell protection mechanisms of the cells are becoming more strategic and their development of resistance to antibiotics is incresing, demanding the need for the continual cycles of discovery and development of new antibiotics. Futures of these work mainly focusing to check the incresing antibiotic resistance. Every company manufacturing

the antibiotics should ensure that the risk to benefit ratio is less. The efficacy of the antibiotics after the expiry will lead to lose its chemical integrity and also play a role in the development of antibiotic resistance .So, the Government should take proper measures that the expired drugs are not in use.

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