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Bacterial Appraisal in Expired and Unexpired Pharmaceutical Products

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ABSTRACT

The microbial quality of expired and unexpired Pharmaceutical products like Metronidazole, Acetylsalicylic acid, Chloramphenicol, Silver sulphadiazine, combination of Polymyxin B Sulphate, Propylene Glycol, Lignocaine, combination of Betamethasone and Neomycinand the combination of Lignocaine, Ethanol and Cetylpyridiniumwere examined by the microbial growth on culture plates. Isolation was performed by serial dilution method and bacterial recovered by streak plate technique on Blood Agar, Chocolate Agar and MacConkey's Agar. Isolates were characterized morphologically by gram staining and biochemical analysis. Microorganisms isolated from expired product are *S.epidermidis* (33.3%), *B. subtilis* (4.7%), *Streptococcus (28.5%), Pseudomonas* (9.5%), *Listeria* (4.7%) *and B.cereus* (19%) while *B. subtilis(*9.5%*), Enterococcus* (9.5%), *E. coli (*9.09%), *S. epidermidis* (27.2%), *Listeria* (9.5%) and *Streptococcus* (9.09%) are isolated from unexpired products. The isolated organism is mostly normal flora but opportunistic and pathogenic bacteria are also isolated which can cause disease especially in immune-compromised person or infants. The isolation of organisms may be due to improper hygiene maintenance or poor packaging techniques as the risk of contamination is higher while processing rather than during use. It revealed that quality of unexpired products have to be improved by producers to minimize the bio-liability so pharmaceutical companies should adopt good manufacturing practice to avoid microbial contamination in unexpired pharmaceuticals as well as the consumer should be educated and are aware of hazardous effect of using expired products.

INTRODUCTION

Health is considered as the major wealth of our life. Present day's system claims huge medical expenditure for individuals as well as for the government or private authorities. Therefore, understanding such economic issues associated with health management and treatment system is of prime importance. Pharmaceutical products are the compounds that are manufactured to use as a medicine or drug (1). The administered of pharmaceuticals to all age-groups are through different routes (oral, topical, otic, ophthalmic) depending upon the form of medicine either tablet, cream and ointment, ear drops, eye drops respectively for the treatment of acute and severe diseases against all type of micro-organisms. They are not only used for the treatment and diagnosis of diseases but also for the prevention of certain diseases (2). But the administration of even low level of contaminated pharmaceutical can be very dangerous to human life (1).

The developmental process of pharmaceutical products is the combined innovation of pharmacist and microbiologist. The process of drug development starts with the invention of a drug molecule that has showed therapeutic value to fight, control, check or cure diseases. The production and characterization of such molecules are also called "Active Pharmaceutical Ingredients" (APIs) and their analysis to generate preliminary safety and therapeutic efficacy data are conditions for the identification of drug (11).

A variety of expired and unexpired pharmaceutical products are susceptible to bacterial invasion resulting in deterioration of products that are not suitable to use. Unexpired pharmaceutical products are manufactured by pharmaceutical industries against number of diseases and are labelled with an expiry date (5). The product before the expiry date is safe and effective for the consumers to intake while the expired pharmaceutical products are not safe for the consumer to use as the expiry date on drugs indicate the last day when the manufacturer guarantee the full potency and safety of the drug (6).

Pharmaceuticals are degraded by three different ways whether physical, chemical or microbial due to environmental factors, microbial contamination, container and closure. Physical instability results in change of color and texture. Chemical instability results in oxidation, hydrolysis, de-carboxylation while microbial growth result in microbial degradation (4). Microorganisms are predominantly present in environment, food, beverages, water even in pharmaceutical products. The presence of microorganisms in pharmaceuticals makes them ineffective or lowers their activity in treating infections (1).

In this research, we have tested the quality of different expired as well as unexpired Pharmaceuticals including Metronidazole, Acetylsalicylic acid, Chloramphenicol, Silver sulphadiazine, combination of Polymyxin B Sulphate, Propylene Glycol, Lignocaine, combination of Betamethasone and Neomycinand the combination of Lignocaine, Ethanol and Cetylpyridinium by examining the microbial count and growth on certain pharmaceuticals.

Mostly, contamination of pharmaceutical products occurs during their production process rather than during use.

The fall in personal hygiene may be due to bacteria that can be transferred to pharmaceutical products at any stage (2). Organisms can be isolated from the used samples are *S.epidermidis,* Streptococcus *spp, Pseudomonas aeruginosa, E.coli, Listeria monocytogenes, Bacillus subtilis, Bacillus cereus* and Enterococcus *spp*. Along with this, there are many other contaminants from the air, water, building equipment which may find their way to contaminate pharmaceutical products thus making them inappropriate for medications (4).

The contamination of expired as well as unexpired Pharmaceuticals with anyof these organisms either the normal flora or the pathogens likeListeriacan be harmful for human health and can cause severe disease. The excess and freely presence of Bacillus in environment interact with pharmaceuticals during production or packaging due to improper hygiene condition leading to the hazardous effect on human health.

The microbial load in expired and unexpired pharmaceutical products can cause serious health hazards and may lethally effect the consumer (3) but the presence of microbial growth either pathogenic or non-pathogenic in unexpired pharmaceutical products forces the consumers to lose faith and not to buy the products from certain manufacturing companies causing decline in the financial status of the manufacturers due to microbial contamination that disrupt the stability of the pharmaceuticals. The hazardous effect on human health by the consumption of contaminated medicine will depend on the type and degree of microbial contamination, extent of deterioration, route of entry and on patient's immune status (2).

The transmission of highly-risked or unpredictable microorganisms from human to life-saving pharmaceuticals products must be limited by improving personal hygiene and quality.The quality of unexpired products have to be improved by producers to minimize the bio-liability.(2) It could be prohibited by development in packaging techniques, managing the production staff, environment, raw materials and by the addition of preservatives to examine the microbial expansion (3).

MATERIALS AND METHODS

Sample Collection:

Seven expired and unexpired samples of each Metronidazole, Acetylsalicylic acid, Chloramphenicol, Silver sulphadiazine, combination of Polymyxin B Sulphate, Propylene Glycol, Lignocaine, combination of Betamethasone and Neomycinand the combination of Lignocaine, Ethanol and Cetylpyridinium were collected from the pharmacies of local markets in Karachi, Pakistan. The labelled information (Type of product, MFG.date and EXP.date) of both expired and unexpired samples was recorded (Table 1 and Table 2) respectively

Isolation of Microorganisms:

Microbial contamination is isolated from all type of expired as well as unexpired Pharmaceutical products.1g or 1ml of drug in 10ml of peptone broth. Serially dilute to 1:100. Streak from both dilutions on BA, CA and MacConkey and incubate the plates at 37° for 24 hours. Next day, observe the plates.

Identification of Microorganisms:

The identification was performed by examine colonial morphology and gram staining. Further identification was performed by biochemical tests.

RESULTS

In this study, 7 different Expired and Unexpired samples ofpharmaceutical products were analyzed to determine their microbial quality. Results showed that 85.7% of the expired as well unexpired pharmaceutical product show microbial growth on CA (Table I), BA (Table II) and Mac.conkey (Table III). Results revealed that microorganisms *S.epidermidis, B.cereus, B.subtilis, Enterococus, Listeria, E.coli, Pseudomonas* and *Streptococcus* are isolated from both expired and unexpired products. The growth of isolated organisms was characterized by their cultural characteristics on Blood Agar (BA), Chocolate Agar (CA) and MacConkey's Agar and by microscopic examination on 100X lens and were further confirmed by catalase test, coagulase test and TSI *Enterococcus* and *E.coli* are not isolated from

any of the expired samples whereas *B. cereus* and *Pseudomonas* are not isolated from unexpired samples. Among these organisms *S. epidermidis* have the highest growth rate of 33.3% in Expired Pharmaceutical Products and 27.2% in Unexpired Pharmaceutical Products (Fig 1). Results showed that microbial isolation was examined in all expired and unexpired samples except sample 5 (Fig 2). Among all the pharmaceutical products the expired product of sample 7 was highly contamination with the rate of 23.8% while the unexpired product of sample 3 was highly contaminated with the rate of 27.2% (Fig 2). Mostly, the isolation rate was higher in expired products than in unexpired products but the contamination rate of unexpired sample 3 is 27.2% which is higher than its expired product.
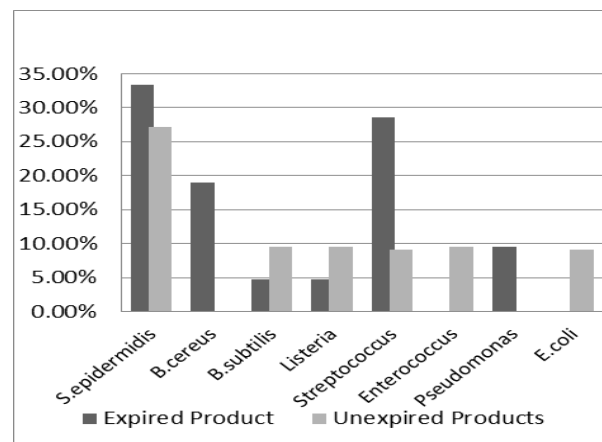

Fig 1: Percentages of Microorganisms isolated from both Expired and Unexpired Pharmaceutical Products
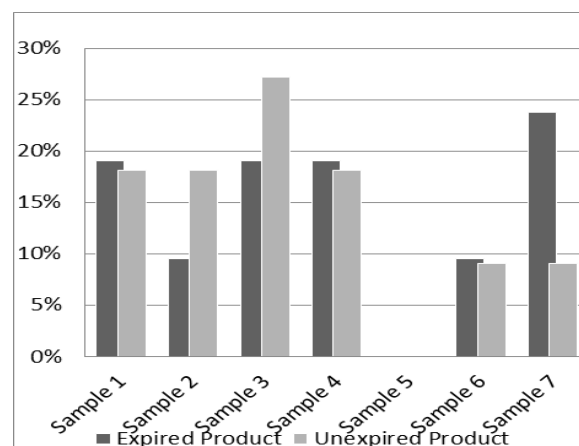

Fig 2: Microbial Contamination in Expired and Unexpired Pharmaceutical Products

Table I: Bacterial contamination on Chocolate Agar (CA)

ORGANISMS	Samples													
	Expired							Unexpired						
	1a	2a	3a	4a	5a	6a	7b	1b	2b	3b	4b	5b	6b	7b
B.cereus	+	-	-	+	-	-	-	-	-	-	-	-	-	-
B.subtilis	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Streptococcus	-	-	+	-	-	+	-	-	-	-	-	-	-	-
Enterococcus	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S.epidermidis	+	+	-	+	-	-	+	+	-	-	+	-	-	-
Pseudomonas	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E.coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Listeria	-	+	-	-	-	-	-	-	-	+	-	-	-	-

Table II: Bacterial contamination on Blood Agar (BA)

Organisms	Samples													
	Expired							Unexpired						
	1a	2a	3a	4a	5a	6a	7a	1b	2b	3b	4b	5b	6b	7b
B.cereus	+	-	-	+	-	-	-	-	-	-	-	-	-	-
B.subtilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Streptococcus	-	-	+	-	-	+	+	-	-	-	-	-	-	+
Enterococcus	-	-	-	-	-	-	-	-	-	-	-	-	+	-
S.epidermidis	+	-	-	+	-	-	-	-	-	-	-	-	-	-
Pseudomonas	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E.coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Listeria	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table III: Bacterial contamination on MacConkey Agar

ORGANISMS	SAMPLES													
	Expired							Unexpired						
	1a	2a	3a	4a	5a	6a	7a	1b	2b	3b	4b	5b	6b	7b
B.cereus	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B.subtilis	-	-	+	-	-	-	-	-	+	-	-	-	-	-
Streptococcus	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Enterococcus	-	-	-	-	-	-	-	+	-	-	-	-	-	-
S.epidermidis	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Pseudomonas	-	-	+	-	-	-	+	-	-	-	-	-	-	-
E.coli	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Listeria	-	-	-	-	-	-	-	-	-	+	-	-	-	-

DISCUSSION

Pharmaceutical products can be contaminated by microbial load at any stage of production, processing, marketing and administration. Administration of pharmaceutical products with bio-burden can be injurious to the recipients such as children, young and elderly patients. Survival and growth of microorganisms can contaminate the product quality as well as the production of metabolites or toxins may be harmful to patient even they are present in minute quantities (2).

There were few researches on microbiological assessment of pharmaceutical products which lead me to carry out this research. The study showed the presence of *S.epidermidis, Streptococcus spp, B.cereus, B.subtilis, Listeria* and *Pseudomonas* is higher in Expired Pharmaceutical while *Enterococcus, Listeria spp, E.coli, S. epidermidis, Streptococcus and B. subtilis*is higher in Unexpired Pharmaceuticals including Metronidazole, Acetylsalicylic acid, Chloramphenicol, Silver sulphadiazine, combination of Polymyxin B Sulphate, Propylene Glycol, Lignocaine, combination of Betamethasone and Neomycin and the combination of Lignocaine, Ethanol and Cetylpyridinium.

Our findings indicate that most of the isolated organisms were normal flora or ubiquitously found in our environment. Like *S. epidermidis* is the normal flora of human body, *Bacillus spp* is commonly found in environment. Isolation of *Bacillus subtilis, Escherichia coli* and *Pseudomonas* suggest that the route of contamination were possibly water, personnel and environment (8). Some recent studies have shown that *B.subtilis* was the most frequent contaminant of non-sterile pharmaceuticals (9) as it is widely spread in air, soil, water and in animal products such as hair, wool and carcasses. The isolation of *E.coli* from some of the pharmaceutical samples indicated faecal contamination which may be principally from production personnel and possibly from the water used as vehicle. *E.coli* are not always confined to the intestine but also persist outside of the body which makes them an ideal indicator organism to test samples for faecal contamination (10).

Pseudomonas is a recalcitrant drug contaminant predominantly contaminates the pharmaceutical product. This indicates that expired and unexpired pharmaceuticals are microbiologically contaminated due to improper handling, improper maintenance of hygiene conditions or lack of packaging techniques which may lead to serious health hazards. Although microbiological quality of expired pharmaceutical products are satisfactory but the quality of unexpired pharmaceutical is not acceptable. So, manufacturers should be rigid in terms of the product manufacturing, packaging and distribution of pharmaceutical product. However, thepresence of pathogenic or opportunistic microorganisms in unexpired pharmaceutical products is not virtuous for health and can seriously affect the health status of people especially of immunocompromised person and infants.

CONCLUSION

In this study, it is concluded that pharmaceutical companies should adopt Good Manufacturing Practice (GMP), packaging practice, proper treatment of water and air; personal hygiene improvement of the production personnel and pre-treatment of natural raw materials should be enforced and maintained. Along with this, proper handling and storage of these products must be carried out to eliminate or reduce microbial factors to ensure reduction in the level of microbial contamination in unexpired pharmaceutical products either the isolated organisms are normal flora or pathogenic. Manufacturers should do their best to achieve 100% compliance and adherence to Good Manufacturing Practice while the consumers should be educated and are well aware of hazardous effect of using any type of expired products so that they may avoid using all types of expired pharmaceutical products.

REFERENCES

1. Nwakile CD, Osonwa UE, Okechi OC, Opurum CC, Nwanyanwu CE. *Microbial and Physicochemical qualities of selected Co-trimoxazole and Metronidazole formulations in South Eastern Nigerian. J Adv Pharm Technol Res. 2011;2: 81-82*

2. Al Mamun A, Shaha TK, Khan M, Kabir S. *Determination of Microbial Load in Multivitamin and Cough Syrups Sold in Dhaka City. Int J Pharm Sci Drug Res. 2014; 6(3): 235*

3. Agbo E, Takon IA and Ajaba MO. *Prevalence Of Contaminating Microorganisms In Anti-Malarial Drugs Sold In Calabar, Cross River State, Nigeria. Int J Pharm Sci Res 2016;7(10): 4272-77*

4. Anderson L. *Current good manufacturing practice in manufacturing for finished pharmaceuticals and expiration dating. The Medical Letter on Drugs and Therapeutics 2012;51:107-108*

5. Anon. *Drugs Past Their Expiration Date. The Medical Letter on Drugs and Therapeutics 2009;51:101-102.*

6. Lyon RC, Taylor JS, Porter DA, et al. *Stability profiles of drug products extended beyond labeled expiration dates. J Pharm Sci 2006;95:1549-60.*

7. Clement A , Felix E, , John A ,Cyril A. *Physical and Microbial Examination of Commonly Sold Over the Counter Drugs, In AsabaMetropoly, Delta State Sch Acad J Pharm. 2013; 2(5):387-390*

8. *Ibezim EC, Esimone CO, Ofoefule SI, Chan KF. Evalution of the Microbiological Quality of Some Commercially Available Syrups and Suspensions in Nigeria. J. Phytomed therap. 2002; 7 (1&2): 18-25.*

9. *Willey JM, Sherwood LM, Woolverton CJ, Prescott, Harley and Klein Microbiology. McGraw Hill Publishers (4th Ed.). New York Press 2008; 936-972.*

10. *Siddiqui MR, AlOthman ZA, Rahman N. Analytical techniques in pharmaceutical analysis: A review. Arab J Chem. 2013;10: 1409-1421*