

**RESEARCH ARTICLE**

# STUDY OF PRESENCE OF MULTI-DRUG RESISTANT ORGANISMS FROM A PHARMACEUTICAL EFFLUENT.

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**Abstract:** One of the wonder discoveries of the 20<sup>th</sup> century is Antibiotics. But the real wonder and a great cause of concern is the rise of antibiotic resistance in hospitals, communities and the environment. There are many mechanisms by which microorganisms can develop resistance. This study aims at finding the role of microorganisms isolated from the effluent of a pharmaceutical industry in spreading drug resistance. The different microorganisms isolated from the effluent were *E.coli*, *Pseudomonas spp*, *Enterobacter spp*, and *S.aureus*. Their antibiotic sensitivity pattern was studied. The results from the study showed the presence of multi-drug resistant organisms in the effluent sample. About 66.66 % isolates were resistant to Ampicillin, Ceftriaxone, and Gentamicin, 33.33% to Ciprofloxacin while 100% showed resistance to Ceftazidime and Erythromycin

**Key words:** Antibiotics, pharmaceutical effluent, multi-drug resistant organisms.

**Introduction:**

The problem of antibiotic resistance is universal. Multi-drug resistance has developed in almost all bacterial pathogens. A variety of nosocomial infections have been reported due to these highly resistant bacteria. Uncon-

trolled and excessive use of antibiotics by humans and animals, results in an increase in antibiotic resistance and cause the spread of resistance genes in environmental samples<sup>1</sup>.

Therefore antibiotic resistance is not only found in pathogenic bacteria but also in environmental organisms inhabiting terrestrial and aquatic habitats. Higher numbers of resistant bacteria occur in polluted habitats compared with unpolluted habitats, indicating that humans have contributed substantially to the increased proportion of resistant bacteria occurring in the environment<sup>2</sup>.

Indian pharmaceutical companies supply 20% of the world's generic drugs<sup>3</sup>. The effluent coming out of these industries needs to be treated well before it is discharged in the natural water bodies. The resistance genes may be transferred from environmental bacteria to human pathogens which is the main risk for public health. The input of resistant bacteria into the environment seems to be an important source of resistance in the environment<sup>4</sup>. Antibiotics exert a selection in favour of resistant bacteria. The resistant bacteria adapt to environmental conditions and serve as vectors for spread of antibiotic resistance<sup>5</sup>.

The effluent coming out of a pharmaceutical industry contains antibiotic and antibiotic resistant bacteria which can transfer drug resistance horizontally to pathogens. To the best of our knowledge not many studies have been undertaken to study this phenomena. This study attempts to generate information regarding the presence of microorganisms in the treated effluent of a pharmaceutical industry located in the outskirts of Mumbai and the drug resistance pattern of these isolates.

**Materials and methods :****Sample collection:**

The treated effluent was collected from a pharmaceutical company located in the outskirts of Mumbai. The sample was collected in sterile conical flask and stored at 4°C till use.

**Isolation and characterization:**

Bacteria were isolated and purified by streak plate method on standard selective and differential media. The plates were incubated at 37°C for 24 hrs. [Fig.1]

Isolated cultures were identified by biochemical tests. Gram staining was carried out. Catalase test, Citrate utilization test, Indole production test, Methyl Red-Voges Proskauer (MR-VP) test, Urease test and fermentation of carbohydrates like glucose, lactose and sucrose were carried out for the identification. [Fig. 2]

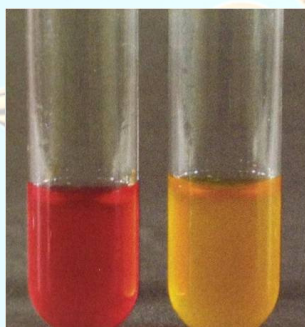
**Fig. 1: Isolation on Mac conkey's agar plate****Antimicrobial Susceptibility Test (AST):**

AST for all the isolates was performed as per Kirby Bauer disc diffusion method<sup>6</sup> using the antibiotics mentioned below. The media used was Mueller Hinton agar prepared as per standard procedure. The density of the culture was adjusted to 0.5 McFarland's standard. With the help of sterile cotton swabs, culture was inoculated on the medium so as to obtain a lawn culture.

Using sterile forceps the discs of various antibiotics obtained from Hi Media were placed on the plate. The plates were incubated overnight at 37°C. The zone sizes were interpreted as per the standard chart and the organisms were classified as sensitive, intermediate and resistant to the various antibiotics. Discs containing Ampicillin/Sulbactam (10/10 µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Ceftazidime (30µg), Erythromycin (15µg), Gentamicin (10µg), Gentamicin (30µg), Norfloxacin (10µg) were used to test Gram negative organisms and discs containing Amoxicillin (30µg), Chloramphenicol (30µg), Cephotaxime (30µg), Cefepime (30µg), Ciprofloxacin (30µg), Ceftizoxime(30µg), Erythromycin (15µg), Penicillin-G (10 units) were used to test Gram positive organisms. All the isolates were maintained at refrigeration temperature on Nutrient Agar slants. Working stocks were maintained and used for further studies.

**Fig. 2: Biochemical tests for identification of organisms.**

Indole test



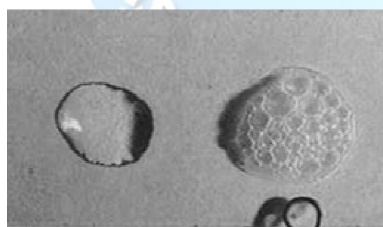
Methy red test



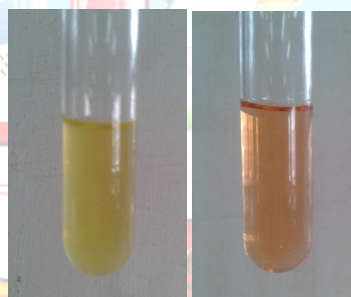
Vogues proskauer test



Citrate utilization



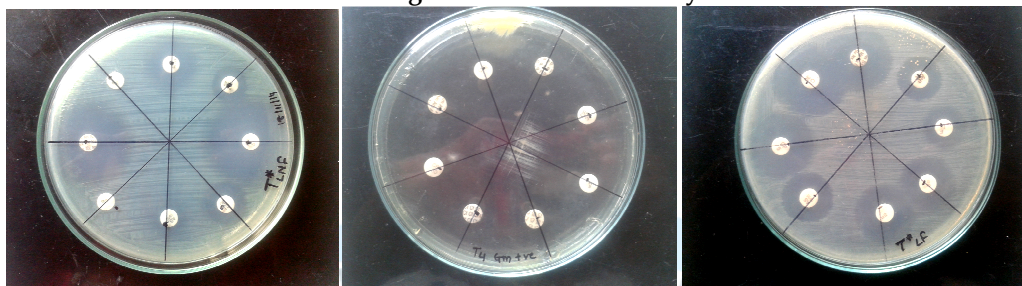
Catalase test



Urease test



Carbohydrate fermentation

**Fig.3: Antibiotic sensitivity test****Result and Discussion:**

A total of four types of bacteria were isolated from the effluent sample collected. Out of these four types, one was Gram positive and three were Gram negative. Using the identification techniques they were confirmed as *Enterobacter spp.* (T1), *Escherichia coli* (T<sub>LF</sub>), *Pseudomonas spp.* (T<sub>LNF</sub>) and *Staphylococcus aureus* (T4).

**Table 1 shows the result of biochemical tests.**

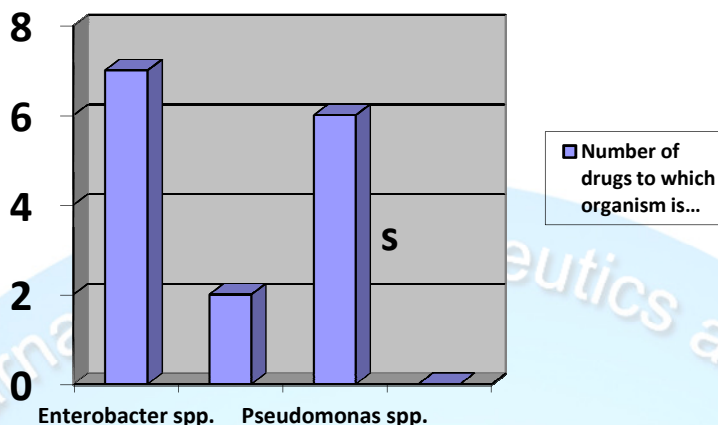
Test culture	Gram's nature	Morphology	Indole production	Methyl red	Voges-proskauer	Citrate utilization	Urease	Carbohydrate fermentation			Organism identified
								Glc	Lac	Suc	
T1	Gram negative	Short rod	+	+	-	-	-	AG	AG	A	<i>Enterobacter spp.</i>
T <sub>LF</sub>	Gram negative	Coccobacilli	+	-	-	-	-	AG	AG	AG	<i>Escherichia coli</i>
T <sub>LNF</sub>	Gram negative	Rod	-	-	-	+	-	-	-	-	<i>Pseudomonas spp.</i>
T4	Gram positive	Cocci	-	+	-	-	-	Catalase			<i>Staphylococcus Aureus</i>
								+			

Legend :- +: positive ; -: negative ; A : acid production ; G : gas production. Glc : glucose ; Lac : lactose ; Suc : sucrose.

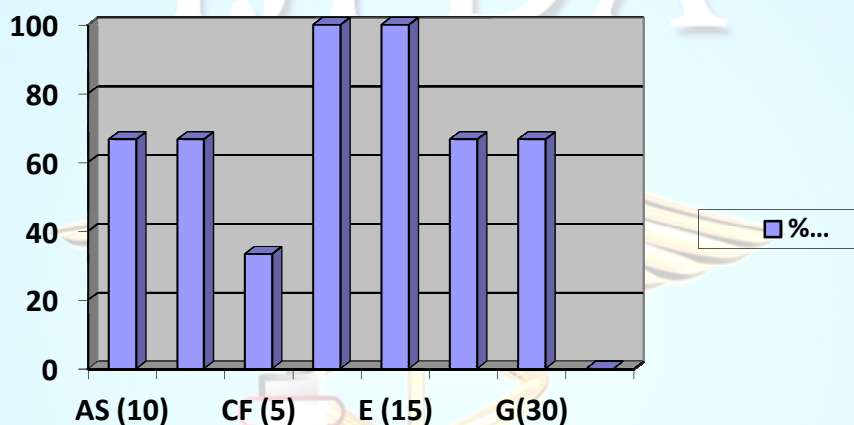
**Table 2 shows the AST results for the various antibiotics used.**

Test culture	ANTIBIOTICS TESTED							
	GRAM NEGATIVE ORGANISMS							
	Ampicillin AS 10/10	Ceftriaxone Ci 30	Ciprofloxacin CF 5	Ceftazidime CAZ 30	Erythromycin E 15	Gentamicin G 10	Gentamicin G 30	Norfloxacin Nx 10
T1	R	R	R	R	R	R	R	I
T <sub>LF</sub>	I	S	I	R	R	S	S	S
T <sub>LNF</sub>	R	R	S	R	R	R	R	S
	GRAM POSITIVE ORGANISM							
	Amoxicillin Am 30	Chloramphenicol C 30	Cephotaxime CE 30	Cefepime CPM 30	Ciprofloxacin Cf 30	Ceftizoxime Ck 30	Erythromycin E 15	Penicillin G P 10
T4	S	S	S	S	S	S	S	S

Legend: R – resistant ; S – sensitive ; I – intermediate.



A graph showing organisms against number of drugs to which they are resistant.



A graph representing the number of organisms (in %) resistant against antibiotics used (with concentrations).

AST was carried out for all the isolates. *Enterobacter spp.* was resistant to Ampicillin (10 $\mu$ g), Ceftriaxone (30 $\mu$ g), Ciprofloxacin (5 $\mu$ g), Ceftazidime (30 $\mu$ g), Erythromycin (15 $\mu$ g), Gentamicin (10 $\mu$ g), Gentamicin(30 $\mu$ g). *Escherichia coli* was resistant to Ceftazidime (30 $\mu$ g) and Erythromycin (15 $\mu$ g). *Pseudomonas spp.* was resistant to Ampicillin (10 $\mu$ g), Ceftriaxone (30 $\mu$ g), Ceftazidime (30 $\mu$ g), Erythromycin (15 $\mu$ g), Gentamicin (10 $\mu$ g), Gentamicin(30 $\mu$ g) and *Staphylococcus aureus* was not resistant to any of the antibiotics used. About 66.66 % isolates were resistant to Ampicillin, Ceftriaxone, and Gentamicin. Ceftazidime and Erythromycin showed 100% resistance. 33.33 % isolates were resistant to Ciprofloxacin. While Norfloxacin showed 0% resistance. [Fig.3]

The results from the present study showed the presence of multi-drug resistant organisms like *Enterobacter spp.*, *Escherichia coli*, and *Pseudomonas spp.* in the treated effluent sample of the pharmaceutical company. The company used activated sludge method for the treatment of the

waste which was then discharged in the water body. Another study carried out by Ashour et al 2011 showed that group of genus *Staphylococcus* contributes to 38.4% of total samples while genus *Micrococci* to 22.4%, *Bacillus spp.* 35% and *Klebsiella spp* 1%<sup>7,8</sup>. All the gram negative strains were resistant to Ceftazidime and Erythromycin.

In a study conducted by Lateef et al (2003) the effluent of a pharmaceutical company was examined microbiologically. The organisms encountered included *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Serratia marcescens* and *Pseudomonas aeruginosa*. The resistances of the 25 bacterial strains isolated from the effluent to the frequently used antibiotics were considered. About 80% of the isolates were resistant to Amoxycillin, 76% to Citrofurantoin, 64% to Cotrimoxazole and Augmentin, 60% were resistant to Nalidixic acid, 52% were resistant to Tetracycline and Ofloxacin, while resistance of 12% was obtained for Gentamicin<sup>9</sup>. Results of their study are in agreement with the present study to some extent.

**Conclusion:**

India has shown to be a breeding ground for around 50 drug resistant microbes due to extensive usage and improper disposal of pharmaceutical drugs into the environment<sup>10</sup>. Constant presence of antibiotics in its environment has led to the gradual adaptation of bacteria to tolerate higher doses of antibiotics. The antibiotic does not technically cause the resistance, but allows it to happen by creating a situation where an already existing variant can flourish.

This study was conducted at a very primary level to find out if there are drug resistant organisms present in the waste generated in the pharmaceutical company. From this study it can be concluded that multidrug resistant microbes are present in the waste which can transmit the resistance to pathogens.

Since not much data is available from this kind of study the need of the hour is to find various ways by which spread of drug resistance by this mechanism can be checked. Taking into consideration its effects, stringent regulation is required from industries and regulatory agencies in India. Monitoring the amount of drugs present in the waste and proper disposal is essential.

**References:**

- Iversen A, Koehn J, Frankin A. High prevalence of vancomycin resistant Enterococci in Swedish wastewater. *Appl. Environmental Microbiology*. 2002; 68:2838-2842.
- Pathak SP, Gaw A, Battacharjee JW. Distribution and antibiotic resistance among aerobic heterotrophic bacteria from rivers in relation to pollution. *J Environmental Science Health A*. 1993; 28:73-87.
- [www.forbes.com/sites/who](http://www.forbes.com/sites/who) is responsible for antibiotic resistance/22Jan 2015.
- Dhundi RS, B Pradhan, SK Mishra (2010). Multiple drug resistance in bacterial isolates from liquid wastes generated in central hospitals of Nepal. Kathmandu. *Uni Med J*. 8 (29): 40-44.
- Kruse H. Indirect transfer of antibiotic resistance genes to man. *Acta Vet Scand*. 1999; 92: 59 – 65.
- Bauer AW, Kirby MM, Sherris JC, Truck M., (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 45: 493-6.
- Sahaab Farooq, Ifra Ghori and Faiqa Abdur Rub. Identification and multiple drug resistance of bacteria, isolated from pharmaceutical industrial effluent (Islamabad, Pakistan). *INT J CURR SCI* 2013. 6: E: 125-132.
- Ashour MS, Mansy MS, Eissa M E (2011). Microbiological Environmental Monitoring in Pharmaceutical Facility. *Egypt Acad Journal Bio Sci*. 3(1): 63- 74.
- Lateef A (2003). The Microbiology of a Pharmaceutical Effluent and Its Public Health Implications. *World J Microb Bio*. 20: 167-171
- Gale J, Narayan A (2012). Drug defying germs from India speed post-Antibiotic era. *Bloomberg Markets magazine*.

