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STUDIES ON ANTIMICROBIAL EFFICACY OF WATER PIPES COATED WITH TRICLOSAN

CUSTOMER : SAVOIR-FAIRE MANUFACTURING CO. PVT. LTD.

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1 Objective

- ✓ To study the anti microbial efficacy of the pipes coated with triclosan and compare it with control (uncoated pipe)
- ✓ To study the efficacy of the triclosan in preventing bio-filming of microorganisms in the inner walls of the pipes.

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2 Sponsor

Prayag Polytech

3 Product Descriptions

Product : 3 sets of Pipes designated A, B and C

Make : A & B (PPR) and C (CPVC)

Appearance : A and B are green coloured with an inner diameter of 13 mm.
C Pipes are Ivory coloured with an inner diameter of 11 mm.

Mfd. Date : Not mentioned

Batch No. : Not mentioned

4 Testing Facility

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5 Study team

5.1. Team Leader	**Dr Rajan Sharma** Medical Advisor Kumar Organic Products Limited Bangalore
5.1 Study Coordinator	**Prof .S. Gouse Basha** Head Department of Microbiology The New College
5.2 Study executives	**Prof . Anzaruddin** **Prof. Iqbal Kasim** Lecturers Department of Microbiology The New College
5.3 Study Monitor	**Mr. S. Gokul Shankar** Microbiologist. Kumar Organic Products Limited Bangalore

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6 Introduction

Water borne diseases represent the major proportion in food borne illness. The water used for domestic and drinking purpose should be free of al pathogens. The presence of coliform bacteria is an indicator of water pollution. Though there are several methods of water treatment to eliminate coliform and other pathogenic bacteria, the contamination of water after treatment s inevitable during transportation across the pipelines. Organisms like *Pseudomonas* sp., *Vibrio* sp etc. colonize the drinking water pipelines making the water unacceptable for human consumption. This problem of contamination of potable water in pipelines could be well handled by coating the inner surface of the pipelines with an antimicrobial agent which is non toxic but also effective in preventing colonization or bio-filming of bacteria. **Triclosan is an effective anti bacterial compound which is commonly used to coat the inner surfaces of pipelines used for potable water systems.**

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7 Study details

7.1 Study Number Micro/NC/ 09/04

8 Study Date

8.1 Start Date 17/08/2004

8.2 Finish Date 25/08/2004

9 Study Design

9.1 Methodology

9.1.1 Cultures

E.coli – a representative of coliform group

Vibrio sp. – a representative of enteric bacteria causing cholera and capable of colonizing and bio-filming in water pipelines

9.1.2 Media

Nutrient broth
MacConkey agar
TCBS agar

9.2.1 Experiment 1

The pipes A, B and C are steam sterilized at the start of the experiment.

The pipes A, B and C are closed at one side with a sterile lid. Sterile nutrient broth is filled into the pipes A, B and C under aseptic conditions. The other end is also tightly sealed with a sterile lid. The pipes are incubated at 37^0 C for a period of 48 hours. After 48 hours the pipes are opened and the turbidity of the broths in the pipes A , B and C are read colorimetrically using standard procedure.

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9.2.2 Experiment 2

The pipes A, B and C are closed on one side with a sterile lid. Sterile water is filled in to the pipes. Inoculate 10^3 cells of *E.coli* in to the water. Close the other end of the pipes aseptically. Incubate the pipes for a period of 7 days to allow the organism to colonize. After Incubation, plate the water sample in to MacConkey agar to enumerate the number of organisms.

The same procedure is carried out with *Vibrio* sp. and plating is done on TCBS agar.

9.2.3 Experiment 3

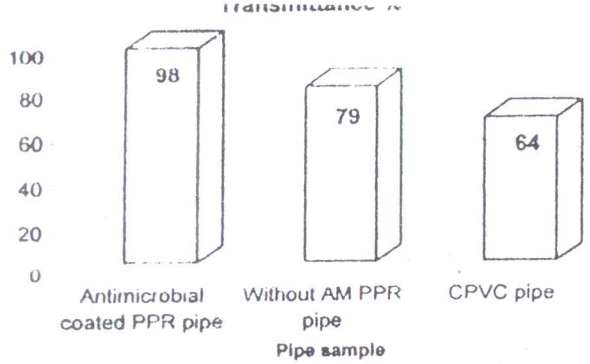
The pipes A, B and C are closed on one side with a sterile lid. Sterile water is filled in to the pipes. Inoculate 10^6 cells of *Vibrio* sp. in to the water. Close the other end of the pipes aseptically. Incubate the pipes for a period of 30 days to allow the organism to colonize and form a bio-film in the pipes. After 1 month swab 1 cm^2 area of the bio-film and transfer the culture in to sterile saline. Serially dilute the sample to the required dilutions and plate the dilutions in TCBS agar using pour plate technique. Calculate the load of organisms in the bio-film per cm^2.

9.3 Observation

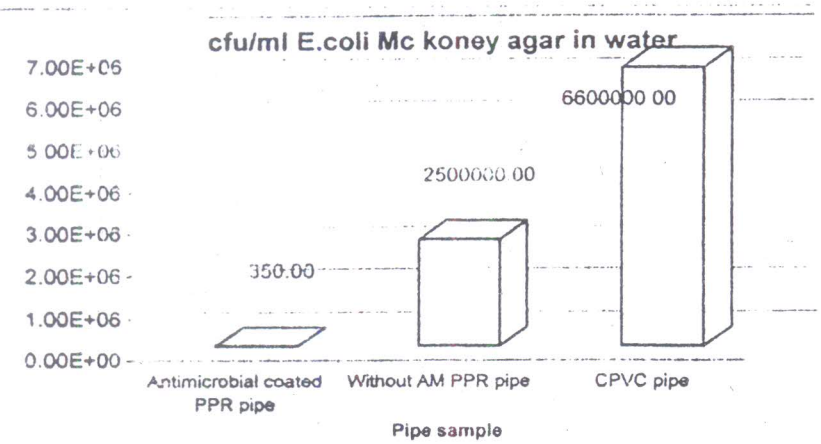
Experiment 1 : Higher the turbidity, more is the contamination (Growth of bacteria) – Read in the colorimeter as transmittance or OD.

Experiment 2 : The load of the microorganisms (*E. coli* and *Vibrio* sp.) is counted on the selective media by pour plate technique.

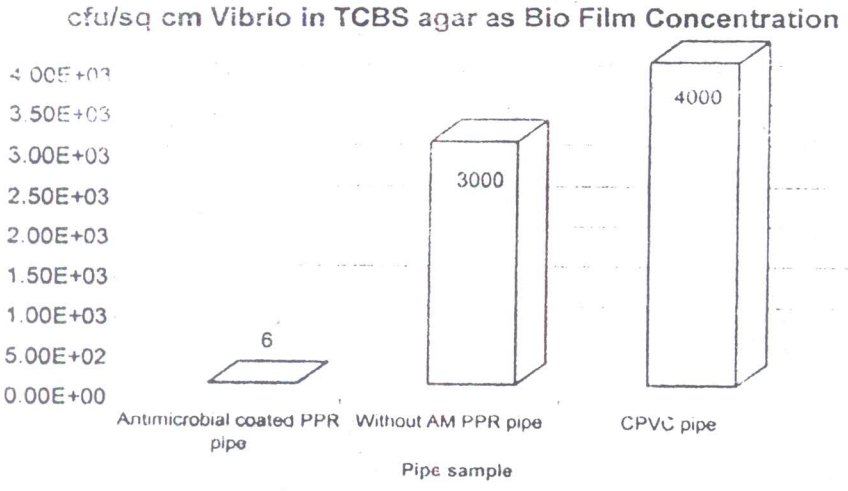
Experiment 3 : The load of the *Vibrio* sp. in the bio-film is counted on the TCBS agar by pour plate technique. If the active ingredient is efficient in preventing bio filming, the load would be less or nil in the coated pipes.



Experiment 2



Experiment 3



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10 Results

10.1 Experiment 1

The turbidity of the broth is high in the Pipes B and C, but the broth inside the pipe A is clear and comparable to the Control (un inoculated broth). Table 1.

10.2 Experiment 2

The load of *E.coli* and *Vibrio* sp. is high in the Pipes B and C, while the load of the organisms is less in the Pipes. Table 2.

10.3 Experiment 3

The load of bio-film is negligible/ nil (6 CFU/ cm^2 in the Pipe A, while the Pipes B and C had a load of $3x10^3$ and $4x\ 10^3$ CFU/ cm^2 respectively. Table 3.

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11 Tables

Table 1 – Experiment 1

S.No	Pipes	Growth (Recorded as turbidity)	Transmittancy (%)
01	A	Nil (-)	98
02	B	Present (+)	79
03	C	Present (++)	64

- Nil
+ Moderate Turbidity
++ High Turbidity

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Table 2 – Experiment 2

S.No	Pipes	Count of E. coli in MacConkey agar in cfu/ml	Count of Vibrio in TCBS agar in cfu/ml
01	A	35x 10	23x 10
02	B	25x 10^5	13x 10^5
03	C	66 x 10^5	34 x 10^5

cfu denotes colony forming units.

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Table 3 – Experiment 3

S.No	Pipes	Count of Vibrio in TCBS agar in cfu/cm2
01	A	6
02	B	$3x\ 10^3$
03	C	4×10^3

cfu denotes colony forming units.

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12. Conclusion and Discussion

The experiments indicate that the Pipe A has antimicrobial efficacy against the water borne pathogens like *E.coli* and *Vibrio* sp. It appears that the pipe A is coated with the antimicrobial which effectively would have prevented the colonization / multiplication of bacteria. Even if there is stagnation of water flow, it is not possible for the microorganisms to form a bio-film on the inner surface of the tubes as the anti microbial coated prevents the adherence of the micro organisms to the inner surface of the pipes. Further the experimental design suggests that since the Pipe A could prevent bio-film formation even when the water is stagnant for a period of one month, its performance would be more effective in pipes systems where there is a constant water flow as in case of the domestic water systems.

While the pipes B and C could not prevent either the multiplication of bacteria or their colonization on the inner surfaces of pipes. This clearly suggests either these pipes are uncoated with the anti microbial compound or the level of the anti microbial used is not sufficient enough to prevent the growth of microorganisms inside them.