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## Evaluation of *in-vitro* efficacy of a water disinfecting tablet, an alcohol-free sanitizer and a detergent of 'Mylchize' Kit (GEA) against environmental bacteria responsible for causing mastitis in milch animals

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### Abstract

India ranks first in terms of population of milch animals and amount of milk production. The quality of milk produced in India has direct relationship with knowledge level and adoption of scientific animal husbandry practices by livestock owners. Mastitis is the main udder/mammary gland affection of milch animals which reduces the quality of milk and has devastating economic impacts on livestock owners. The proportion of bacterial mastitis caused by environmental pathogens is less as compared to infectious mastitis. Moreover, the present era is facing challenges of antimicrobial resistance (AMR). Therefore, it is important to look after possible preventive strategies. A minor effort to reduce incidence of environmental mastitis can also lead to reduction in economic losses. With a similar view, the present investigation was carried out to identify common bacterial pathogens present in water trough, on milker's hands and on milking utensils. Additionally, a chemical-based water disinfectant (AQUA PRO), an alcohol-free hand sanitizer (DERMA PRO) and a utensil detergent (CIRCO BLUE) provided within Mylchize kit (a commercial farm hygiene kit) were evaluated for their *in-vitro* efficacy against the bacterial pathogens recovered from samples. The present paper highlights the results and places special emphasis on newer aspects to promote clean milk production for economic benefit of the livestock owners.

**Keywords:** mastitis, Mylchize kit, water disinfectant, alcohol-free hand sanitizer, utensil detergent, *in-vitro* efficacy

### Introduction

India holds a reputable position with regards to number of milch animals and quantity of milk production throughout the world. The total livestock population in India is 535.78 million as per the 20<sup>th</sup> Livestock Census and total milk production is 187.7 million tonnes (2019) as per data provide by the National Dairy Development Board (NDDB) [1, 2]. However, the milk produced in the country is not exported to many countries being unable to meet certain standards.

Mastitis is the major culprit which is responsible for deleterious effects on quality and quantity of milk causing huge economic losses [3]. It can be broadly classified as infectious mastitis and environmental mastitis [4, 5]. Here, the environmental mastitis is often caused by pathogens prevalent in areas where milch animals are being raised [6]. Additionally, hygienic practices (such as washing of hands, cleaning/disinfecting water sources and water troughs and cleaning of utensils with suitable detergents) are also important to reduce the incidence of environmental mastitis. For this, efforts can be made to produce economically feasible kits which can be provided to marginal farmers and commercial milch animal farm owners to improve hygiene standards. With a similar vision, an agency named Bharat Biogas Pvt. Ltd., Ahmedabad had prepared a commercial animal farm hygiene kit – Mylchize Kit which contains different components including a chemical-based water disinfectant (*viz.*, AQUA PRO), an alcohol-free hand sanitizer (*viz.*, DERMA PRO) and a utensil detergent (*viz.*, CIRCO BLUE) to be used at milch animal farms.

The components are chemical-based compounds and are yet to be explored for *in-vitro* efficacy against bacterial pathogens present in water sources, milker's hands and milking utensils. The present study was undertaken to evaluate *in-vitro* efficacy of AQUA PRO, DERMA PRO and CIRCO BLUE components of the Mylchize kit.

**Materials & Methods**

The present investigation was conducted at 05 commercial milch animal farms nearby Rajpur (Nava), Himmatnagar adopting similar hygienic practices, *i.e.*, not using hand sanitizers before and after milking an animal, not performing cleaning/disinfection of water using chemical compounds and not washing milking utensils with specific detergents.

Swab samples were collected from individual water troughs, milking utensils and milker’s hands at all farms (Figure-1). Later, all the swabs were subjected to bacteriological cultural isolation on different medias [including Nutrient agar, Blood agar, Eosin Methylene Blue (EMB) agar, MacConkey Agar and Brilliant Green Agar (BGA)] followed by staining with Gram’s stain and identification tests as per standard methods.



**Fig 1:** Collection of swab samples from (A) Water trough; (B) Milking utensils & (C) Milker’s hands

Additionally, standard solutions of AQUA PRO, DERMA PRO and CIRCO BLUE components of the Mylchize kit (Figure-2) provided by Bharat Biogas Pvt. Ltd., Ahmedabad were prepared and poured on plain antibiotic discs (HiMedia®) to evaluate their efficacy against bacteria

identified from all swab samples using Disc Diffusion Method on Mueller Hinton Agar (MHA). The *in-vitro* efficacy of different components was evaluated on the basis of Zone of Inhibition (ZoI) developed on MHA.



**Fig 2:** (A) Mylchize Kit; (B) AQUA PRO tablet; (C) DERMA PRO hand sanitizer & (D) CIRCO BLUE detergent

**Results and Discussion**

In the present study, five bacterial pathogens, *viz.*, *Staphylococcus spp.*, *Streptococcus spp.*, *Escherichia coli*, *Klebsiella spp.* and *Proteus spp.* were identified in swab samples collected from water source while the swab samples of milker’s hands and milking utensils revealed presence of *Staphylococcus spp.*, *Streptococcus spp.* and *Escherichia coli* only as shown in Table-1. The minimum & maximum ZoI of AQUA PRO against

*Staphylococcus spp.*, *Streptococcus spp.*, *Escherichia coli*, *Klebsiella spp.* and *Proteus spp.* were 13 & 31, 12 & 29, 11 & 20, 9 & 13 and 8 & 10, respectively. The minimum & maximum ZoI of DERMA PRO against *Staphylococcus spp.*, *Streptococcus spp.* and *Escherichia coli* were 13 &16, 12 & 14 and 10 & 13, respectively. The minimum & maximum ZoI of CIRCO BLUE against *Staphylococcus spp.*, *Streptococcus spp.* and *Escherichia coli* were 10 & 17, 11 & 19 and 9 & 13, respectively (Table-2).

**Table 1:** Different bacterial pathogens isolated from different swab samples

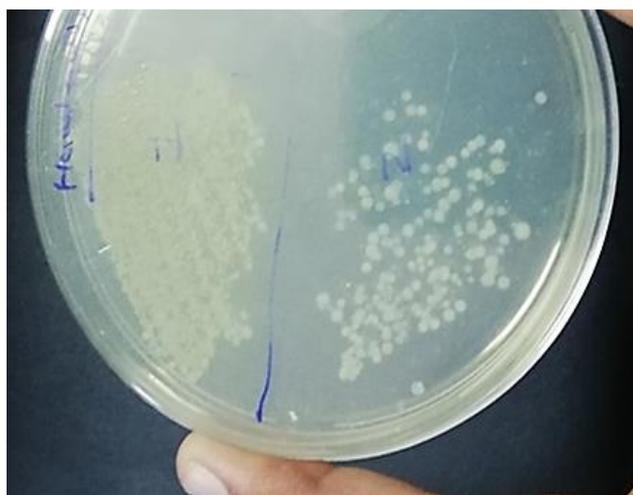
Bacteria isolated and identified	Type of swab samples collected from 05 farms					
	Milker’s hands		Utensils		Water	
	n=10	n=10	n=10	n=10	n=37	n=37
<i>Staphylococcus spp.</i>	6	60.00%	4	40.00%	32	86.49%
<i>Streptococcus spp.</i>	6	60.00%	3	30.00%	19	51.35%
<i>Escherichia coli</i>	4	40.00%	2	20.00%	21	56.76%
<i>Klebsiella spp.</i>	-	-	-	-	2	5.41%
<i>Proteus spp.</i>	-	-	-	-	1	2.70%

**Table 2:** Zone of Inhibition (ZoI; in mm) of different components against bacteria

Sample Component	Milker's hands DERMA PRO		Utensils CIRCO BLUE		Water AQUA PRO	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
<i>Staphylococcus</i> spp.	13	16	10	17	13	31
<i>Streptococcus</i> spp.	12	14	11	19	12	29
<i>Escherichia coli</i>	10	13	9	13	11	20
<i>Klebsiella</i> spp.	-	-	-	-	9	13
<i>Proteus</i> spp.	-	-	-	-	8	10

The AQUA PRO is a tablet provided within the Mylchize kit and it is claimed to have properties for disinfection of water. The tablet is required to be used @ One tablet (20gm) for one water tank (source tank) having >2000 lit. capacity as per recommendation provided in the kit. AQUA PRO contains sodium chloride, sodium sulfate, sodium carbonate, sodium chlorite, tetra sodium pyrophosphate and fumed silica as per the available description. Most of the farmers neither used any such water sanitation/disinfectant agents nor cleaned the tanks on daily basis before the project. Farmers became aware about water-based contamination of udder/teat canal and milk. This component may be subjected to extensive trials covering a greater number of samples, more bacterial isolates and wide geographic coverage for *in-vivo* application.

The DERMA PRO provided within the Mylchize kit is a hand sanitizer to be used by milkers before and after milking each animal as per the recommendation given in the kit. DERMA PRO does not contain alcohol. Additionally, the DERMA PRO hand sanitizer was also evaluated to observe immediate visible effects on bacterial colonies after application which revealed visible reduction in development of bacterial colonies as shown in Figure-3. Remarkably, none of the farmers/milkers used such a hand sanitizer before initiating the project. This component has not resulted in any hazards while using *in-vitro* which could be due to absence of alcohol content. DERMA PRO may be subjected to extensive trials covering a greater number of samples, more bacterial isolates and wide geographic coverage for *in-vivo* application.



**Fig 3:** Initial bacterial colonies ('1' on Petri dish) and noticeable reduction in bacterial colonies immediately after application of DERMA PRO ('2' on Petri dish)

The CIRCO BLUE provided within the Mylchize kit is a detergent to be used @ 65 gm/10 lit. of water to clean milking utensils as per the recommendation given in the kit. CIRCO BLUE contains silicates, phosphates, non-ionic surfactants and chlorine-based chemicals as per description given on the

product. Contamination of milk because of bacterial contaminants of utensils may be reduced if farmers use safe detergents on regular basis. Moreover, farmers do not have to purchase detergents separately as it has been provided within the kit itself. This component can replace commonly available costly detergents in the market because it is available within the kit itself. This component may be subjected to extensive trials covering a greater number of samples, more bacterial isolates and wide geographic coverage for *in-vivo* application. Based on the study, it could be observed that AQUA PRO, DERMA PRO and CIRCO BLUE components of the Mylchize kit have variable *in-vitro* efficacy against environmental bacteria isolated from different samples. Extensive trials including greater number of samples for a prolonged evaluation period may be conducted in future.

### Conclusion

The water disinfection agent (AQUA PRO), alcohol-free hand sanitizer (DERMA PRO) and detergent to clean milking utensils (CIRCO BLUE) provided within the Mylchize kit developed variable zone of inhibition against bacteria isolated from water source, milker's hands and milking utensils in the study. Such chemical-based compounds and kits are required to be explored at larger scale to prevent occurrence of environmental mastitis which will eventually benefit livestock owners and nation economically.

### Conflict of Interest

Authors declare no conflict of interest with regards to funding. The project was funded by Bharat Biogas Pvt. Ltd., Ahmedabad and approved by the Hon'ble Vice Chancellor, Kamdhenu University, Gandhinagar. Necessary permissions were received before initiating the work.

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