

Research article

Bactericidal, protozoacidal, and algicidal efficacy of Sanodrink: a complete water sanitizer in poultry farmLabanyamoy Kole¹, Sujan Maity¹, Nensina Murmu², Sananda Dey², Rimee Dutta¹, Biplob Giri²¹RR Animal Health Care Ltd. Adarsh Nagar Colony, Nagole, Hyderabad-500068, India²Department of Physiology, University of Gour Banga, Mokdumpur, Malda 732103, India

(Received: December 2021

Revised: July 2022

Accepted: August 2022)

Corresponding authors: **Labanyamoy Kole**. Email: dr.kole@rrahc.in; **Biplob Giri**. Email: bgiri.emscl@gmail.com**ABSTRACT**

Introduction and Aim: The growth in poultry industries due to increasing demand for meat and egg production has set off the establishment of various poultry farms on a commercial basis. But with an increase in demand for production from poultry farms, the need for maintenance of a healthy and clean environment has also become a necessity for disease free and quality production. As these pose a greater challenge in the production management, the development of a versatile compound that could be beneficial in overcoming all the microbial challenges faced in a poultry farm is also necessary.

Materials and Methods: Sanodrink is a complete water sanitizer that is a highly effective bactericidal, protozoacidal and algicidal agent which could be used for the drinking water storage tanks of poultry farm. It is effective in killing all the commonly encountered microbes like *E. coli*, *Giardia spp.* etc., and algae that are commonly found in the poultry farm water tank.

Results: It contains quaternary ammonium compounds which are microbicidal and potent inhibitors of both pathogenic and non-pathogenic bacterial, algal, and protozoal growth and survival. Quaternary ammoniums are also good surfactants as they lower the superficial tension of water. Furthermore, Sanodrink maintains its action in hard water also and in the presence of organic matter.

Conclusion: Our present study aims at evaluating the microbicidal efficacy of Sanodrink as a water sanitizer that can be used at poultry farms.

Keywords: Poultry farm disinfectant; antimicrobial; bactericidal; water sanitizer; protozoacidal; algicidal.

INTRODUCTION

Poultry farming is one of the commonest types of animal husbandry practiced in India along with dairy farming, sheep and goat rearing and aquaculture. Poultry farms contribute to a greater part of the Indian economy consisting of 45% of the total meat production and ranking 7th worldwide. It ranks 3rd in the world for total egg production of which chicken accounts 95% of total eggs produced (1,2). The major challenges that poultry industry in our country faces include product quality, safety standards, nutrition and hygienic management. Thus, maintenance of a healthy and well-sanitized environment in poultry farms is inevitable for both the flock and the workers for a better and disease-free production (3). Besides, organic materials composing of fecal matters, droppings and litters from the birds, their feeds and feathers and dust aerosols harbors a huge microbial load that might consist of pathogenic bacteria, fungi, spores and toxic gases.

Poultry farm microbiota mainly consists of certain Gram-positive bacteria including *Bacillus*, *Staphylococci sp.*, *Enterococcus sp.*, *Actinomyces* and Gram-negative bacterial species including

Escherichia coli, *Salmonella sp.*, *Pseudomonas sp.*, *Shigella sp.*, *Campylobacter sp.*, *Enterobacter sp.* and *Klebsiella sp.* (4). This might sum up in spreading diseases among the fowl and sustained unpredicted death and incurred losses. Among the fungi species *Aspergillus penicillioides*, *Eurotium chevalieri*, *Mucor fragilis*, *Absidia glauca*, *Fusarium sp.*, *Trichosporon sp.*, *Alternaria sp.*, *Geotrichum sp.* and *Cladosporium sp.* and pathogenic variants *Aspergillus fumigatus* inhabit the poultry environment. Pathogenic yeasts including *Candida albicans* and *Cryptococcus neoformans* and parasites such as *Cryptosporidium* and *Giardia spp.* are also found in the poultry microflora. These gram-negative pathogenic bacteria and pathogenic fungi release endotoxins and mycotoxins respectively which further worsen the poultry sanitation (5). Endotoxins which form a part of the outer membrane of gram-negative bacteria are released into the air when a particular bacterial cell ruptures and thus comprise a major component of the organic dust present in a poultry farm. The endotoxins can exhibit proinflammatory responses and induce respiratory disorders including asthma and organic dust toxic syndrome (ODTS). These endotoxin levels were found to be higher in the poultry farm dust as compared to the normal threshold levels (6). Not only

the flock but humans as compared to other mammals are more susceptible and at a greater risk of being infected with zoonotic diseases. The pathogens and toxins present in poultry air, litter, droppings, feathers and livestock itself can easily be transmitted to humans through inhalation, ingestion of infected poultry products, during transport, slaughtering for meat and injuries. Certain studies have already indicated a high prevalence of chronic bronchitis, obstructive pulmonary disorders and avian influenza infection among poultry workers (7-9). Since water is the most suitable medium for most of the microorganisms to enter the living body and establish themselves to the host, water sanitation and hygiene should be among the most important aspects in terms of bio-security and overall productivity in poultry farms. It is important to use a sanitized water source for the preparation of poultry feed and drink and also in the disinfection of farm. A well must be sanitized twice a year irrespective of being a new or an old one which is used as water source in many poultry farms. Birds also consume twice to thrice the amount of water compared to feed and hence a prime focus should be on the assessment of water quality (10). Even the farms with good management practices find it difficult to keep the diseases at bay owing to multifactorial dimensions in water quality management. Water sanitizers and disinfectants are the most commonly used tools for maintaining water quality and keeping microbial load to a minimal level.

Quaternary ammonium compounds (QACs) are cationic surfactants commonly used as disinfectants in hospitals. Though QACs are poor detergents, they easily ionize in solutions to produce cations and hence contribute to their surface-active property and better stability. The Centers for Disease Control and Prevention (CDC) of United States has defined QACs as low-level disinfectants which are bactericidal, fungicidal, virucidal for certain enveloped viruses and effective against mycobacteria (11). Bacterial cell membrane is composed of phospholipid bilayer that imparts a negative charge to its surface and maintains the molecular integrity of the membrane. The bactericidal property is due to the cationic surface-active nature of QACs that causes damage to the bacterial cell membrane by disruption of the molecular interactions and cellular surface proteins leading to inactivation of membrane functions and energy producing enzymes (12). Of the many QACs available, the commonly used disinfectants are the di-alkyl-quaternaries namely di-decyl- di-methyl-ammonium chloride (DDAC) and di-octyl- di-methyl- ammonium chloride (DODAC). Research studies on efficacy of DDAC on *E. coli* have found the minimum inhibitory concentration (MIC) value to be around 1-1.3 mg/L (13,14). Other prevalent QACs include alkyl- di-methyl-benzyl ammonium chloride or benzalkonium chloride (13). In this study, we have shown the efficacy of our QACs formulated water

sanitizer, Sanodrink, as a potent antimicrobial and bactericidal compound that can be used in low concentrations. During our standardization of manufacturing disinfectants, we assayed the performance of different batches. We uncovered different bacterial loads associated with poultry farms and this information prompted us to develop an effective sanitizer specially formulated for the poultry. In order to develop the product a series of experiments were conducted and different factors were thoroughly examined to prove the superior efficacy of Sanodrink.

MATERIALS AND METHODS

Bacterial culture

The study was designed to test the bactericidal efficacy of Sanodrink using *Escherichia coli* as a model system.

Samples collection for antimicrobial activity

Surface swab was collected from different poultry farms (Layer and Broilers) as the source of microorganism.

Protozoan culture

Antiprotozoal efficacy of Sanodrink was assessed by using *Giardia lamblia* as the model organism.

Sanodrink water sanitizer composition

Each 1000 ml of Sanodrink water sanitizer was formulated of DDAC (22.50gm), DODAC (22.50gm), Octyl-decyl di-methyl ammonium chloride (ODDAC) (45.00gm), Benzalkonium chloride IP (60gm) and Azorubine (0.30gm). Sanodrink has been created as complete sanitization of water that prevents the growth of bacteria, algae and giardia in pipelines and water storage tanks. It is non-toxic and tasteless in recommended dosage.

Assessment of kinetics and dilution dependent bactericidal activity of Sanodrink

Pre-determine counts (CFU) of *E. coli* were incubated in Mueller-Hinton Broth (MHB, Oxoid, CM0405) at 37°C (18–20 h) for different time and cultures were inoculated overnight to observe the number of CFU per plate. Counted CFU/ml were compared against the dilution of media containing Sanodrink by standard microbiological efficacy evaluation method (according to United States Pharmacopeia. Retrieved 2015) (15). In order to standardize the inoculums, different CFUs (starting from 100 to 10⁶ CFU/ml) of *E. coli* were used for the enumeration of bactericidal activity.

Determination of pH dependent bactericidal activity of Sanodrink

To determine the bactericidal activity of Sanodrink using *E. coli* as model system, pre-determine counts (CFU) of *E. coli* were incubated in different pH containing water for 2 hours and cultures were

inoculated overnight in an agar plate to observe the number of CFU per plate. Experiment was carried out according to United States Pharmacopeia. Retrieved 2015 (16).

Antimicrobial activity of Sanodrink from poultry farm

Surface swab was collected from different poultry (Layer and Broilers) as the source of microorganisms. All antimicrobial disks and Mueller-Hinton agar plates (90 mm) were prepared according to the methods provided by manual (12). Different dilutions of Sanodrink soaked disks were placed on to agar plate which was pre-inoculated with saturated culture using swab taken from poultry farm. All zones of inhibition were measured. Standard antibiotic was used as positive control.

Antibacterial activity of Sanodrink from sample collected from different farm

Samples were collected from different poultry farm. Diluted Sanodrink was added using the collected water and incubated at 37°C incubator for 2 hrs. Total content was centrifuged and plated onto agar plates and incubated overnight. Bacterial counts (CFU) were tabulated and compared to determine the antibacterial activity of Sanodrink.

Preparation of the protozoan culture

Giardia lamblia isolates trophozoites (GS/H7) (17) were grown in screw-cap glass culture tubes (Fisher Scientific) maintaining a neutral pH (pH 7.0) anaerobically in modified TYI-S-33 complete culture media (supplemented with 0.05% bovine bile (Sigma-Aldrich) and 10% heat-inactivated bovine serum (Gibco) (18,19). 85-90% of the culture tube volume was filled and incubated at 37°C (no stirring conditions) under low pressure conditions reach. Cultures were maintained by sub-culturing trophozoites (2×10^5 per tube) three times a week. The cultured trophozoites were dislodged from the wall of the culture tube by cooling the cultures in ice for 20 minutes and used for assays.

Protozoacidal effect of Sanodrink and determination of minimum lethal concentration (MLC)

To determine MLC of Sanodrink against *G. lamblia*, trophozoites were seeded in a 96-well culture plates in duplicates (Corning Incorporated). Cultured trophozoites were transferred to 8 ml culture tubes (Fisher Scientific) for proliferation in with or without Sanodrink in different dilutions followed by the addition of 10,000 organisms in 10 µl of growth medium. 100 µM Metronidazole was dissolved in DMSO and diluted with the culture media which was used as a positive control, and the media-diluted DMSO was taken as a negative control. All culture plates were incubated at 37°C in an anaerobic environment and observed under the microscope after

3 days to analyze survival, mobility, and attachment of *G. lamblia* trophozoites. The plates were chilled using ice for about 30 minutes, and the total contents of similar treatment wells were transferred into the different 8 ml culture tubes containing complete growth media. The culture tubes were kept in an anaerobic environment at 37°C for 3 days, observed under the microscope and the percentage of growth was calculated.

Algicidal effect of Sanodrink

Algicidal activity was assessed against three representative Cyanobacterial algae, *M. aeruginosa*, *S. obliquus*, and *C. Pyrenoidosa* at different time levels and different concentrations. After 7 days of inoculation of the three algae species, the density variation was noted continuously. Algal cultures were collected locally from water bodies, clonal axenic cultures were regularly maintained using modified SWM-III medium prepared using water as the base. The water used was filtered through a 0.22 µ filter (Corning) and stored in the dark at 4°C. Algal cultures were maintained and grown at 20°C under an illumination of 35 microeinsteins m⁻² s⁻¹ on a 12-h light–12-h darkness schedule (20). Wells of control samples contained only unialgal culture. The CuSO₄ solution and Diuron were used as a reference.

RESULTS

Bactericidal activity of Sanodrink

A wide range of Sanodrink concentrations (1:5000 to 1:30000) was taken in 5 ml of media and incubated for 2 hrs prior to the inoculation of bacteria onto the plate. There was no growth up to 1: 20000 Sanodrink containing media in a repeated observation. But incubation of Sanodrink for one hour at a dilution of 1:30000 did not halt the complete growth of *E. coli* and leaving average 16.7 ± 0.7 (N=3) CFU/ml of *E. coli* in the experimental culture plate. Dilution dependent and time dependent antimicrobial activities of Sanodrink were represented in Figure-1 and Fig.2 (Table1 and 2). Sanodrink thus exhibits a dose dependent and time dependent bactericidal efficacy even at lower dilution (1:10000). Surprisingly, at 5 min of exposure bactericidal effects can be identified.

Bactericidal activity of Sanodrink at different pH

From the experimental data it is evident that Sanodrink is very much effective at wide range of pH exposure (pH 7-12). The control buffers showed average 10³ CFU/ml *E. coli* counts. But there was no colony found after 2 hours of incubation with Sanodrink. Sanodrink works well even at 1:10000 dilutions (1 ml/10 lit of water). However, in the pH dependent study we have tested both at 1:10000 and at 1:20000 dilution of Sanodrink to show the effectivity of Sanodrink in lower working concentration during pH dependent study (Fig. 3).

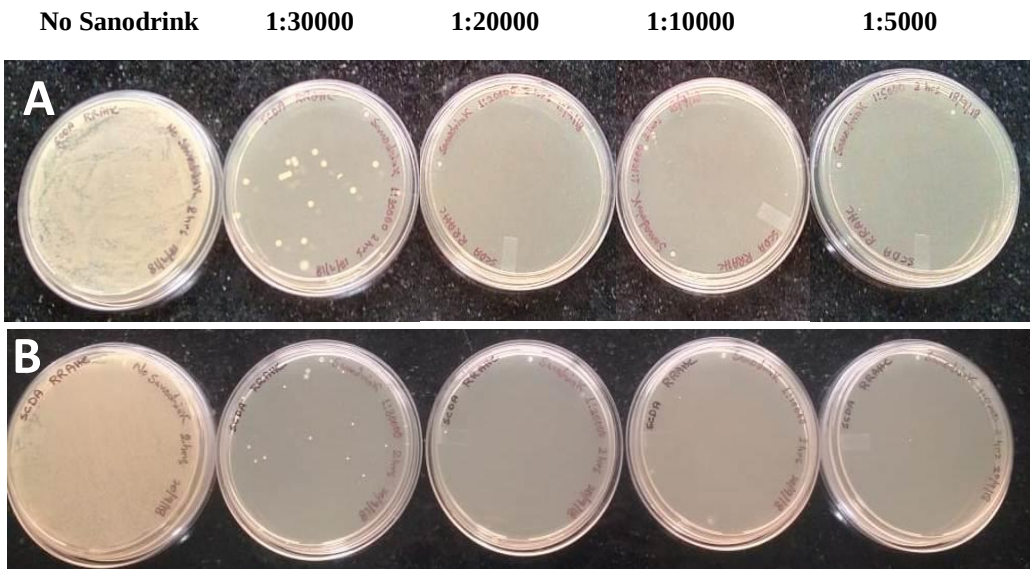


Fig. 1: Dose dependent Bactericidal activity of Sanodrink: Demonstration of colony count (CFU/ml) at different dilution of Sanodrink using *E. coli* as model system

Table 1: Determination of *E. coli* colony count (CFU/ml) at different dilutions of Sanodrink

Sanodrink dilutions		CFU / ml			Average	SD
		Experiment 1	Experiment 2	Experiment 3		
A	0.0	>10 ⁶	>10 ⁶	>10 ⁶	>10 ⁶	0.0
E	1:5000	0.0	0.0	0.0	0.0	0.0
D	1:10000	0.0	0.0	0.0	0.0	0.0
C	1:20000	0.0	0.0	0.0	0.0	0.0
B	1:30000	17	15	18	16.7	0.7

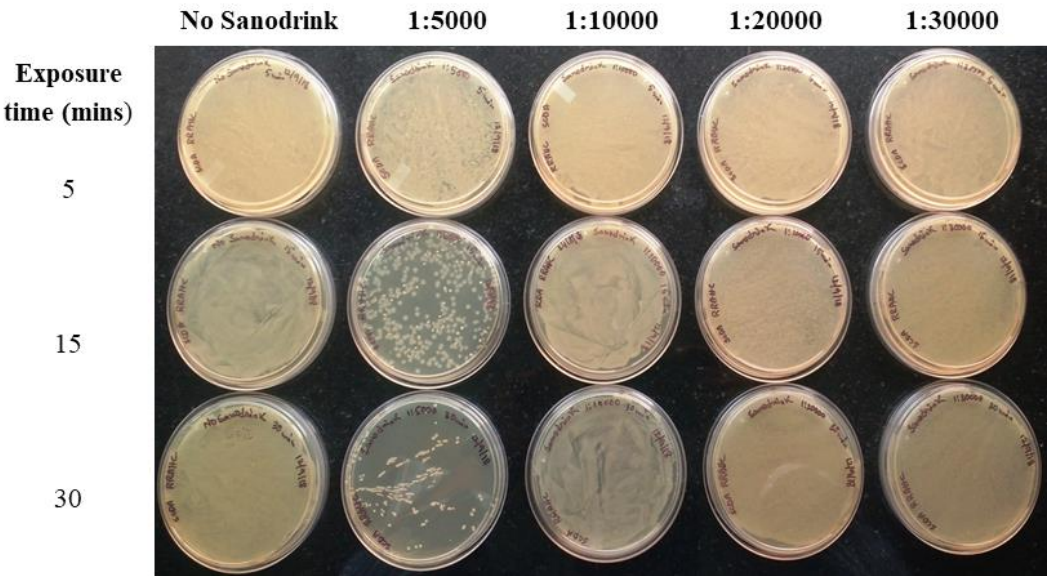


Fig. 2: Time dependent Bactericidal activity of Sanodrink: Effect of Sanodrink at different dilutions (No Sanodrink, 1:5000, 1:10000, 1:20000, 1:30000) at different time points (5min, 15 min & 30 mins).

Table 2: Bactericidal activity of Sanodrink at different dilutions and different time point

Exposure with Sanodrink	Control (CFU/mL)	1:5000 (CFU/mL)	1:10000 (CFU/mL)	1:20000 (CFU/mL)	1:30000 (CFU/mL)
5 min	>10 ¹⁰	<10 ⁴	<10 ⁶	<10 ⁷	<10 ⁸
15 min	>10 ¹⁰	5000	<10 ⁵	<10 ⁶	<10 ⁶
30 min	>10 ¹⁰	1500	<10 ⁴	<10 ⁵	<10 ⁵

Bactericidal activity of Sanodrink from the sample (swab) collected from poultry farm bed

Collected sample was used to observe bactericidal efficacy of Sanodrink. It is evident that Sanodrink showed a dose dependent inhibition of microbial load. This *in vitro* experimental model may not be

ideal to evaluate potency of a water sanitizer for a poultry farm but it is evident that Sanodrink has an accountable inhibitory effect on bacterial load of the farm sample. Hence, Sanodrink could be an effective antimicrobial agent that can be used in poultry farms (Fig.4; Table 3).

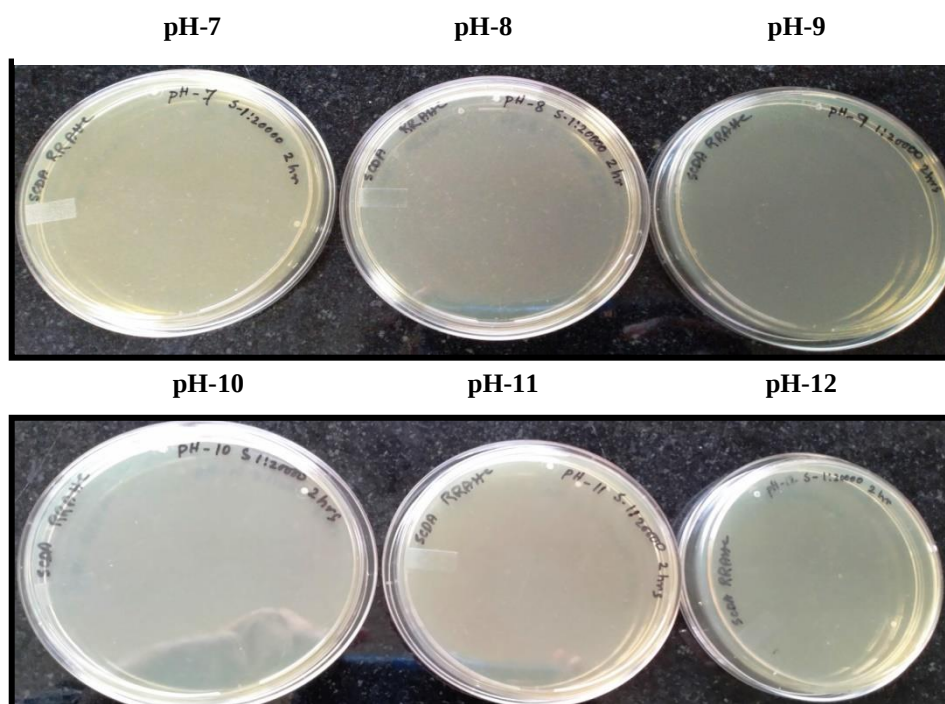


Fig. 3: Bactericidal activity of Sanodrink at different pH: Bactericidal effect of Sanodrink at 1:20000 dilution at different pH (pH-7, pH-8, pH-9, pH-10, pH-11 & pH-12) of buffers with 10^3 CFU/ml microorganisms. The growth in control plates in respective pH without Sanodrink was uniform (data not shown).

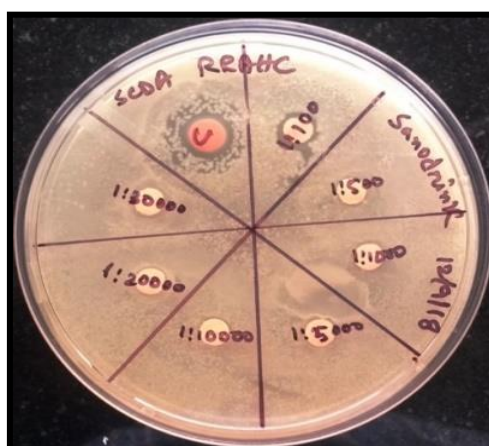


Fig. 4: Effect of Sanodrink in zone inhibition using the sample collected from representative poultry farm in agar diffusion assay

Table 3: Determination of zone of inhibition of different dilutions of Sanodrink using agar diffusion assay.

Sanodrink Dilution	A Crude	B 1:100	C 1:500	D 1:1000	E 1:5000	F 1:10000	G 1:20000	H 1:30000
Zone(mm)	22	12	8	7	6	5	5	5

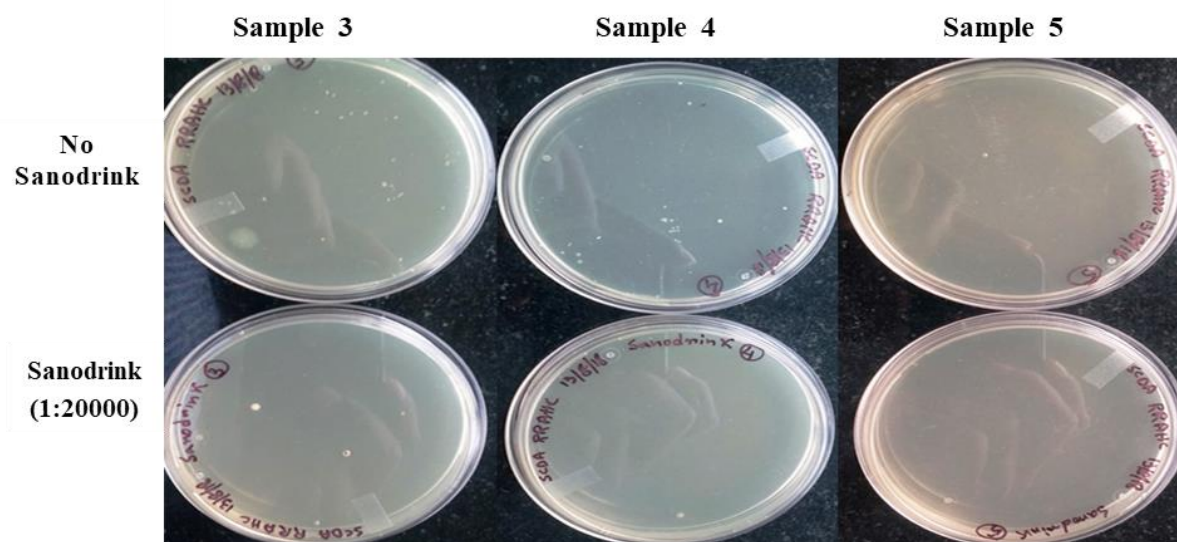


Fig. 5: Antimicrobial effect of Sanodrink in water: Antimicrobial effect of Sanodrink (1:20000) in water sample collected from representative farms (Sample 3, 4, 5) after 30 min of incubation. Data was calculated by counting CFU/ml as compared with the control (without Sanodrink)

Bactericidal activity of Sanodrink from the water sample collected from different farm

Different bacterial load was observed at different farms. Irrespective of the sources of sample, Sanodrink effectively reduced the bacterial load. Available data generated from the sample collected from farm strongly supports the efficacy of Sanodrink. (Fig. 5, Table 4).

Table 4: Comparing CFU/ml of water samples from farms against control (without Sanodrink)

Sample collected from farm	Without Sanodrink (CFU/ml)	Sanodrink (1:20000) (CFU/ml)
Sample 3	500	10
Sample 4	300	0
Sample 5	200	0

Protozoacidal effect of Sanodrink

There is a clear dose dependent growth pattern was observed across the Sanodrink treated sample. The lowest percentage of growth was observed at 1:5000 dilution of Sanodrink treated water when samples were incubated for 3hrs. Subsequently, different percentage of growth were furnished as shown in the Table-5.

Table 5: Protozoacidal effect of different doses of Sanodrink

Sanodrink dilutions	Growth %	±SD
Positive control	0	0
Untreated control	100	0
1:30000	78.5	2.64
1:20000	52.5	5.56
1:10000	29.75	2.5
1:5000	2.62	1.25

Algicidal effect of Sanodrink

To evaluate the algicidal activity we have incubated Sanodrink diluted water system and found effective algicidal properties against a variety of algal species, indicating that Sanodrink has a wide killing capacity of algal species. The representative experiment also showed that Sanodrink showed antialgal activity in time dependent fashion (Table-2). We have assessed capacity of antialgal activity of Sanodrink and its other two products (Competitor I & II) available in the market. Surprisingly, Sanodrink provided a potential promising algicidal capacity over the respective competitors in dose and time dependent fashion (Tables 6 and 7).

Table 6: Algicidal activity of Sanodrink (1:5000) against time (5 min to 3 h)

	Control 0 min	5 min	10 min	15 min	20 min	30 min	1 h	2 h	3 h
Sanodrink	0%	12%	31%	46%	62%	84%	96%	100%	100%
Competitor I	0%	7%	18%	28%	45%	52%	65%	70%	72%
Competitor II	0%	10%	22%	30%	42%	49%	58%	65%	70%

Table 7: Algicidal activity over different dilution of Sanodrink after 15 min incubation

	Control	1:30000	1:20000	1:10000	1:5000
Sanodrink	0%	12%	23%	35%	47%
Competitor I	0%	5%	16%	21%	30%
Competitor II	0%	18%	20%	24%	28%

DISCUSSION

The poultry industry is one of the flourishing one and growing ever since. With lot many cases arising regarding the biosafety of workers and transmission of zoonotic diseases due to unhygienic management and practices in poultry farms, several measures have been employed to disinfect the farm (21). The farm waste which mostly consists of flock fecal matter, droppings, feathers, feeds and litters contribute to the overall microbial load consisting of pathogenic bacteria, fungi and yeast. To establish a clean hygienic environment in the poultry farm and ensure quality production, antibiotics, nutritional supplements, vaccines, sanitizers and acidifiers have been used (22,23). Water which plays an important role in poultry farms can easily be contaminated with microorganisms and transmitted to the livestock and human system through inhalation and ingestion. It is thus important to provide a microbial contamination free water source. Certain chemicals such as hydrogen peroxide and chlorine are used as water disinfectant and purifiers but these compounds are often not compatible in combinations and are temperature sensitive, making them less stable and less efficient. Sanodrink, a formulated water sanitizer which contains quaternary ammonium compounds (DDDAC, DODAC, ODDAC, benzalkonium chloride and azorubine) have been found to be highly effective against bacterial growth and has cumulative properties including bactericidal, protozoacidal and algicidal. Present study indicates that Sanodrink exhibits a dose dependent bactericidal activity even at low exposure time of 5 min in a wide pH range (pH 7- pH 12) at very low concentrations of dilution 1:10000 (1 ml/10 lit of water). Sanodrink also showed inhibitory effect against microbial growth of samples collected from poultry farm as observed in the assessment of antimicrobial activity using disk diffusion method. In the colony count assay, water samples collected from different poultry farms showed negligible CFU/ml as compared to the control plates without Sanodrink at dilutions 1:20000 indicating that Sanodrink can act as a potent microbicidal agent. The rationale for designing pH dependent study is to cover the pH variability of the drinking water exposed to different environmental conditions for different animal farm industries. It is evident that different biotic environmental conditions created due to differential growth of different microorganisms in exposed water which can change the pH of the water. To examine protozoacidal effect of Sanodrink *G. lamblia* trophozoites were used as a model system. It has been found that Sanodrink significantly reduces the percentage of growth after 3 hr of treatment and minimum lethal concentration (MLC) was noted at 1:5000 dilution. Sanodrink also showed algicidal effects too. Our experimental data revealed that, Sanodrink, provides a potential promising algicidal capacity over the respective commercial competitor

products in dose and time dependent way. From the present laboratory, another microbicidal disinfectant of the marketed agent RH5+ has been evaluated and found to be effective against different multidrug resistant bacteria and showed promising microbicidal effect against the microbes found in representative poultry bed soil (24).

CONCLUSION

The present study provides an insight that, our QACs formulated water sanitizer, Sanodrink has superior potency to lower different types of microbial load associated with farms and it can be used in poultry farm as an effective bactericidal, protozoacidal and algicidal agent as well as for water disinfection and sanitization.

ACKNOWLEDEMENTS

Authors are thankful to the R.R Animal Health Care Ltd, Adarsh Nagar Colony, Nagole, Hyderabad and University of Gour Banga, Malda for providing necessary infrastructure and support.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Chatterjee, R.N., Rajkumar, U. An overview of poultry production in India. Indian J Anim Sci. 2015; 54(2): 89-108.
- National Action Plan for Egg & Poultry- 2022. Department of Animal Husbandry, Dairying & Fisheries Ministry of Agriculture & Farmers Welfare Government of India. Available from: <https://www.dahd.nic.in/sites/default/files/Seeking%20Comments%20on%20National%20Action%20Plan-%20Poultry-%202022%20by%2012-12-2017.pdf>.
- Pandey, M. Doubling farmer's income-issues and challenges in egg production by Manjari Pandey. Life Sci. leaflet. 2019; 17:10-15.
- Skóra, J., Matusiak, K., Wojewódzki, P., Nowak, A., Sulyok, M., Ligocka, A., et al., Evaluation of microbiological and chemical contaminants in poultry farms. Int. J. Environ. Res. Public Health. 2016; 13(2): 192(1-16).
- Witkowska, D., Sowińska, J. Identification of microbial and gaseous contaminants in poultry farms and developing methods for contamination prevention at the source. Poultry Sci. 2017; 51-72.
- Huneau-Salaün, A., Le Bouquin, S., Bex-Capelle, V., Huonnic, D., Balaine, L., Guillam, M.T., et al., Endotoxin concentration in poultry houses for laying hens kept in cages or in alternative housing systems. Br. Poult. Sci. 2011;52(5):523-530.
- Zuskin, E., Mustajbegovic, J., Schachter, E.N., Kern, J., Rienzi, N., Goswami, S., et al., Respiratory function in poultry workers and pharmacologic characterization of poultry dust extract. Environ. Res. 1995;70(1):11-19.
- Viegas, S., Faisca, V. M., Dias, H., Clérigo, A., Carolino, E., Viegas, C. Occupational exposure to poultry dust and effects on the respiratory system in workers. J. Toxicol. Environ. Health Part A. 2013;76(4-5):230-239.
- MacMahon, K.L., Delaney, L.J., Kullman, G., Gibbins, J.D., Decker, J., Kiefer, M.J. Protecting poultry workers from exposure to avian influenza viruses. Public Health Rep. 2008;123(3):316-322.
- McCreery, D. Water consumption behavior in broilers. University of Arkansas; 2015. <https://scholar.google.com/>

- scholar?hl=en&ssdt=0%2C5&q=McCreery+D+Water+Consumption+Behavior+in+Broilers+University+of+Arkansas%3B+2015&btnG=.
11. Rutala, W.A., Weber, D.J. Disinfection, sterilization, and control of hospital waste. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 2015:3294-3309.
 12. McKeen, L. Introduction to food irradiation and medical sterilization. The Effect of sterilization on plastics and elastomers. 2012:1-40.
 13. Fu, E., McCue, K., Boesenberg, D. Chemical disinfection of hard surfaces—household, industrial and institutional settings. In Handbook for cleaning/decontamination of surfaces 2007: 573-592. Elsevier Science BV.
 14. Yoshimatsu, T., Hiyama, K.I. Mechanism of the action of didecyldimethylammonium chloride (DDAC) against *Escherichia coli* and morphological changes of the cells. Biocontrol Sci. 2007;12(3):93-99.
 15. United States Pharmacopeia. 61 Microbiological examinations of nonsterile products: microbial enumeration tests. Rockville: United States Pharmacopeial Convention, 2016.
 16. Wayne, P.A. National committee for clinical laboratory standards. Performance standards for antimicrobial disc susceptibility testing. NCCLS document M27-A. 2002; 12: 1-53.
 17. Nash, T.E., McCutchan, T., Keister, D., Dame, J.B., Conrad, J.D., Gillin, F.D. Restriction-endonuclease analysis of DNA from 15 *Giardia* isolates obtained from humans and animals. J. Infect. Dis. 1985;152(1):64-73.
 18. Chen, C.Z., Kulakova, L., Southall, N., Marugan, J.J., Galkin, A., Austin, C.P., et al., High-throughput *Giardia lamblia* viability assay using bioluminescent ATP content measurements. Antimicrob Agents Chemother. 2011;55(2): 667-675.
 19. Keister, D.B. Axenic culture of *Giardia lamblia* in TYI-S-33 medium supplemented with bile. Trans. R. Soc. Trop. Med. Hyg. 1983;77(4):487-488.
 20. Lee, S.O., Kato, J., Takiguchi, N., Kuroda, A., Ikeda, T., Mitsutani, A., et al., Involvement of an extracellular protease in algicidal activity of the marine bacterium *Pseudoalteromonas* sp. strain A28. Appl. Environ. Microbiol. 2000; 66(10):4334-4339.
 21. Gerber, P., Opio, C., Steinfeld, H. Poultry production and the environment—a review. Animal production and health division, Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla. 2007;153: 1-27.
 22. Agyare, C., Boamah, V.E., Zumbi, C.N., Osei, F.B. Antibiotic use in poultry production and its effects on bacterial resistance. Antimicrob Resist-A Global Threat. 2018:33-51. DOI: 10.5772/intechopen.79371
 23. Manwar, S.J., Awandkar, S.P., Munde, V.K. and Waskar, V.S. Effect of different sources of drinking water and commercially treated water on the performance of broiler chicken. Indian J. Poult. Sci. 2012;47(1):60-63.
 24. Banerjee, J., Dey, S., Rizowana, H.N., Samanta, S., Dutta, R., Kundu, P. et al., Evaluation of antimicrobial activities of commercial product (RH5+) and its competitor products against clinically isolated bacterial strains and bacterial population present in poultry bed. Int. J. Health Sci. Res. 2019; 9(3): 105-118.