International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

*Original Research Article*

# Evaluation of Antimicrobial Activities of Commercial Product ($RH^{5+}$) and its Competitor Products against Clinically Isolated Bacterial Strains and Bacterial Population Present in Poultry Bed

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## ABSTRACT

The gradual increase of antibiotic resistance in microbes has become the greatest health threat for human beings and animals. Disinfectants which are generally used in the farming industry effected on animal health and reduced productivity. Therefore, the aim of the present study was to evaluate antimicrobial activity of newer marketed disinfectant product $RH^{5+}$ and its three competitor products (D1, D2 and D3) against clinically isolated multidrug resistant bacteria *Escherichia coli (EC-1), Pseudomonas aeruginosa (PA-1), Klebsiella oxytoca (KO-1), Proteus vulgaris (PV-1),* and *Staphylococcus aureus (SA-1) and* also investigate their efficacy against the bacteria found in Poultry bed. $RH^{5+}$, D1, and D3 showed excellent antimicrobial efficacy against clinical isolates as well as against the bacterial populations found in the soil of poultry bed. The MIC, MBC values were noted at very low concentration (1µl/mL) of the tested compounds and inhibition zones were also significant as observed from agar diffusion assay. The $RH^{5+}$, D1 and D3 also reduced bacterial colony formation from poultry bed soil. The D2 did not show any antimicrobial property against clinical isolates up to 100µl/mL dose. The quaternary ammonium, glutaraldehyde and other active ingredients of the commercial compounds present in those formulations were noted as potent bacteriostatic against multidrug resistant bacteria and against the bacterial populations found in the poultry bed soil. Thus, it was concluded that the commercial products i.e. $RH5^{+}$ and competitor products could be used as an effective disinfectant in poultry farm to eliminate the pathogenic infections of the birds.

***Keywords:*** Commercial antimicrobial, RH5+, minimum inhibitory concentration, minimum bactericidal concentration, poultry bed

## INTRODUCTION

In recent time, multidrug resistant bacteria are a great threat to the world. Now-a-days, it has been found that abundant use of antibiotics without maintaining the real prescription policies develops massive emergence of multidrug-resistant bacterial strains. [1] This is not only the biggest risk for human health but also producing a remarkable threat to animals. According to the research reports, severe crisis of new antibiotics for the treatment of those superbugs is the most shocking issue in this time. [2] Abundant use of

antimicrobial drugs results a selective amplification of resistant bacteria, having capability to endure and replace the sensitive bacteria as well as greatly affect normal microbial flora. [3] From the last 30 years, alarming spreads of zoonotic diseases produce a great challenge to global health. Zoonotic diseases are not the latest conception, they have an extensive periphery in human disease and most communicable diseases e.g. influenza, tuberculosis, yellow fever, anthrax etc., spreads from the domestic animals, poultry and farm animals. [4,5] Over the world, poultry industry is one of the most pervasive food industries, and on the other hand chicken is commonly consumed food item. Approximately, 90 billion tons of chicken meat produced in every year. [6] Various antibiotic resistant bacteria isolated from the poultry soil, and these enteric bacteria draw attention because of its direct exposure on public health, through uplifting the morbidity, mortality of infection caused by cross resistant to drugs applied in human medicine. [7] The most frequent causative microbes of wound infection , urinary tract infection, nosocomial and various other types of infections are *Staphylococcus* family, *Enterobacteriaceae* family and other bacterial species, [8] which have a prominent connection with the poultry industry . Many kind of opportunistic bacteria have been reported from the poultry, including *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Klebsiella oxytoca*. In the *Enterobacteriaceae* group *Escherichia coli is a* facultative anaerobe bacterium and thin layer of peptidoglycan indicate the gram negative phenomena of bacteria. [9] Importantly, *Escherichia coli* are present in intestinal tract of animals and widely used in the research field to examine antimicrobial resistant in food products found from animal sources. [10] Some *Escherichia coli* stains hosted by poultry farms are potential source of antimicrobial resistant genes that may transmit to humans. [11] Staphylococci family's *Staphylococcus aureus* is a gram positive coccus, non-motile and non-spore producer. A stain of *Staphylococcus* i.e. *Staphylococcus aureus* is one of the typical inhabitants of skin and nares (man and animals) which act as pathogen under various conditions. [12] In the Pseudomonas genus, *Pseudomonas aeruginosa* has high significance in poultry industry, is an opportunistic bacterium capable of infecting all tissues in poultry. [13] *Proteus vulgaris* is a zoonotic human pathogen and have a great importance in public health. It is a member of enterobacteriaceae group found in intestinal tract of mammals, soil, water and they have been identified or isolated from the poultry. [14]

As a protective agent, antibiotics played a great role at sub therapeutic level in farm animals at the time of their discovery. [15] Extensive and unidirectional use of antibiotics lead to the rush in widespread and uncontrolled disease causes, related to antibiotic resistant bacterial infection. [16] The use of antibiotics as a growth enhancer was subsequently prohibited soon after the increase in prophylactic usage of therapeutic antibiotics against bacterial infections in animals. [17] Researcher found the alternative way out for the control of bacterial disease in poultry in a post-antibiotic era. A harmless sterilizer used continually in poultry production where a diminution in bacterial loads has been recorded. [18] Previous reports strongly suggested that for the management and elimination of pathogenic organisms, very little progress has been for finding of alternatives to the use of chemical disinfectants, which are generally applied, such as quaternary ammonium based compounds, glutaraldehyde, sodium hypochlorite, and a variety of organic acids. Those agents have multiple cellular targets such as the cell wall (or the outer membrane), the cytoplasmic membrane, functional and structural proteins, DNA, RNA and other cytosolic components. [19,20] Quaternary ammonium compound, which are USDA (United States Department of

Agriculture) permitted and EPA (The Environmental Protection Agency) approved non-toxic sanitizers for food processing utensils , are exceedingly used in the poultry industry due to the flourishing efficacy. [21] Quaternary ammonium compounds (QACs) served as antimicrobials agent with a cationic surfactants and an extensive spectrum of activities. [22] Predominant action of QACs is disruption of the bacterial cell membrane. [23] The length of the N-alkyl chain of QACs made it a potent antimicrobial agent. In case of gram-positive bacteria the chain contain 14 carbon whereas 16 carbons for gram-negative bacteria. [24] A quaternary ammonium blend alkyl dimethyl benzyl ammonium chloride (ADBAC), and a dialkyl agent, didecyldimethylammonium chloride (DDAC) performed as bactericidal, contributed to cell death of *S. aureus*. These agents caused immediate leakage of intracellular constituents by interacting with the cytoplasmic membrane of gram positive S. aureus. [25] Sublethal dose of DDAC showed a synergistic bactericidal effect against S. aureus [26] Glutaraldehyde served as a biocidal agent against glutaraldehyde (GTA)-sensitive and -resistant strains of *Mycobacterium chelonae.* [27] Glutaraldehyde-didecyldimethylammonium bromide (GA-DDAB) a newly discovered bactericide components damaged the *E. coli* cell membrane by leakage of intracellular components and inactivation of $H^+$ - ATPase. [28] Benzalkonium chloride and QAC was tested against 77 resistant bacterial strains and QAC performed more efficiently rather than the other two disinfectants. [20]

In the application field, disinfectants are regularly mixed with water in a container and exposed out to the severe environmental situation before their definite appliance. Disinfectants may have a small lifespan after their initial dilution and it is possible that heat, sunlight, time, organic matter and adulterants may reduce their efficacy. In this study we used Quaternary ammonium and other compounds (like glutaraldehyde, didecyl dimethyl ammonium chloride, benzalkonium chloride are) against gram positive and gram negative both bacterial stain, which was found in the poultry soil.

## 2. MATERIALS AND METHODS

### 2.1. Materials

*2.1.1. Culture Media and chemicals*

Nutrient agar, Mueller-Hinton agar, Nutrient broth, Luria broth and peptone broth were used for antibacterial activity respectively. All the culture media were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India) and Merck Ltd. (India).

*2.1.2. The antimicrobial Compounds*

The experimental compounds are composed of antimicrobial components i.e., Di-decyl di-methyl ammonium chloride, Di-octyl di-methyl ammonium chloride, Octyl-Decyl di-methyl ammonium chloride, Benzal konium chloride, Glutaraldehyde, Pine oil, and Terpineol oil, cumulatively designated as $RH^{5+}$ which is used as disinfectant in market, whereas, D2 and D3 were the competitor products. All the test compounds (in liquid state) were supplied by R.R Animal Health Care Ltd, Hyderabad, Telangana 500068 and were stored at room temperature until further use.

*2.1.3. Test microorganisms*

Five clinically isolated multidrug resistant bacterial strains including four gram negative bacteria *Escherichia coli (EC-1), Pseudomonas aeruginosa (PA-1), Klebsiella oxytoca (KO-1), Proteus vulgaris (PV-1),* and one-gram positive bacteria *Staphylococcus aureus (SA-1)* were collected from department of Microbiology, Malda Medical college and Hospital, Malda, West Bengal, India. Species specific confirmation and antibiotic susceptibility pattern of those strains were confirmed by VITEK® 2 system, BioMérieux India. All the above mentioned strains were multidrug resistant strains and were subcultured accordingly and used throughout the study.

### 2.2. Methods

*2.2.1. Determination of minimum inhibitory concentration (MIC) and* minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) was determined by micro-dilution method according to the National Committee for Clinical Laboratory Standards (currently known as CLSI) with some modifications. Briefly, in 1ml Luria broth, 10µl bacterial suspension was added containing $2.5X10^5$ CFU/mL strains. Different concentrations (1, 5, 10, 25, 50 and 100 µl/mL) of test antimicrobial compounds ($RH^{5+}$, D1, D2 and D3) were added to the test tubes containing the bacterial strains and incubated for 24 h at 37 °C. The MIC value of each compound against each bacterial strain was noted at the lowest concentration inhibiting visible growth of bacteria after 24 h of incubation. [29]

The minimum bactericidal concentration (MBC) of the $RH^{5+}$, D1, D2 and D3 were determined according to the method described elsewhere. [29] MBC is an addition part of the MIC experiment. In case of MBC, all MIC dilutions were sub-cultured on the sterile nutrient agar plates incubated at 37°C for 24 hours. The minimum concentration of the tested compounds ($RH^{5+}$, D1, D2 and D3) required for completely killing of the multidrug resistant bacterial strains were noted as MBC value of the compound. The MBC value was shown as 100% bacterial killing, compared with the positive control (without treatment) and negative control (without bacterial inoculums).

*2.2.2. Determination of Tolerance*

The tolerance levels of the bacterial strain against $RH^{5+}$, D1, D2 and D3 were determined according to the standard method using the following formula. [30]

Tolerance = MBC/MIC.

*2.2.3. Agar diffusion assay*

The antimicrobial susceptibility test using agar diffusion of four test compounds ($RH^{5+}$, D1, D2 and D3) against five multidrug resistant bacterial strains was also determined according to the technique. [31] Muller Hinton agar (MHA) plates were prepared into sterile Petri plates by pouring 15 ml of molten media. The bacterial inoculums were taken from freshly grown culture (5 h) having $10^6$ CFU/ml cells (compared with MacFarland's standard solution). By using sterile swab sticks (HiMedia, India) a bacterial lawn was prepared with on Muller Hinton agar media plate and was allowed to dry at 37°C incubator. Using 1ml micropipette tips, five holes on the MHA agar were done. Then 40 µl of different concentrations of the test compounds (crude, 1:2, 1:4, 1:8 and 1:16) were poured in each hole. Then the plates were kept for incubation at 37˚C for 24 hours. The diameter of zone of bacterial growth inhibition was measured using zone scale (HiMedia, India). [29]

*2.2.4. The onsite antibacterial activities of compounds* $RH^{5+}$, D1, and D3

At first 50 gm of poultry soil (collected from poultry bed of nearby firm) was mixed with 200 ml autoclaved distilled water and waited until all the solid precipitates deposited at the bottom of the container and clear supernatant found at the upper part. Then, 100 µl of that supernatant was mixed with peptone broth and incubated for 4 hrs in shaking condition for bacterial growth. For each set 100 µl of the fresh supernatant was mixed in peptone broth according to the following Table 1.

**Table 1: Experimental setup of onsite antimicrobial study of compounds D1, D2, and D4**

| Drug dilution | (+) control | (+) control (Diluted) (200 times) | (-) control | 1:1000 dilution | 1:2000 dilution |
|---|---|---|---|---|---|
| Bacteria | 100 µl | 20 µl (+ control) | - | 100 µl | 100 µl |
| Antimicrobial compounds ($RH^{5+}$, D1 and D3) | - | - | - | 7 µl | 3.5 µl |
| Peptone broth | 6900 µl | 1980 µl | 7000 µl | 6893 µl | 6896 µl |
| Total | 7000 µl | 2000 µl | 7000 µl | 7000 µl | 7000 µl |

After the specified incubation period, the antimicrobial compounds ($RH^{5+}$, D1, and D3) were added to each test tube maintaining dilution of 1:1000 and 1:2000

for each compounds and allowed to incubate for 30 min and 60 min. After that treatment 100 µl of bacterial culture were taken from each of the test tube including positive control (diluted 200 times) and were spread on nutrient agar and incubated overnight at 37º C incubator. After overnight incubation period, the colonies were counted from each plate. The antimicrobial compound D2 was not used for the study as it showed no antimicrobial activity against clinical isolates.

## 3. RESULT

In the present study, commercial disinfectant compound $RH^{5+}$, and competitor products D2 and D3 were examined against five clinical isolates of multidrug resistant bacterial strains *viz. E. coli, S. aureus, K. oxytoca, P. vulgaris,* and *P. aeruginosa* and certain parameters of antimicrobial activities were determined.

### *3.1. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)*

As presented in Table 1, $RH^{5+}$ and competitor product were tested displayed against multidrug resistance bacteria at different concentrations, revealed a strong dose-dependent antimicrobial activity. Among the four compounds, D1 was the most active as it showed the highest inhibitory effects against all five clinical isolates of multidrug resistant bacterial strains (MIC value of 1µl/mL). Although $RH^{5+}$ and D3 also displayed same MIC value i.e. 1µl/ml but there had a moderate result for *K. oxytoca, P. aeruginosa* and *S. aureus.* MIC values of *K. oxytoca, P. aeruginosa* were 5µl/ml against $RH^{5+}$ whereas *S. aureus* exhibited 5µl/ml MIC value against D3. D2 did not show any significant inhibition of microbial growth (Table 2). The negative controls were used to compare with the inoculated tubes where no growths were present (Fig.1A, Fig.1B).

**Table-2: Determination of MIC, MBC and Tolerance values**

| Bacteria | Commercial compounds | MIC value (µl/ml) | MBC value (µl/ml) | Tolerance level |
|---|---|---|---|---|
| E. coli | $RH^{5+}$ | 1 | 1 | 1 |
| | D1 | 1 | 1 | 1 |
| | D2 | >100 | >100 | ≥1 |
| | D3 | 1 | 1 | 1 |
| S. aureus | $RH^{5+}$ | 1 | 1 | 1 |
| | D1 | 1 | 1 | 1 |
| | D2 | >100 | >100 | ≥1 |
| | D3 | 5 | 5 | 1 |
| K. oxytoca | $RH^{5+}$ | 5 | 5 | 1 |
| | D1 | 1 | 1 | 1 |
| | D2 | >100 | >100 | ≥1 |
| | D3 | 1 | 1 | 1 |
| P. vulgaris | $RH^{5+}$ | 1 | 1 | 1 |
| | D1 | 1 | 1 | 1 |
| | D2 | >100 | >100 | ≥1 |
| | D3 | 1 | 1 | 1 |
| P. aeruginosa | $RH^{5+}$ | 5 | 5 | 1 |
| | D1 | 1 | 1 | 1 |
| | D2 | >100 | >100 | ≥1 |
| | D3 | 1 | 1 | 1 |

The MBC values were also found at the same dose of MIC value of the respective compounds. Against all clinical isolated bacteria, D2 was showed no growth inhibition upto 100 µl/ml dose. (Table 2).

*3.2. Determination of Tolerance*

The Tolerance level of each multidrug resistance bacterial strain against the compounds $RH^{5+}$, D1, D2 and D3 was calculated from the respective MIC and MBC value. In most of the multidrug resistance bacterial stains *E. coli, S. aureus, K. oxytoca, P.vulgaris,* and *P. aeruginisa* the tolerance level is 1. The MBC/MIC ratio is a parameter that reflects the bactericidal capacity of the test compound. As seen in Table 1, there is no difference between the bactericidal effects of $RH^{5+}$, D1, D2, and D3

on all drug-resistant microorganisms (Table 2).

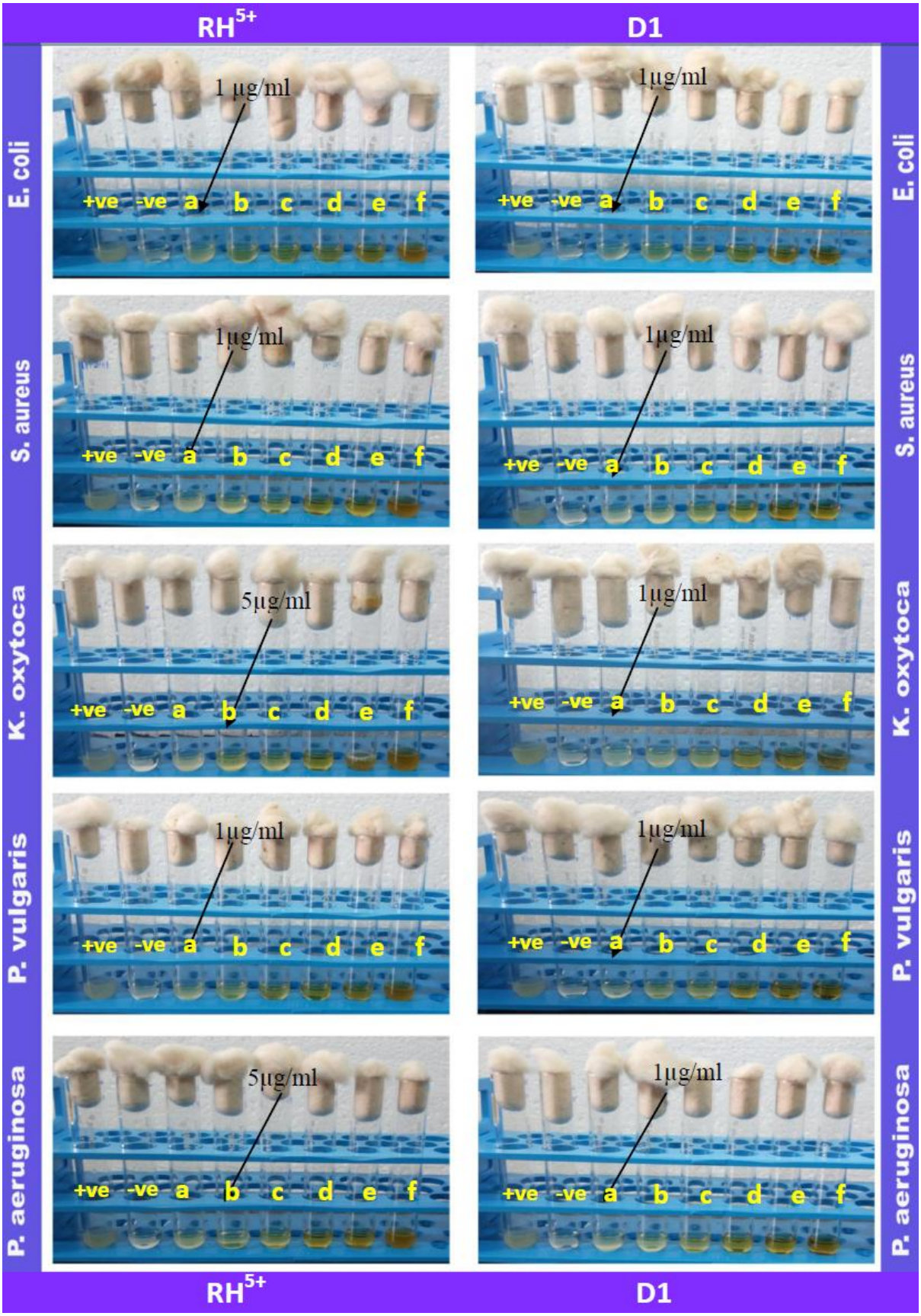


**Figure 1A**


D2
D3
E. coli
S. aureus
K. oxytoca
P. vulgaris
P. aeruginosa
>100µg/ml
1µg/ml
5µg/ml
+ve -ve a b c d e f
D2
D3

**Figure 1B**

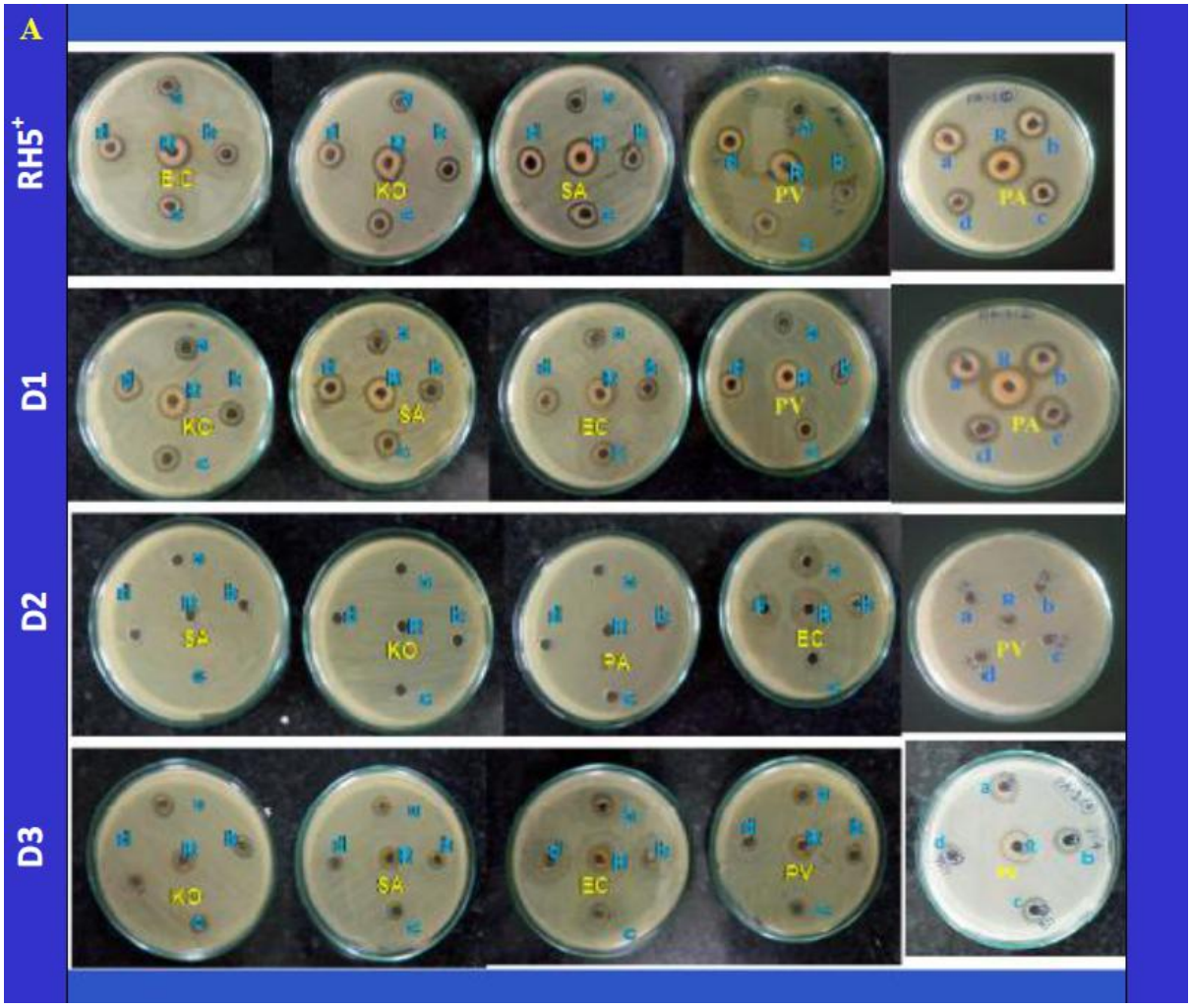


**Figure 2 A**

### *3.3. Determination of zone of inhibition by agar diffusion assay*

The antibiotic resistance profile of the pathogenic gram positive and gram negative bacteria was determined by the agar diffusion test. During the assessment of antibacterial activity the diameter of zone of inhibition obtained as significant value against those selective tested compounds. The zone of inhibition of bacterial strain against the $RH^{5+}$ and competitor product D1, and D3 within the range of 6 mm to 22 mm at concentration of undiluted (raw) were, 1:2, 1:4, 11:8, and 1:16 respectively, whereas the drug D2 showed the partial zone of inhibition against *E. coli*. The compounds $RH^{5+}$, D1, and D3 showed better antibacterial activity than D2 against all multidrug resistant bacterial strains (Fig.2).

### *3.4. Onsite antibacterial activities using poultry soil bed*

Zoonotic disease can be caused by bacteria present in poultry industries. So antibacterial activity of $RH^{5+}$, D1, D3 against those zoonotic bacterial strains were determined using poultry bed soil. The different dilutions of the sample were spread on nutrient agar plate and the colonies of the bacteria were counted after 24 hrs of incubation at 37ºC. The counted colonies are tabulated in Table 3. Due to low antimicrobial efficacy, D2 was not considered for the onsite antimicrobial activity study. The remaining three antimicrobial compounds were highly active against the all bacterial populations even at very low concentration (1:2000) and very short period of treatment time (30 mins and 60 mins). (Table-3, Fig.3 (A-F).

**Table-3: Time and dose dependent antimicrobial activity using colony count assay of D1, D2 and D4 against the bacterial populations found in poultry bed soil.**

| Incubation time | Untreated | | D 1 | | D 2 | | D 4 | |
|---|---|---|---|---|---|---|---|---|
| | Undiluted (uncountable) | Diluted (200 times) | 1:1000 | 1:2000 | 1:1000 | 1:2000 | 1:1000 | 1:2000 |
| 30 minutes | 3,249 | (63X200) =12,600 | 6 | 7 | 0 | 5 | 2 | 8 |
| 60 minutes | 4,142 | (76X200) =15,200 | 0 | 2 | 2 | 6 | 4 | 7 |

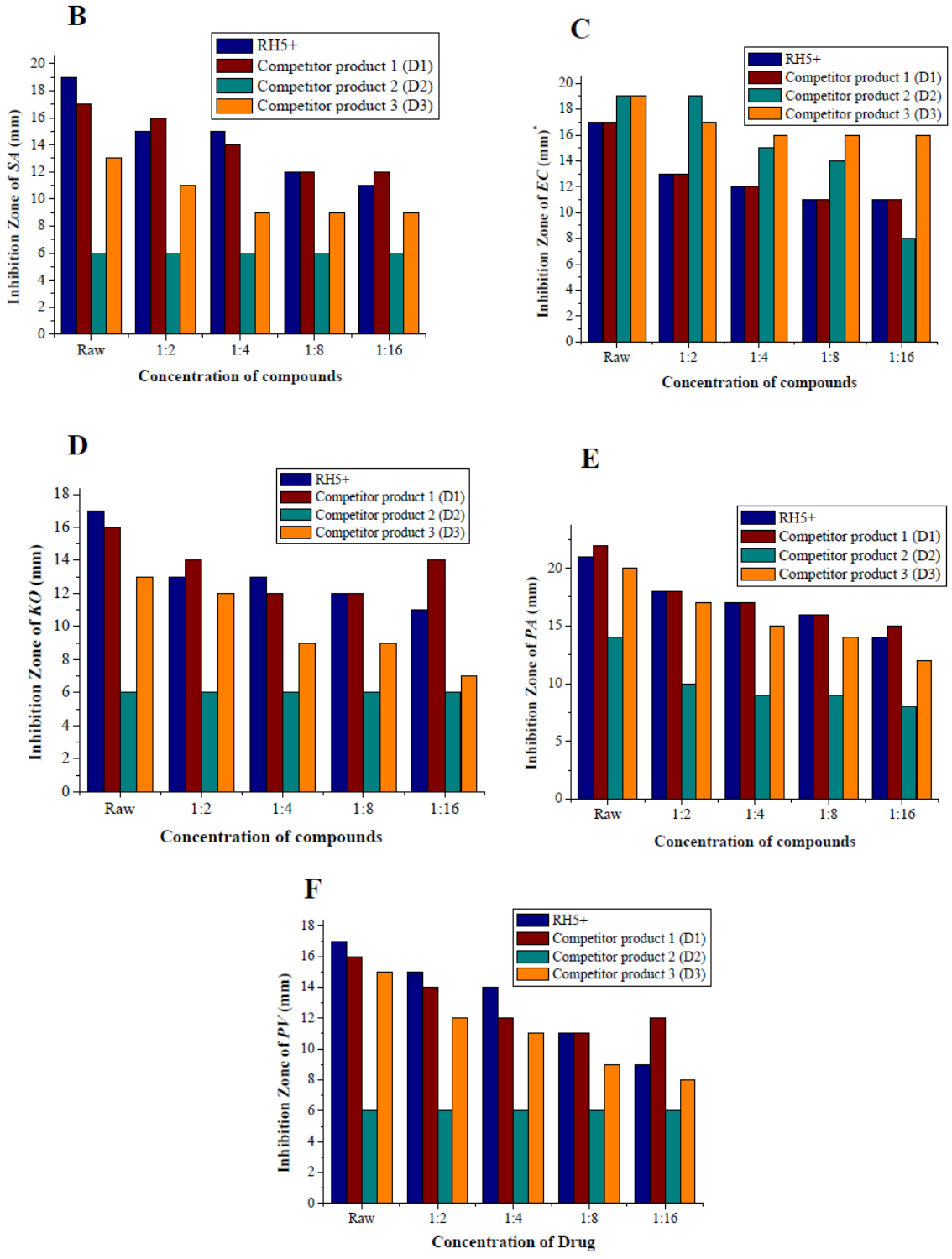


**Figure 2**

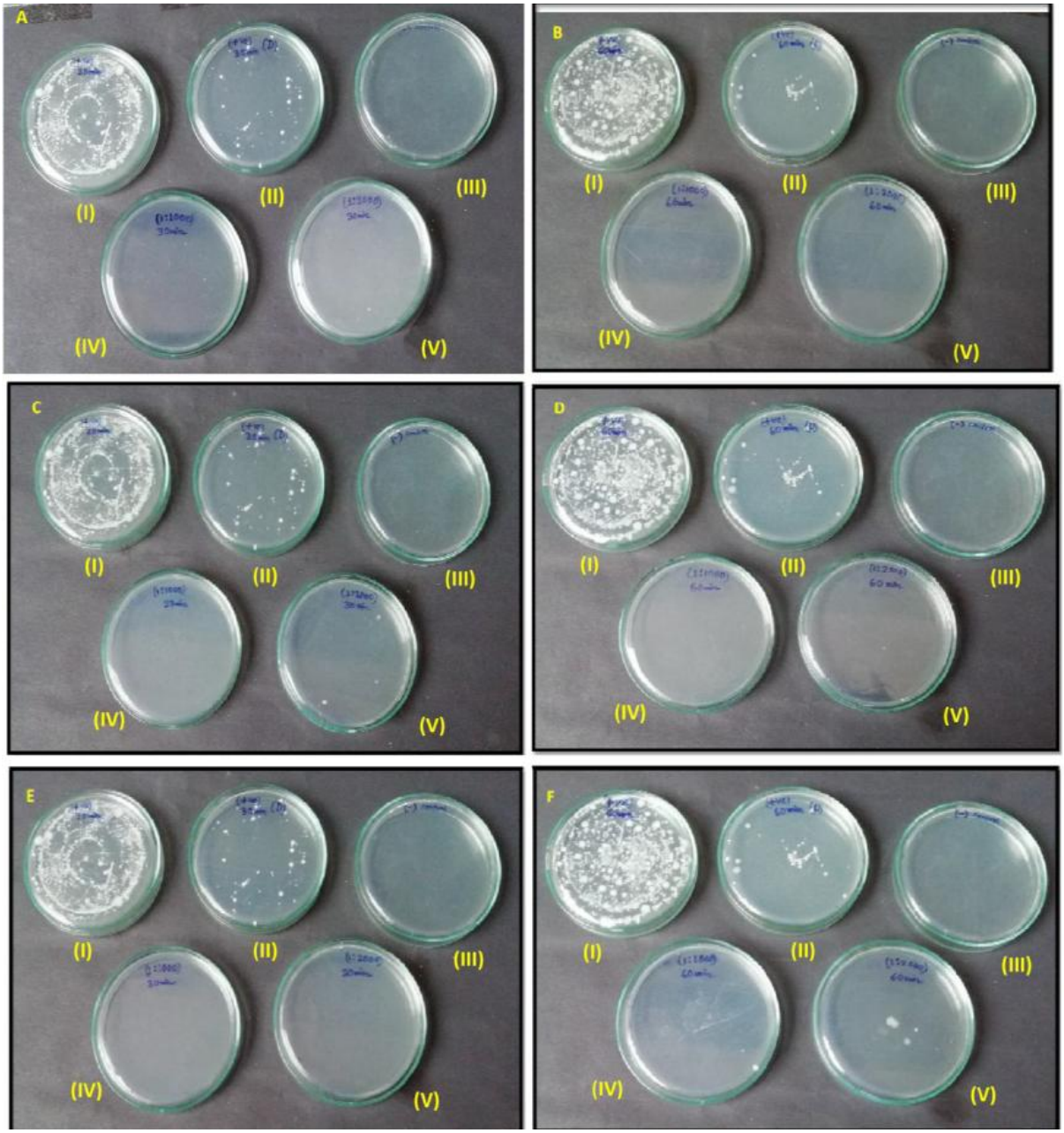


**Figure 3**

## 4. DISCUSSION

In the last year U.S. Geological Survey (USGS) investigated wildlife diseases, including zoonoses, at places where wildlife, domestic animals, humans, and the environment interacted with each other. [32] Along with the animal, food products such as eggs, meat, and milk are considered as vehicles for one or more of pathogens, causing food borne illnesses. In last few years, increasing number of organisms has been becoming resistant to several antibiotics and patients normally did not respond to high doses of antimicrobial agents even if administered for prolonged periods. The poultry farms leading to a proliferation of resistant bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Klebsiella oxytoca* etc. which are now spreading in the environment because of insecure disposal of poultry litter and waste in agricultural fields, has a potential to infect human beings. [33, 34] Workers in poultry farm and farmers in agricultural field suffer most as they directly come in contact with most hazardous pathogen. Disinfectants are commonly used in poultry

farm to prevent spreading of pathological diseases caused by bacteria. Chemical disinfectants are rich in quaternary ammonium compounds, glutaraldehyde, sodium hypochlorite, and organic acids. QAC was showed bactericidal activity against *S. mutans* and other microorganisms present in dental material. [35] In a recent study QAC immobilized onto silica-decorated membrane surface which exhibited long-lasting antibiofouling and antibacterial efficacy against *Escherichia coli, Staphylococcus aureus*. [36] An in vitro antimicrobial assays demonstrated that pine needle essential oil exhibits remarkable inhibitory and sterilizing activity against typical food-borne microorganisms such as *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Saccharomyces cerevisiae, Aspergillus niger, Penicillium*. [37] Condensation of glycine metal complexes with glutaraldehyde were screened for their antimicrobial activities against selected microorganisms like *Streptococcus aureus, Escherichia coli, Bacillus sphaericus, and Salmonella sp. (Bacteria) and also Fusarium oryzae, Candida albicans, and Aspergillus niger* (Yeast). [38] In this experiment the commercial disinfectants were mainly contained of a class of QACs. So in our study the marketed antimicrobial compounds $RH^{5+}$, and three competitor products (D1, D2 and D3) which were composed of mainly Di-decyl di-methyl ammonium chloride, Di-octyl di-methyl ammonium chloride, Octyl-Decyl di-methyl ammonium chloride, Benzal konium chloride, Glutaraldehyde, Pine oil, and Terpineol oil were used against five multidrug resistant bacteria (*Escherichia coli (EC-1), Pseudomonas aeruginosa (PA-1), Klebsiella oxytoca (KO-1), Proteus vulgaris (PV-1), Staphylococcus aureus (SA-1)* to determined antibacterial activities. Onsite antibacterial potency also was examined by using poultry bed soil. From the observed MIC value and MBC values (Table-1), it can be stated that the $RH^{5+}$, D1, and D3 are highly active at low concentration while D2 did not show any significant antibacterial activity. Most of the research reviewed here shows that whole plant extracts or combinations of compounds are more effective antimicrobials than isolated constituents. [39-43] Combinations of non-specific mechanisms of action might create a more effective antimicrobial activity than a traditional antibiotic. [43]

The MBC/MIC ratio is a parameter that reflects the bactericidal and bacteriostatic capacity of the test compound. The high tolerance value indicates that the test compounds is less effective and have chances to get resistance. But here, it was observed that the tolerance values of $RH^{5+}$, D1, and D3 were 1, against all multi drug resistant bacteria which indicates low chances for obtaining resistance property by the microorganisms. In disc agar diffusion study, the measurements of the diameter of zone of inhibition of bacterial strain against $RH^{5+}$, D1, and D3 (Table-2), exhibiting diameters of zones of inhibition greater than 10 mm each (>10 mm) which was considered as active antimicrobial activity. [40] D2 showed the partial zone of inhibition against *E. coli.* and displayed zone of inhibition against other bacterial strain was <10 mm. Thus, it is assumed that the remaining compounds were highly effective towards the multidrug resistant bacterial strains. In the onsite antimicrobial study, shows high sensitivity towards the bacteria present in poultry soil bed. In onsite study it also demonstrated that minimum amount of drugs showed significantly high efficacy towards zoonotic bacteria present in poultry soil. It was also found that the antimicrobial components of $RH^{5+}$ and competitor products were versatile disinfectant with a wide spectrum of antimicrobial action and safe for animals.

## 5. CONCLUSION

Gradual unresponsiveness of commercially available antibiotics becomes a major threat for not only the human health but also for the animal farming industries.

Moreover, unregulated use of antibiotics increases the chances of resistance property and also minimizes the quality of animal products. Considering the present alarming situation, the present study was carried out to investigate the antibacterial property of some unique formulations ($RH^{5+}$ and competitor products) containing quaternary ammonium, Glutaraldehyde and other active ingredients. It was noted that $RH^{5+}$ and its other two competitor products i.e. D1, and D3 were found as potent bacteriostatic agents against multidrug resistant bacteria and also against the bacterial populations found in the poultry bed soil. The D2 competitor product was observed to be non-effective against multidrug resistant bacterial strains and thus it was not used for further studies. So, it may be concluded that, the commercial products i.e. RH5+ and its variants could be used as an effective disinfectant in poultry firm to eliminate the pathogenic infections of the birds as well as for industrial and hospital areas to minimize the spreads of pathogenic bacterial strains from environment to human.

**Conflict of Interests:**

All authors contributed in the article declare no potential conflicts of interests.

## ACKNOWLEDGEMENT

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

## REFERENCES

1. Hsueh PR. New Delhi Metallo-β-lactamase-1 (NDM-1) &58; An Emerging Threat Among Enterobacteriaceae. Journal of the Formosan Medical Association. 2010 Jan 1;109(10):685-7.
2. Carlet J. The fight against bacterial resistance, a public health priority. Soins; la revue de reference infirmiere. 2015(797):21-3.
3. Delsol AA, Halfhide DE, Bagnall MC, Randall LP, Enne VI, Woodward MJ, Roe JM. Persistence of a wild type Escherichia coli and its multiple antibiotic-resistant (MAR) derivatives in the abattoir and on chilled pig carcasses. International journal of food microbiology. 2010 Jun 15;140(2-3):249-53.
4. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P. Global trends in emerging infectious diseases. Nature. 2008 Feb;451(7181):990.
5. Woolhouse ME, Haydon DT, Antia R. Emerging pathogens: the epidemiology and evolution of species jumps. Trends in ecology & evolution. 2005 May 1;20(5): 238-44.
6. Food and Agriculture Organization of the United Nations. FAOSTAT: Live Animals Data. (2017). Available from: http://www.fao.org/faostat/en
7. Warren RE, Ensor VM, O'neill P, Butler V, Taylor J, Nye K, Harvey M, Livermore DM, Woodford N, Hawkey PM. Imported chicken meat as a potential source of quinolone-resistant Escherichia coli producing extended-spectrum β-lactamases in the UK. Journal of Antimicrobial Chemotherapy. 2008 Jan 25;61(3):504-8.
8. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature reviews microbiology. 2015 May;13(5):269.
9. Nhung NT, Chansiripornchai N, Carrique-Mas JJ. Antimicrobial resistance in bacterial poultry pathogens: a review. Frontiers in veterinary science. 2017 Aug 10;4:126.
10. Hui YZ. Serotypes and antimicrobial susceptibility of Salmonella spp. isolated from farm animals in China. Frontiers in microbiology. 2015 Jun 22;6:602.
11. Kheiri R, Akhtari L. Antimicrobial resistance and integron gene cassette arrays in commensal Escherichia coli from human and animal sources in IRI. Gut pathogens. 2016 Dec;8(1):40.
12. Licitra G. Etymologia: Staphylococcus. Emerging Infect. Dis.. 2013; 19(9):1553.
13. Abdel-Aziz NA, Salem AZ, El-Adawy MM, Camacho LM, Kholif AE, Elghandour MM, Borhami BE. Biological treatments as a mean to improve feed utilization in agriculture animals- An overview. Journal of Integrative Agriculture. 2015 Mar 1;14 (3):534-43.
14. Ogunleye AO, Carlson S. Drug resistant Proteus mirabilis and Proteus vulgaris isolated from rats captured from some poultry houses in Ibadan, Oyo State, Nigeria

and their Public health importance. African Journal of Biomedical Research. 2016; 19(3):261-6.
15. Feighner SD, Dashkevicz MP. Subtherapeutic levels of antibiotics in poultry feeds and their effects on weight gain, feed efficiency, and bacterial cholyltaurine hydrolase activity. Applied and Environmental Microbiology. 1987 Feb 1;53(2):331-6.
16. Threlfall EJ. Antimicrobial drug resistance in Salmonella: problems and perspectives in food-and water-borne infections. FEMS microbiology reviews. 2002 Jun 1;26(2): 141-8.
17. Casewell M, Friis C, Marco E, McMullin P, Phillips I. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. Journal of antimicrobial chemotherapy. 2003 Aug 1;52(2):159-61.
18. Bragg RR, Plumstead P. Continuous disinfection as a means to control infectious diseases in poultry. Evaluation of a continuous disinfection programme for broilers. Onderstepoort J. Vet. Res. 2003 Sep 1;70:219-29.
19. Creyghton MP, Cheng AW, Welstead GG, Kooistra T, Carey BW, Steine EJ, Hanna J, Lodato MA, Frampton GM, Sharp PA, Boyer LA. Histone H3K27ac separates active from poised enhancers and predicts developmental state. Proceedings of the National Academy of Sciences. 2010 Dec 14;107(50):21931-6.
20. Bridier A, Briandet R, Thomas V, Dubois-Brissonnet F. Comparative biocidal activity of peracetic acid, benzalkonium chloride and ortho-phthalaldehyde on 77 bacterial strains. Journal of Hospital Infection. 2011 Jul 1;78(3):208-13.
21. Taormina PJ, Dorsa WJ. Evaluation of hot-water and sanitizer dip treatments of knives contaminated with bacteria and meat residue. Journal of food protection. 2007 Mar;70(3):648-54.
22. Marcotte L, Barbeau J, Lafleur M. Permeability and thermodynamics study of quaternary ammonium surfactants-phosphocholine vesicle system. Journal of colloid and interface science. 2005 Dec 1;292(1):219-27.
23. Haldar J, An D, de Cienfuegos LA, Chen J, Klibanov AM. Polymeric coatings that inactivate both influenza virus and pathogenic bacteria. Proceedings of the National Academy of Sciences. 2006 Nov 21;103(47):17667-71.
24. Kong M, Chen XG, Xing K, Park HJ. Antimicrobial properties of chitosan and mode of action: a state of the art review. International journal of food microbiology. 2010 Nov 15;144(1):51-63.
25. Ioannou CJ, Hanlon GW, Denyer SP. Action of disinfectant quaternary ammonium compounds against Staphylococcus aureus. Antimicrobial agents and chemotherapy. 2007 Jan 1;51(1): 296-306.
26. Gomi M, Osaki Y, Mori M, Sakagami Y. Synergistic bactericidal effects of a sublethal concentration of didecyldimethylammonium chloride (DDAC) and low concentrations of nonionic surfactants against Staphylococcus aureus. Biocontrol science. 2012;17(4):175-81.
27. Walsh SE, Maillard JY, Russell AD, Hann AC. Possible mechanisms for the relative efficacies of ortho-phthalaldehyde and glutaraldehyde against glutaraldehyde-resistant Mycobacterium chelonae. Journal of applied microbiology. 2001 Jul;91(1):80-92.
28. Lin W, Guan X, Cao J, Niu B, Chen Q. Bactericidal mechanism of glutaraldehyde-didecyldimethylammonium bromide as a disinfectant against Escherichia coli. Journal of applied microbiology. 2017 Mar;122(3):676-85.
29. Das B, Dash SK, Mandal D, Ghosh T, Chattopadhyay S, Tripathy S, Das S, Dey SK, Das D, Roy S. Green synthesized silver nanoparticles destroy multidrug resistant bacteria via reactive oxygen species mediated membrane damage. Arabian Journal of Chemistry. 2017 Sep 1;10(6): 862-76.
30. May J, Shannon K, King A, French G. Glycopeptide tolerance in Staphylococcus aureus. The Journal of antimicrobial chemotherapy. 1998 Aug 1;42(2):189-97.
31. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology. 1966 Apr 1;45(4_ts):493-6.
32. Meteyer CU, Rogall GM. Information to Prevent Human Exposure to Disease Agents Associated with Wildlife- US Geological

Survey Circulars on Zoonotic Disease. US Geological Survey; 2018 Mar.
33. Blaak H, van Hoek AH, Hamidjaja RA, van der Plaats RQ, Kerkhof-de Heer L, de Roda Husman AM, Schets FM. Distribution, numbers, and diversity of ESBL-producing E. coli in the poultry farm environment. PLoS One. 2015 Aug 13;10(8):e0135402.
34. Chika E, Ifeanyichukwu I, Carissa D, Thomas A, Okoro O, Joshua E, Michael A, Charles E. Occurrence of Metallo-Beta-Lactamase-Producing Enterobacteriaceae from a Local Poultry Farm in Abakaliki, Nigeria. International Journal of Applied Pharmaceutical Sciences and Research. 2016 Sep 5;1(2):70-5.
35. Makvandi P, Jamaledin R, Jabbari M, Nikfarjam N, Borzacchiello A. Antibacterial quaternary ammonium compounds in dental materials: A systematic review. Dental Materials. 2018 Apr 17.
36. Zhang X, Wang Z, Tang CY, Ma J, Liu M, Ping M, Chen M, Wu Z. Modification of microfiltration membranes by alkoxysilane polycondensation induced quaternary ammonium compounds grafting for biofouling mitigation. Journal of Membrane Science. 2018 Mar 1;549:165-72.
37. Zeng WC, Zhang Z, Gao H, Jia LR, He Q. Chemical composition, antioxidant, and antimicrobial activities of essential oil from pine needle (Cedrus deodara). Journal of food science. 2012 Jul;77(7):C824-9. Zeng WC, Zhang Z, Gao H, Jia LR, He Q. Chemical composition, antioxidant, and antimicrobial activities of essential oil from pine needle (Cedrus deodara). Journal of food science. 2012 Jul;77(7):C824-9.
38. Nishat N, Hasnain S, Dhyani S, Asma. Coordination polymers of glutaraldehyde with glycine metal complexes: synthesis, spectral characterization, and their biological evaluation. Journal of Coordination Chemistry. 2010 Nov 10;63 (21):3859-70.
39. Junio HA, Sy-Cordero AA, Ettefagh KA, Burns JT, Micko KT, Graf TN, Richter SJ, Cannon RE, Oberlies NH, Cech NB. Synergy-directed fractionation of botanical medicines: a case study with goldenseal (Hydrastis canadensis). Journal of natural products. 2011 Jun 10;74(7):1621-9.
40. Ncube B, Finnie JF, Van Staden J. In vitro antimicrobial synergism within plant extract combinations from three South African medicinal bulbs. Journal of ethnopharmacology. 2012 Jan 6;139(1):81-9.
41. Wang SY, Sun ZL, Liu T, Gibbons S, Zhang WJ, Qing M. Flavonoids from Sophora moorcroftiana and their synergistic antibacterial effects on MRSA. Phytotherapy research. 2014 Jul;28(7):1071-6.
42. Garvey MI, Rahman MM, Gibbons S, Piddock LJ. Medicinal plant extracts with efflux inhibitory activity against Gram-negative bacteria. International journal of antimicrobial agents. 2011 Feb 1;37(2):145-51.
43. Kourtesi C, Ball AR, Huang YY, Jachak SM, Vera DM, Khondkar P, Gibbons S, Hamblin MR, Tegos GP. Suppl 1: Microbial efflux systems and inhibitors: approaches to drug discovery and the challenge of clinical implementation. The open microbiology journal. 2013;7:34.
44. Usman H, Osuji JC. Phytochemical and in vitro antimicrobial assay of the leaf extract of Newbouldia laevis. African Journal of Traditional, Complementary and Alternative Medicines. 2007;4(4):476-80.

How to cite this article: Banerjee J, Dey S, Rizowana HN et. al. Evaluation of antimicrobial activities of commercial product ($RH^{5+}$) 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.

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