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***In vitro* Efficiency of Disinfectants Against *Salmonella enteritidis* Samples Isolated from Broiler Carcasses**

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■ Keywords

Broiler carcasses, disinfectants, *in vitro* resistance, *Salmonella enteritidis*.

ABSTRACT

The threat to public health represented by *Salmonella* is at least partially a consequence of its ecology in poultry hosts. Good manufacturing practices in the processing plant can reduce the contamination of poultry products, and critical control point principles are essential throughout the chain production. One procedure adopted in critical points control to prevent and to reduce *Salmonella* in farms and poultry products is the use of disinfectants. This study aimed at evaluating disinfectant efficiency against *Salmonella enteritidis* samples isolated from broiler carcasses in Rio Grande do Sul State between 1995 and 1996. The tested disinfectants were: phenol 1:256, quaternary ammonium 1:2500, glutaraldehyde 1:200, and iodine 1:500, with contact times of 5, 10, 15, and 20 in an *in vitro* test. Phenolic compounds showed better results, iodine and glutaraldehyde showed intermediary results, and quaternary ammonium presented efficiency at all contact times evaluated in the *in vitro* test.

INTRODUCTION

Many food-borne pathogens pose risk to public health, and the genus *Salmonella* is particularly important due to its ecology in the birds that carry them. *Salmonella paratyphus* is typically associated to asymptomatic diseases, and present different colonization patterns in the intestinal tract of birds.

Good manufacturing practices in processing plants may reduce final product contamination, and the control of critical points is essential along the entire production chain (Bailey *et al.*, 2001). One of the mechanisms adopted in the control of critical points to control and to reduce the presence of *Salmonella* spp during broiler processing and in the final product is the use of disinfectants. A wide range of active principles, such as chlorine, quaternary ammonium, glutaraldehyde, and iodine, is available for the use in poultry production, both during rearing and processing.

The expected efficacy of disinfectants depends on their correct application, considering recommended dilutions, time of contact with the surface to be sanitized, previous removal of organic matter, the quality of the water used for cleaning, and general characteristics of the action of disinfectants (Berchieri Jr. & Barrow, 1995; Davison *et al.*, 1996; Martinez *et al.*, 1999), in addition to product concentration, and environment temperature and pH (Merianos, 1991).

Improper food handling and neglecting water treatment in the presence of microorganisms – pathogenic or not – can increase equipment contamination problems due to the formation of biofilms, which protect these microorganisms and increase up to 100 fold their resistance to disinfectants (Lillard, 1993 *apud* Davies & Wray, 1995).



The present study aimed at evaluating the efficiency of disinfectants commonly used in poultry processing plants in the presence of *Salmonella enteritidis* samples isolated from broiler carcasses in the state of Rio Grande do Sul in 1995 and 1996.

MATERIAL AND METHODS

Eighty *Salmonella enteritidis* samples isolated from carcasses of broilers processed in Rio Grande do Sul, Brazil, between 1995 and 1996, were collected. Carcasses were processed according to the method established in Ordinance 08 (Brasil, 1995), at the Avian Pathology Diagnosis and Research Center (CDPA-UFRGS), and the final identification of serovars was carried out at Oswaldo Cruz Institute.

The disinfectant efficiency evaluation test was carried out according to the Microbiological Methods Manual for Foods (Brasil, 1992). The collected *Salmonella enteritidis* samples were placed in BHI broth (Brain Heart Infusion, Merck) and incubated for 18-24h. Samples were then diluted at 10^{-1} to 10^{-10} in peptone water at 0.1%, and colony-forming units (CFU) per milliliter (ml) were counted in BHA medium (Brain Heart Agar, Difco). The 1:100 dilution, with 10^5 to 10^7 CFU/ml, was selected for efficiency tests. The following disinfectants and their respective concentrations were tested: phenol 1:256, quaternary ammonium 1:2500, glutaraldehyde 1:200, and iodine 1:500, which are the concentrations commonly used in poultry processing companies. Disinfectants were diluted in distilled water, and 9ml were distributed in sterile tubes. To each tube, 1ml organic matter (whole milk submitted to UHT treatment) and 10 μ L of the 1:100 dilution of stationary-phase culture were added. Solutions were homogenized, and 5, 10, 15, and 20 minutes of exposure were counted, seeding 10 μ L in BHI broth. Tubes were incubated at 37°C for 96 hours. Tubes were checked for turbidity and film formation on the surface or precipitation in the bottom of the tube, and those considered positive were seeded in *Salmonella* selective media for confirmation.

The results were submitted to the test of Cochran (Siegel, 1975).

RESULTS AND DISCUSSION

Table 1 shows the efficiency of disinfectants against the 80 *Salmonella enteritidis* (SE) samples isolated from broiler carcasses in 1995 and 1996, in Rio Grande do Sul, Brazil. In the present study, glutaraldehyde was

the most efficient (93.75%) after 15 minutes of contact against SE samples. Both phenol and iodine started to act after 10 minutes, but phenol presented 100% efficiency, whereas iodine, 91.25%. Quaternary ammonium acted only on 8.75% of SE samples, even after 20 minutes of contact.

Table 1 - Performance of disinfectants after 5, 10, 15, and 20 minutes of contact against 80 *Salmonella enteritidis* samples isolated from broiler carcasses between 1995 and 1996, in Rio Grande do Sul, Brazil.

Commercial product	Contact time	n. of resistant strains	Resistance percentage
*Glutaraldehyde 1:200	5'	35	43.75%
	10'	15	18.75%
	15'	5	6.25%
	20'	4	5.00%
*Iodine 1:500	5'	33	41.25%
	10'	9	11.25%
	15'	7	8.75%
	20'	4	5.00%
*Phenol 1:256	5'	16	20.00%
	10'	5	6.25%
	15'	0	0%
	20'	0	0%
*Quaternary ammonium 1:2500	5'	79	98.75%
	10'	76	95.00%
	15'	74	92.00%
	20'	73	91.25%

(*) Significance: $p < 0.05$.

Berchieri Jr. and Barrow (1995, 1996) found that phenolic compounds and glutaraldehyde are efficient against *Salmonella* spp., and also reported that quaternary ammonium compounds were inefficient.

In high concentrations, phenol breaks the cell wall and precipitates proteins, and in low concentration, it inactivates essential enzymes, being considered bacteriostatic in small quantities (O'Connor & Rubino 1999). The bacteriostatic capacity of phenol may have contributed for the better performance of this product in 15 minutes of contact with SE samples.

Glutaraldehyde has wide spectrum and rapid action, even in the presence of organic matter. In addition, it does not corrode plastic and metal. Its biocidal activity is due to its effect on protein synthesis and to its capacity of destroying spores (Heit & Riviere, 1995; Scott & Gorman, 1991).

The low reactivity of iodine with protein and its disinfection capacity in a wide range of pH (it maintains its activity in the presence of organic matter and at $pH < 4$) are the main reasons for its utilization (Gottardi, 1991; Heit & Riviere, 1995). However, some disadvantages limit its use, such as changes of metal surfaces not resistant to oxidation and its absorption by some plastics, resulting in brown spots (Gottardi, 1991). In the present study, iodine presented similar or



higher efficiency as compared to glutaraldehyde at 5, 10, 15, and 20 minutes of contact. Also, it was more efficient than quaternary ammonium, but less efficient than phenols at these same times.

Conner and Eckman (1997) compared alkaline and acid phenolic compounds, and observed that alkaline phenols were less efficient than acid phenols. On the other hand, bactericide activity was not reduced when those disinfectants were applied in a shuttle program.

In the present study, quaternary ammonium presented low efficiency at all tested times against SE. However, when Leite (2002) tested the efficiency of quaternary ammonium and sodium hypochloride against samples of *Salmonella enteritidis*, *S. typhimurium*, *S. hadar*, and *S. heidelberg* isolated from broiler carcasses, he observed that quaternary ammonium had better performance as compared to sodium hypochloride. In addition, among the tested samples, SE was the serovar that showed the highest resistance to quaternary ammonium.

In the present study, disinfectants were individually evaluated; however, the use of a single active principle for long periods of time may impair its efficiency. Therefore, *in vitro* tests with disinfectants may aid in the control of the efficiency levels obtained with different active principles, and underscores the importance of using the proper concentrations of products with proven efficiency against the targeted microorganisms.

CONCLUSION

It was concluded that disinfectants based on glutaraldehyde, phenol, and iodine were 95% efficient or more against *Salmonella enteritidis*; however, disinfectants based on quaternary ammonium presented very low efficiency.

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