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EVALUATION OF SOME COMMERCIAL DISINFECTANTS AGAINST SOME PATHOGENS IN PRESENCE OF INTERFERING SUBSTANCES

(With 4 Tables)

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تقييم بعض المطهرات التجارية على بعض الميكروبات المرضية في وجود المواد المعاكسة

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في هذه الدراسة تم تقييم خمسة أنواع من المطهرات التجارية الحديثة واسعة الأنتشار في الحقل البيطري بإستخدام طرق مختلفة في هذا التقييم والتي تعتمد على معادلة المطهرات ببعض المواد التي تتميز بعدم تأثيرها على الميكروبات تحت الأختبار . المركبات التي أستخدمت هي مركبات الأمونيا الرباعية ومركبات الأمونيا الرباعية مع الجلوتزالدهيد ومركب فينولي ونوعين من مركبات اليود وذلك على أنواع من البكتريا الممرضة وهي الميكروب العنقودي الذهبي وميكروب القولوني السبحي وميكروب المسالونيلا لورم. وقد أظهرت النتائج أن كل أنواع المطهرات تحت الدراسة قد قتلت الميكروبات في توقيتات متباينة عند التركيز الموصي به من الشركة المنتجة. وقد وجد أن المواد العضوية لم تؤثر في مركبات الأمونيا الرباعية والفينولات بينما أثرت بشكل واضح على كفاءة مركبات اليود. من ذلك يوصى بإزالة كل المواد العضوية من الأسطبلات قبل عملية التطهير خصوصا إذا أستخدمت مركبات اليود مع ضرورة تعرض الميكروبات للمطهرات بوقت كافي.

SUMMARY

The bactericidal activities of five chemical disinfectants against Gram-positive and Gram-negative bacterial species were conducted at various concentrations in absence and presence of 2% bovine albumin as an interfering substance. The evaluated disinfectants are, quaternary ammonium compound (Quaternary active sterilizer); quaternary ammonium compounds with glutaraldhyde (TH4+); phenolic compound (Tek-Trol) and two iodine compounds (Biocide-30 and Iodine active sterilizer). The obtained results revealed that, the neutralizer of choice

for TH4+ and quaternary active sterilizer was letheen broth where its inhibition percentages of the tested organisms were non-significant and in the same time it, neutralize the disinfectant efficiency. Moreover, a combination of 0.3% lectihin and 3% Tween 80 was the neutralizer of choice for phenolic compound (TekTrol) while iodine compounds were effectively neutralized by 0.5% Sod. Thiosulphate. Concerning the bactericidal efficiencies, the results indicated that the recommended concentration of all used disinfectants completely destroyed *S.pullorum* within 5 minutes except iodine active sterilizer which required 5 minutes more. While, *E.coli* was completely destroyed within 10 min. by Iodine active sterilizer and 15 min. by both TH4+ and Biocide-30; Tek-Trol required 20 min., and quaternary active sterilizer required 30 min., 30 min. On the other hand, *S.aureus* was completely cleared out by TH4+ after 15 min.; Tek-Trol after 25 min. while quaternary active sterilizer, Biocide-30 and Iodine active sterilizer were required 30 min. While quaternary active sterilizer, Biocide-30 and Iodine active sterilizer were required 30 min. These results indicate that, Gr-ve bacteria were highly sensitive to the action of disinfectants under test (*S.pullorum*). While G+ve organism showed less sensitivity. The results showed that quaternary ammonium ocmpounds, phenolic compounds are not affected by the presence of organic matter in contrast to the iodine compound which their efficiencies were drastically reduced in presence of the organic matter. In conclusion, removal of the organic materials and other interfering substances are of primary consideration before application of disinfectants in animal and poultry enclosures.

Key words: *Disinfectants; E.coli; S.pullorum; S. aureus; Organic matter, quatemary ammonium compounds.*

INTRODUCTION

Proper management; improving programs and efficient measures for disease control are the principals for obtaining maximum productivity from the livestock. Poultry industry is the most hygiene conscious sector of all livestock. It has emphasized in recent years that there is an over riding demand under intensive system of poultry industry. Sanitation and good hygiene are necessary insurance policy for prevention of disease.

From the earliest days of recorded history, we have evidence of the application of various types of disinfectants as a matter of regular

routine. Nowadays there are many types of disinfectants widely used in the veterinary practice. Iodine was used for the first time for wound dressing at 1839 while pure phenol used for the same purpose at 1860 (Block, 1991).

There are many compounds available for sanitation of livestock units (Berchier and Barrow, 1996), each type has a characteristic feature of its anti-microbial activity under the optimum conditions (Van Impe et al., 1966; Ismail, 1967; Borick, 1968; Bergan et al., 1972a; El-Falaha et al., 1985 and Power & Russel, 1989).

The present work was conducted to evaluate the bactericidal efficiencies of some commercial disinfectants in the veterinary practice tested against some pathogenic bacteria in presence of interfering substances.

MATERIAL and METHODS

A) Disinfectants:

Five commercial disinfectants were used in the present study. Each one was used at three concentrations including the recommended concentration as well as one higher and one lower. The disinfectants were:

1- TII4+ (12.5% quaternary ammonium compounds + 6.3% Glutaraldhyde) used at 1; 0.5 & 0.33%.
2- Quaternary active sterilizers (25% quaternary ammonium compounds) used at 0.25; 0.2 & 0.16%.
3- Tek-Trol (26% phenolic compounds) used at 1; 0.39 and 0.25%.
4- Iodine active sterilizers (2.3% available iodine +28% phosphoric acid) used at 0.33; 0.25 and 0.2%.
5- Biocide-30 (2.75% available iodine + 9.5% phosphoric acid + 9.3% sulphoric acid + 24.2% non-ionic surfactants) used at 0.33; 0.25 and 0.2%.

B) Pathogenic bacteria:

The bacterial species were selected for the test included:

1- *E.coli* O_{78}.
2- *S.pullorum.*
3- *Staph. aureus.*

The bacterial species were obtained from the Animal Health Institute, Giza, A.R.E. A loopfull from 24 h nutrient slope was transferred into 10 ml broth and incubated at 37°C for 18-24 h. The total colony count was determined by the plating technique (Cruickshank et al., 1980).

C- Neutralizers used in the evaluation of the disnfectants:

Six neutralizers were used against the previously mentioned disinfectants to determine the most suitable one for each including:

1- 3% lecithin.
2- 10% Tween 80.
3- 0.3% lecithin + 3% Tween 80.
4- 2% lectihin + 4% tween 80.
5- Letheen broth (2.07% letheen broth + 0.05 Tween 80).
6- 0.5% Sod. Thiosulphate.

The toxicity of the neutralizers on the bacterial strains was checked at room temperature where the total colony count was determined for each organism suspension. The bacterial suspension was then added to each neutralizer (at the previous concentrations) and left for 30 minutes before determining the total colony count/ml again (Russel, 1981).

In order to determine the neutralizing efficiency of the neutralizer, a disinfectant-neutralizer mixture (5 ml disinfectant + 4.5 ml neutralizer solution) was prepared. 0.5 ml of the microbial suspension then added to the mixture and incubated at room temperature. The total colony count was determined before and after incubation by 15 minutes.

4- Efficiency of the disinfectants against bacterial species:

0.1 ml of the microbial suspension under test was added to 9.9 ml of the disinfectant solution. 1 ml of the mixture was removed at time intervals (5; 10; 15; 20; 25 and 30 min.) and added to 9 ml neutralizer (suitable for the disinfectant), left for 3-5 min. to stop the effect of the disinfectant and then the total colony count was conducted (Cruickshank et al., 1980 and Tuncan, 1993).

5- Effect of interfering substances:

The efficiency of the disinfectants was conducted at the recommended concentrations in presence of 2% bovine albumin (Gelinas and Goulet, 1983).

RESULTS

Results were illustrated in Tables 1; 2; 3 and 4.

DISCUSSION

Data presented in Tables (1 & 2), revealed that TH4+ and QAS were efficiently neutralized by letheen broth where the inhibition

percentages of non significant. It was stated that, the inhibition percentages were 1.7; 6.1. & 6.7% for *E.coli*; *S pullorum* and *S.aureus* respectively. On the other hand, the neutralizer has no toxic effect on each of the tested organisms. The inhibition percentages of letheen broth were of non significant where *E.coli* was inhibited by 7.8% (5.1 and 2.9%) for *S.pullorum* and *S.aureus*, respectively. These results indicated that letheen broth was the most suitable neutralizer for the quaternary ammonium compounds. The reached data were in agreement with those recorded by Davis (1960); Ceglowski & Lear (1992); Weiner et al. (1965); Prickett & Rawal (1972) and Russel et al. (1979).

It can concluded that Tek-Trol was efficiently neutralized by 0.3% lecithen + 3% Tween 80. While, 0.5% Sod. Thiosulphate was the most effective neutralizer for iodine compounds, Biocide-30 and iodine active sterilizer (Tables 1 & 2). These results were coincided with those recorded by Hugo & Newton (1964); Newton & Vickers (1964); Bergan & Lystand (1972a) and Linton et al. (1987). Data in Table (3) showed the the recommended concentration of TH4+ (1:200) was effective to destroy *E.coli* and *S.pullorum* within 15 minutes. But, *S. pullorum* was highly sensitive to the quaternary ammonium compounds with glutaraldhyde and completely destroyed within 5 minutes even at a lower concentration. Concerning QAS, results revealed that, Gram-negative bacteria were quickly destroyed than Gram-positive one. In this respect, it was stated that, *S.pullorum* was firstly destroyed (5 min.) followed by *E.coli* (20 min.) and then *S. aureus* (30 min.). The reached results indicated that, the quaternary ammonium compounds were highly effective against *E.coli*; *S.pullorum* and *S.aureus*. Which inturn nearly similar to those recorded by Ansari (1984) and Bergan and Lystand (1972b). There is no doubt that addition of glutaraldhyde to quaternary ammonium compounds in TH4+, increased its bactericidal properties on the vegetative bacteria (Stonehill et al., 1963; Borick, 1968; Bergan and Lystand, 1972b and Power & Russel, 1989).

Data illustrated in Table (3), showed that, the recommended concentration of phenolic compound, Tek-Trol (1:256) was quite enough to destroy *E.coli* and *S.pullorum* at 20 and 5 min., respectively. While, *S.aureus* needed a little more time to be completely destroyed (25 min.). These results were more or less similar to those recorded by Van Impe et al. (1966); Hegna (1977) and Band (1990). On contrast, they were differ than those obtained by Ismail (1967) and Ansari (1984) who used higher concentrations of phenolic compounds against *E.coli*. It noticed that *S.aureus* showed a greater resistance to phenolic compounds than did

Gram-negative bacteria (Hegna, 1977 and Block, 1991). On the other hand, Hugo and Bloomfield (1971) stated that *E.coli* was less affected by phenolic disinfectants due to the lipid-rich nature cell wall.

Concerning iodine compounds, Table (3) showed that both Biocide-30 and iodine active sterilizer showed strongly bactericidal against Gram-negative bacteria. In this respect, both *E.coli* and *S.pullorum* were completely destroyed within 15 and 5 min., respectively. But, *S.aureus* required more time up to 30 min. to be destroyed. These results were more or less similar with those obtained by Varga (1972) and Zorawski and Shwarek (1984).

Organic matter occurs in various forms as serum, blood, food, residues, milk, and faecal matter. These materials may interfere with the bactericidal activity of the used disinfectants. This interference gradually takes the form of "reaction" between the compound and the organic matter, thus leaving a reduced concentration of antimicrobial agent for attacking microorganisms. Data in Table (4) indicated that the bactericidal effectiveness TH4+, quaternary active sterilizer and Tek-Trol were not affected by the presence of 2% bovine albumin. The recommended concentrations of these compounds were able to destroy more or less completely both Gram-positive and Gram-negative bacteria within the time recorded in absence of organic matter (Table 4). Data showed that all these compounds were able to destroy *S.pullorum* within 5 min. On the other hand, both *E.coli* and *S.aureus* were required little more time to be destroyed (Table 4). These results are similar to those recorded by Miner et al. (1977); Gelinas & Goulet (1983) and Gjorman & Scott (1983) who stated that quaternary ammonium compounds are not affected by the organic matter. Contrary results were reported by Linton et al. (1987) who found that, the organic matter adversely affect the antimicrobial activity of quaternary ammonium compounds. Concerning phenolic compounds, our results were in contrast to the results of Bergan & Lystad (1971); Sainsbury & Sainsbury (1982) and Block (1991) who found that, the organic matter interfered with the efficiency of phenolic compounds. This difference could be attributed to the type and structure of phenol (Russel et al., 1982 and Linton et al., 1987). However, Band (1990) revealed that, phenolic compounds have broad activity and retained their efficiency in the presence of organic matter. Concerning Iodine compounds the reached results showed that, the bactericidal action of Biocide-30 and iodine active sterilizer were greatly affected by presence of organic matter. It was revealed that the inhibition percentages of Biocide-30 and iodine active sterilizer (at the

recommended concentrations) were quite reduced in presence of organic matter to 20% and 43.1% for *E.coli* and *S.pullorum*, respectively. On the other hand, *S.aureus* was inhibited by 99.9%.

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Table (1) Toxicity and efficiency of the neutralizers on the disinfectants in case of Gram-negative bacteria

Disinfectants	Neutralizers	Micro-organism											
		E.coli						*S. Pullorum*					
		Toxicity			Efficiency			Toxicity			Efficiency		
		A	B	Inh. %	C	D	Inh. %	A	B	Inh.%	C	D	Inh. %
TH4+ 2%	Letheen broth	$3.9x10^5$	$3.7x10^5$	7.8	$4.2x10^5$	$4.1x10^5$	1.7	$5.9x10^5$	$5.6x10^5$	5.1	$4.9x10^5$	$4.6x10^5$	6.1
QAS 0.33%	Letheen broth	$5.2x10^5$	$5.0x10^5$	3.8	$4.0x10^5$	$3.8x10^5$	5.0	$3.8x10^5$	$3.5x10^5$	5.4	$6.5x10^5$	$5.8x10^5$	10.8
Tek-Trol 2%	0.3% lecithin + 3% Tween 80	$3.3x10^5$	$3.1x10^5$	3.7	$2.9x10^5$	$2.8x10^5$	3.1	$3.8x10^5$	$3.6x10^5$	5.3	$4.2x10^5$	$4.0x10^5$	4.8
Biocide-30 0.5%	0.5% sod. Thio-sulphate	$6.0x10^5$	$5.8x10^5$	3.3	$4.6x10^5$	$4.3x10^5$	6.5	$7.2x10^5$	$6.9x10^5$	4.2	$6.3x10^5$	$6.1x10^5$	3.2
IAS 0.5%	0.5% sod. Thio-sulphate	$5.8x10^5$	$5.5x10^5$	5.2	$4.6x10^5$	$4.3x10^5$	6.5	$7.1x10^5$	$6.9x10^5$	2.8	$5.3x10^5$	$4.9x10^5$	7.6

QAS; quaternary active sterilizer, IAS; Iodine active sterilizer, Inh., Inhibition percentage.
Toxicity (A & B), inhibition% of the organism that obtained after 30 min. incubation with the neutralizer.
Efficiency (C & D), inhibition% of the organism obtained after 15 min. incubation with the disinfectant-neutralizer mixture.
Statistical analysis by t-test.

Table (2): Toxicity and efficiency of the neutralizers on the disinfectants in case of Gram-positive bacteria.

Disinfectants	Neutralizers	*S.aureus*					
		Toxicity			Efficiency		
		A	B	Inh.%	C	D	Inh.%
TH4+ 2%	Letheen broth	$3.4X10^5$	$3.3X10^5$	2.9	$1.5X10^5$	$1.4X10^5$	6.7
QAS 0.33%	Letheen broth	$4.5X10^4$	$4.3X10^4$	4.4	$4.8X10^5$	$4.4X10^5$	8.3
Tek-Trol 2%	0.3% lecithin + 3% Tween 80	$1.4X10^5$	$1.3X10^5$	7.1	$1.3X10^5$	$1.2X10^5$	6.9
Biocide-30 0.5%	0.5% sod. Thio-sulphate	$2.3X10^4$	$2.1X10^4$	8.7	$2.7X10^4$	$2.3X10^4$	4.4
IAS 0.5%	0.5% sod. Thio-sulphate	$5.5X10^5$	$5.1X10^5$	7.3	$4.4X10^4$	$4.1X10^4$	6.8

QAS; quaternary active sterilizer, IAS; Iodine active sterilizer, Inh., Inhibition percentage.
Toxicity (A & B), inhibition% of the organism that obtained after 30 min. incubation with the neutralizer
Efficiency (C & D), inhibition% of the organism obtained after 15 min. incubation with the disinfectant-neutralizer mixture.
Statistical analysis by t-test.

Table (3): Bactericidal effectiveness of disinfectant on pathogenic bacteria.

Organism	Time (min.)	Inhibition % of the organisms under the effect of Disinfectants														
		TH4+			QAS			Tek-Trol			Biocide-30			IAS		
		1:100 (1%)	1:200* (0.5%)	1:300 (0.33%)	1:400 (0.25%)	1:500* (0.2%)	1:600 (0.16%)	1:100 (1%)	1:256* (0.39%)	1:400 (0.25%)	1:300 (0.33%)	1:400* (0.25%)	1:500 (0.2%)	1:300 (0.33%)	1:400* (0.25%)	1:500 (0.2%)
E.coli	05	100	99.80	99.5	99.80	98.60	92.80	96.60	96.00	95.20	99.70	98.90	92.60	100	99.00	97.60
	10	100	99.96	99.7	100	99.10	97.90	99.30	98.30	96.90	99.90	99.80	95.80	100	100	99.30
	15	100	100	99.8	100	99.99	98.30	100	99.20	97.40	100	100	97.40	100	100	100
	20	100	100	99.9	100	100	99.40	100	100	98.70	100	100	99.50	100	100	100
	25	100	100	100	100	100	99.90	100	100	99.30	100	100	100	100	100	100
	30	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
S. pullorum	05	100	100	100	100	100	100	100	100	100	100	100	100	100	99.98	99.96
	10	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	15	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	20	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	25	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	30	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
S. aureus	05	99.10	99.00	76.4	91.70	71.60	70.70	96.00	91.30	87.10	88.60	78.60	71.40	98.30	91.90	90.10
	10	99.80	99.70	89.1	96.70	79.40	78.10	97.20	96.50	89.00	92.70	85.30	76.10	99.40	93.40	92.90
	15	100	100	91.3	98.00	83.30	81.30	99.90	97.80	96.40	96.90	93.90	83.40	99.80	96.90	96.00
	20	100	100	97.7	99.30	98.90	97.00	100	99.98	98.00	99.00	98.30	92.70	100	98.50	97.80
	25	100	100	99.8	100	99.90	99.30	100	100	99.10	100	99.20	96.20	100	99.20	98.30
	30	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

QAS, quatemary active sterilizer; IAS, Iodine active sterilizer
* recommended concentration by the producer.

Table (4): Inhibition percentages of the disinfectants in presence of 2% bovine albumin.

Disinfectants	Bacterial species											
	E.coli /time (min.)				*S. pullorum*/time (min.)			*S. aureus* /time (min.)				
	10	15	20	25	5	10	15	15	20	25	30	35
TH4+ (0.5%)	Nc	99.90	99.90	Nc	100	100	100	99.96	100	Nc	Nc	Nc
QAS (0.2%)	Nc	Nc	100	100	100	100	Nc	Nc	Nc	Nc	96.20	96.80
Tek-Trol (0.39%)	Nc	Nc	99.90	100	100	100	Nc	Nc	Nc	99.90	99.90	Nc
Biocide-30 (0.25%)	Nc	Nc	16.10	20.00	40.40	43.10	Nc	Nc	Nc	99.90	99.90	Nc
IAS (0.25%)	50	54.30	Nc	Nc	Nc	50.90	59.50	Nc	Nc	Nc	35.20	42.20

Nc, not conducted; QAS, quaternary active sterilizer; IAS, iodine active sterilizer.