Research Paper

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Primary Physiological Effect of the Sub-Inhibitory Concentrations of Formalin, TH4+ and Virkon-S on *Escherichia coli* Serotype O55: K39

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The primary physiological effect of TH4+ and Virkon-S on *E. coli* serotype O55: K39 cells in comparison to formalin was investigated. The *in vitro* activity of the disinfectants indicated that TH4+ and Virkon-S caused a leakage of sodium (Na), potassium (K) ions and UV-absorbing materials. The leakage level was directly proportional to disinfectant concentration and to the exposure time. Virkon-S recorded higher efflux of Na^+ and K^+ ions than TH4+ . The X-ray microanalysis for *E. coli* cells treated with Virkon-S recorded a low elemental level for P, S, K, Na, Cu, Zn and Mg. TH4+ caused a lowering in Na, K, S, Cl, Fe and P levels, whereas Cu and Zn levels were similar to the control. On the other hand, TH4+ showed a superior effect on the leakage of UV-absorbing material compared to Virkon-S. The amount of total protein of *E. coli* serotype O55: K39 decreased with the increase of disinfectant concentration. Virkon-S recorded the highest inhibition for protein synthesis, followed by formalin compared to TH4+ . The electrophoretic profiles demonstrated a considerable difference in the protein pattern of the treated cells as compared to the control. Formalin treated cells (0.015 and 0.007%) revealed that the absence of most of the protein bands between 18.5-36 kDa and all bands above 36 kDa. Virkon-S treated cells (at 0.015%) masked the bands at 36-330 kDa, whereas Virkon-S (0.007%) and TH4+ (0.007 and 0.015%) addition showed a typical protein banding which matched that of the control.

Key words: *Escherichia coli*, formalin, membrane potential, protein pattern, TH4+ , Virkon, X-ray-microanalysis

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Introduction

Escherichia coli infection in chickens is manifested in various forms. The most common disease syndrome is colisepticemia, which is characterised by airsacculitis, pericarditis, and perihepatitis. Bacteremia commonly occurs after primary infection of the upper respiratory tract with viral agents (Charleston *et al.*, 1998) and subsequent extension into the lower respiratory tract. *E. coli* infections account for significant morbidity and mortality in the poultry farms. The sprevention of *E. coli* infection through the bird's inhalation of dust and the control of colisepticemia are crucial problems in poultry industry. Several disinfectants are currently used in poultry farms and it is important to establish the activity of the emerging industrial biocides against pathogenic strains. Formalin has been extensively used with a considerable success in fumigation and disinfection practices in poultry farms. TH4+ is a patented formula of glutaraldehyde and a blend of 4 different quaternary ammonium biocides. Although glutaraldehyde and quaternary ammonium were individually proved to be effective disinfectants and the former is currently used in sterilizing medical devices (Sagripanti, 1992), little attention is directed towards the effect of the new combination. On the other hand, Virkon is a new type of disinfectants based on the acid per oxygen system, with multipurpose for use in hospitals and laboratories that carry no legal requirement for exposure monitoring (Anon, 1991). Recently, Virkon was used for chemical sterilization and high –level disinfection of dental instruments (Angelillo *et al.*, 1998). Although Virkon has been widely investigated with regards to its antimicrobial activity towards several strains of micro-organisms (Gasparini *et al.*, 1995; Coates, 1996; Ares-Mazas *et al.*, 1997), a little attempt has been made to establish a detailed mechanism by which this agent acts on *E. coli.* Direct comparative studies using these disinfectants under controlled conditions are also lacking.

In a previous work, electron microscope investigation demonstrated the morphological alterations of *Escherichia coli* serotype O55: K39 after the treatment with TH4+ and Virkon-S, whereas, Formalin treatment resulted in no changes in the morphology of this serotype except low pilli frequency (El-Naggar *et al.*, 2001). Therefore, this paper extends to deals with the study of the possible primary physiological changes in *Escherichia coli* serotype O55: K39 brought about by sub-inhibitory concentrations of TH4+ and Virkon-S in comparison to formalin.

Materials and Methods

Isolation of *Escherichia coli:* *E. coli* serotype O55: K39 was isolated from broiler chicken with symptoms of chronic respiratory disease lesions (pericarditis, perihepatitis and air saculitis) from poultry farm in Behera Governorate, Egypt. The isolated strains was then purified and identified as 055: K39 serotype using serological typing, antibiotic sensitivity assays, hemolysin test, *in vivo* and *in vitro* pathogenicity testing
(Sahaly, 1995). For the maintenance of this serotype, semisolid agar (4%) medium was used and the culture was kept at 5°C until use.

Disinfectants: Formalin (28%) was diluted with a sterile distilled water so as to obtain formalin concentrations of 0.06, 0.03, 0.015, and 0.007% respectively. TH4+ (Laboratories Sogeval Laval Cedex -France) is a patented formula contains a powerful hydrophilic biocide (Glutaraldehyde) activated by a specific blend of 4 different lipophilic quaternary ammonium biocides in a stable detergent solution with plant extracts (Pin oil, Terpineol). It was diluted with sterile distilled water to obtain a series of TH4 + concentrations (0.06, 0.03, 0.015 and 0.007%). Virkon-S (Antec International Ltd. Sudbury, UK) is a balanced, stabilized blend of per oxygen compounds, surfactant, organic acids and inorganic buffer system. This disinfectant was freshly diluted in a sterile distilled water immediately before use to obtain Virkon-S concentrations of 0.06 and 0.03, 0.015 and 0.007%.

Leakage of Na^+, K^+ and UV-absorbing materials: The cells in a logarithmic phase culture grown in trypticase soya broth (0.4 optical density at 540nm) were harvested by centrifugation at 2°C for 10 minutes at 1,700 rpm (Chilspin MSE Fisons centrifuge), washed with 0.05M HCl bis tris- buffer (pH; 7.0). The cells were then suspended in the same buffer to which the disinfectant was added and incubated with shaking at 37° C for 10 hours. The concentrations for each disinfectant were 0.06, 0.03, 0.015 and 0.007%. The control experiment was conducted without addition of disinfectant. Samples (10ml) were removed at zero time and after 2, 4, 6 and 8 hours. Samples were then filtered by passing through a membrane filter (Millipore filter, 0.45µm). The 5 ml filtrate was washed three times with an equal volume of ethyl acetate ($CH_3COOC_2H_5$). The organic layer was separated and then measured spectrophotometrically at 260nm using Perkin Elemer Lambda UV/VIS 4B spectrophotometer for the possible leakage of UV_{260} - absorbing material. For the determination of K^+ and Na^+ ions, 5-ml filtrate were used to estimate K^+ and Na^+ concentrations (ppm) using Cornning Clinical Flame photometer 410C according to Tanaka *et al.* (1988).

X-Ray-Microanalysis: The cells in a logarithmic phase culture grown in trypticase soya broth (TSB) of optical density of 0.4 (at 540nm) were harvested by centrifugation at 2° C for 10 minutes at 1,700 rpm, washed with 0.05M HCl bis- tris buffer (pH; 7.0) and then suspended in the same buffer to give an optical density of 0.2. To a set of 250ml Erlenmeyer flasks each contained 100ml bacterial suspension, Virkon-S was added to reach the final concentrations of 0.015 and 0.007%. To another set of bacterial suspension, TH 4+ was added to reach the final concentrations of 0.015 and 0.007%. The control suspension was without disinfectant addition. The flasks were incubated at 37° C with shaking for 10 hours. Cultures were centrifuged at 4000 rpm, 2°C for 15 minutes. The supernatant was then discarded and the cells were washed twice with glass distilled water to remove salts and debris. Centrifugation was performed as mentioned above after each wash. Samples were placed on the electron microscope stub and air-dried in vacuum-desiccator for 24 hr. at 25° C. Samples were analyzed using a scanning electron microscope (JOEL JSM 5300) with associated LINK AN 10000 analyzer (Sigee and Hodson, 1993). The analysis of cells was carried out using the following parameters: lifetime, 100sec, accelerating voltage, 15 KeV; detector- specimen working distance, 25mm; detector take off angle, 35°. Probe current was normally adjusted to give a count rate of approximately 10^3cps and was typically about 500 A. For each sample, the mean of the elemental mass fractions were calculated from analyses of 10 randomly chosen cells.

Determination of the total protein content: A loopful of *E. coli* cells was transferred into 250ml Erlenmeyer flask containing 50ml trypticase soya broth and incubated at 37° C for 20 hours to give a seed culture. One ml of this culture was transferred into each Erlenmeyer flask (250ml) each containing 100ml of TSB to make an optical density (at 540nm) of 0.4 and incubated at 37° C for 24 hours. To investigate the effect of disinfectants on protein level, disinfectants were individually added to the cultures after 2 hours

of growth (concentrations were 0.007 and 0.015% for each disinfectant). Five ml amount of each culture was removed at 2 hours time intervals and centrifuged at 3500rpm, 4° C for 10 minutes, the supernatant was discarded and the cells were resuspended in Tris-HCl, buffer (pH; 8.0) containing (O.1mg ml^{-1}) a cocktail of protease inhibitors (pepstatin, antipain, leupeptin) according to Dickson (1991). followed by lysis of cells to release of cellular proteins by sonication for 1 minute using the small probe of Ultrasonic Homogenizer (4710 series Cole-Parmer Instrument Co. Chicago, Illinois 60648). The samples were centrifuged as before and the total amount of protein preparation was determined using the procedure of Lowry *et al.* (1951) with bovine serum albumin (BSA) as the standard.

Polyacrylamide gel electrophoresis: The control and treated *E. coli* serotype O55: K39 cells were analyzed for their total protein to determine which proteins were lost upon disinfectant treatment. The total proteins from control and treated cells were subjected to SDS-PAGE (10% T) to determine if any particular protein species were preferentially removed or if the protein banding pattern had changed as a result of disinfectant treatment. The discontinuous sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) procedure of Laemmli (1970) was used to analyze protein preparations. Protein was mixed with sample buffer (3:1), which contained 0.5M Tris-HCl (pH 6.8), 17 % glycerol, 10% (w/v) SDS, 2.0% (v/v) 2-mercaptoethannol and 0.05% (w/v) bromophenol blue. Samples from the previous experiment were heated in a boiling water bath for 5 minutes and 40µl (approximately 4µg of protein) of this mixture was loaded onto a 3% stacking gel which overlay a 15% separating gel. Samples were electrophoresed at 30 mA until the blue indicator dye reached the bottom of the gel (approximately 4 hours). Following electrophoreses, gels were stained with Coomassie brilliant blue R250 and destined with 10% acetic acid (CH_3COOH) in 50% methanol (CH_3OH). Protein molecular- weight determination was made using protein standard marker.

Results

A wide range of concentrations for each disinfectant was tested (data not shown) and sub-inhibitory concentrations were only considered for this study. The growth of *E. coli* O55: K39 serotype was determined as turbidity measurements (Table 1) for the untreated (control) and disinfectant treated cultures. It is worthy to mention that Formalin showed a very close and quite similar turbidity record to TH4+ .

Release of UV- absorbing materials: The leakage of UV- absorbing materials (Table 2) was monitored for a period up to 8 hours for *E. coli* O55: K39. It was found that the leakage of UV_{260} - absorbing material is concentration dependent i.e., as the disinfectant concentration increases with the leakage of UV_{260} - materials did. This holds true for both TH4+ and Virkon-S. TH4+ at 0.06% recorded 4.8-fold increase in the leakage compared to control and 1.7-fold increase compared to the same concentration of Virkon-S. At 0.03% TH4+ , 2.1-fold- increase was recorded compared to Virkon-S after 6 hours. After 8 hours, TH4+ (at 0.015%), recorded 4.5- fold increase, whereas Virkon-S recorded 1.6 fold increase compared to control. Virkon-S had no effect at all on the leakage process at 0.007% concentration, whereas TH4+ recorded 4.1, fold increase in the leakage compared to control. It is worthy to note that formalin failed to induce any leakage from the *E. coli* cells at any of the concentrations used.

Ion leakage: The actual level of K^+ ions from *E. coli* cells was also proportional to the concentration of the disinfectant added (Table 3). The highest efflux of K^+ ions was recorded after 6hr. of incubation with TH4+ and Virkon-S at all concentrations used. After 4 hr. of incubation with Virkon-S at 0.06% recorded 6.3 fold increase compared to control and 2.7-folds compared to TH4+ at the same concentration. At 0.015% Virkon-S showed 1.7 fold increase compared to TH4+ at the same concentration after 8hr. of incubation. Virkon-S showed 1.5 fold increase compared to TH4+ at 0.03 and 0.007% after 8 hr. of incubation. The efflux of Na^+ ions from *E. coli* cells was directly proportional to the concentration and incubation time up-to 6 hours of incubation. The maximum % of Na^+ ion efflux for Virkon-S and TH4+ was recorded after 4 and 8 hr., respectively. Compared to control, Virkon-S at 0.06% recorded 13.7-fold increase after 4 hr compared to 5-folds increase for TH4+ after 8 hours (Table 4). At 0.03% Virkon-S recorded 8.2 fold increase compared to 2.8-folds for TH4+ at the same concentration. While Virkon-S at 0.015%, the release of Na^+ ions was 5.7 fold increase, whereas, TH4+ recorded 2.8-folds increase at the same concentration. Formalin was unable to release Na^+ or K^+ ions from the *E. coli* cells at any concentration.

X ray microanalysis: X-ray emission spectra from the control (untreated) cells of *E. coli* O55: K39 serotype had clear peaks of atomic P with the percentage occurrence of 38.7%, S (9.64%), plus monovalent [Na (16.5%), K (8.6%)] and divalent [Mg (5.32%)] cations (Fig. 1a). Small peaks of Cl (1.9%), Zn (5.0%), Cu (6.6%) were also identified. TH4+ treated cells at 0.007% (Fig. 1b) showed a reduction in S amounted to 58.5%, P (78%), K (95%), Na (94%) and Mg (81%). When TH4+ concentration of 0.015% was applied, further reduction [K (98%), Na (98%), Mg (96%), P (95%) and S (98%)] occurred. At both concentrations of TH4+ , Cu and Zn level remained constant (Fig. 1c). The use of Virkon-S at concentration of 0.007% resulted in a decrease in the levels of P (68%), S (17%) K (83%), Na (97%), Mg (82%), Cu (30%) and Zn (20%) as compared to control (Fig. 1d). Using the concentration of 0.015%, further decrease in elemental composition was also recorded (Fig. 1e) especially for K (97%), Na (99%), Mg (96%), P (68%), Cu (51%) and Zn (62%). The aluminum peak appeared in all traces is derived from stub preparation.

Total protein content: Data in Table 5 showed that increasing disinfectant concentration and its exposure time resulted in an extended inhibitory effect on the protein synthetic machinery. Virkon-S exhibited a superior inhibitory effect on protein synthesis compared to formalin and TH4+ . In a comparison to control culture, treatment of *E. coli* serotype O55: K39 for 90 min. with 0.007% Virkon-S, caused a reduction in the total protein amounted to 41.8%, whereas formalin and TH4+ recorded 31.97 and 7.93%, respectively. Doubling the concentration (0.015%) and exposure time (360 min) resulted in an extended reduction amounted to 37, 39 and 74% for formalin, TH4+ and Virkon-S, respectively.

Protein Pattern: The electrophoretic profiles (Fig. 2) showed a considerable differences in the protein content of the treated cells as compared to untreated control. Formalin treated cells (at 0.015 and 0.007%) for 360 min showed the deletion of protein bands between 36-330 kDa with the removal of most

Table 1: Growth pattern of *E. coli* 055: K39 serotype in absence (control) and in the presence of disinfectants TH4+ (T) and Virkon-S (V)

	Optical Density at 540nm for								
		0.06%		0.03%		0.015%		0.007%	
Incubation time (hr)	Control	T	V	T	V	T	V	T	V
0	0.127	0.127	0.127	0.127	0.127	0.127	0.127	0.127	0.127
2	0.273	0.106	0.120	0.165	0.177	0.240	0.237	0265	0.247
4	0.500	0.122	0.140	0.246	0.275	0.359	0.403	0.376	0.486
6	0.540	0.110	0.145	0.254	0.290	0.370	0.423	0.484	0.516
8	0.584	0.106	0.150	0.280	0.355	0.391	0.199	0.500	0.525

Table 2: Effect of TH4 + (T) and Virkon-S (V) on the leakage of the UV-absorbing materials from *E. coli* serotype 055: K39

	Absorbance at 260nm								
		0.06%		0.03%		0.015%		0.007%	
Incubation time (hr)	Control	T	V	T	V	T	V	T	V
0	0.140	0.140	0.140	0.140	0.140	0.140	0.140	0.140	0.140
2	0.150	0.420	0.270	0.365	0.254	0.340	0.225	0.310	0.158
4	0.160	0.610	0.380	0.589	0.350	0.540	0.268	0.510	0.163
6	0.170	0.816	0.490	0.799	0.373	0.731	0.270	0.719	0.170
8	0.170	0.807	0.580	0.781	0.370	0.770	0.277	0.703	0.170

Table 3: Effect of TH4 + (T) and Virkon-S (V) on the electrolyte leakage (K^+ ions) from *E. coli* serotype 055: K39

	Absorbance at 260nm								
		0.06%		0.03%		0.015%		0.007%	
Incubation time (hr)	Control	T	V	T	V	T	V	T	V
0	13	13	13	13	13	13	13	13	13
2	15	32	60	27	44	25	37	22	32
4	16	37	100	35	60	24	46	31	36
6	17	40	88	38	60	36	46	30	36
8	17	33	85	32	60	27	46	22	34

Table 4: Effect of TH4+ (T) and Virkon-S (V) on the electrolyte leakage (Na^+ ions) from *E. coli* serotype 055: K39

	Absorbance at 260nm								
		0.06%		0.03%		0.015%		0.007%	
Incubation time (h)	Control	T	V	T	V	T	V	T	V
0	3	3	3	3	3	3	3	3	3
2	4	8	68	6	66	4	48	4	29
4	7	16	96	14	58	12	40	10	25
6	9	28	68	26	52	24	34	22	24
8	10	50	68	36	52	28	34	24	24

Table 5: Effect of Formalin, TH4+ and Virkon-S concentrations and exposure time in relation to control (untreated cells) on protein content on *E. coli* 055: K39 serotype

		Protein concentration ($\mu g\ ml^{-1}$) after	
disinfectants	Disinfectant concentration (%)	90 min	360min
Formalin	0.015	39.65	80.42
	0.007	51.40	114.23
	0.015	51.56	100.96
	0.007	69.57	150.75
Virkon-S	0.015	25.18	43.56
	0.007	43.97	63.39
	-	75.56	165.75

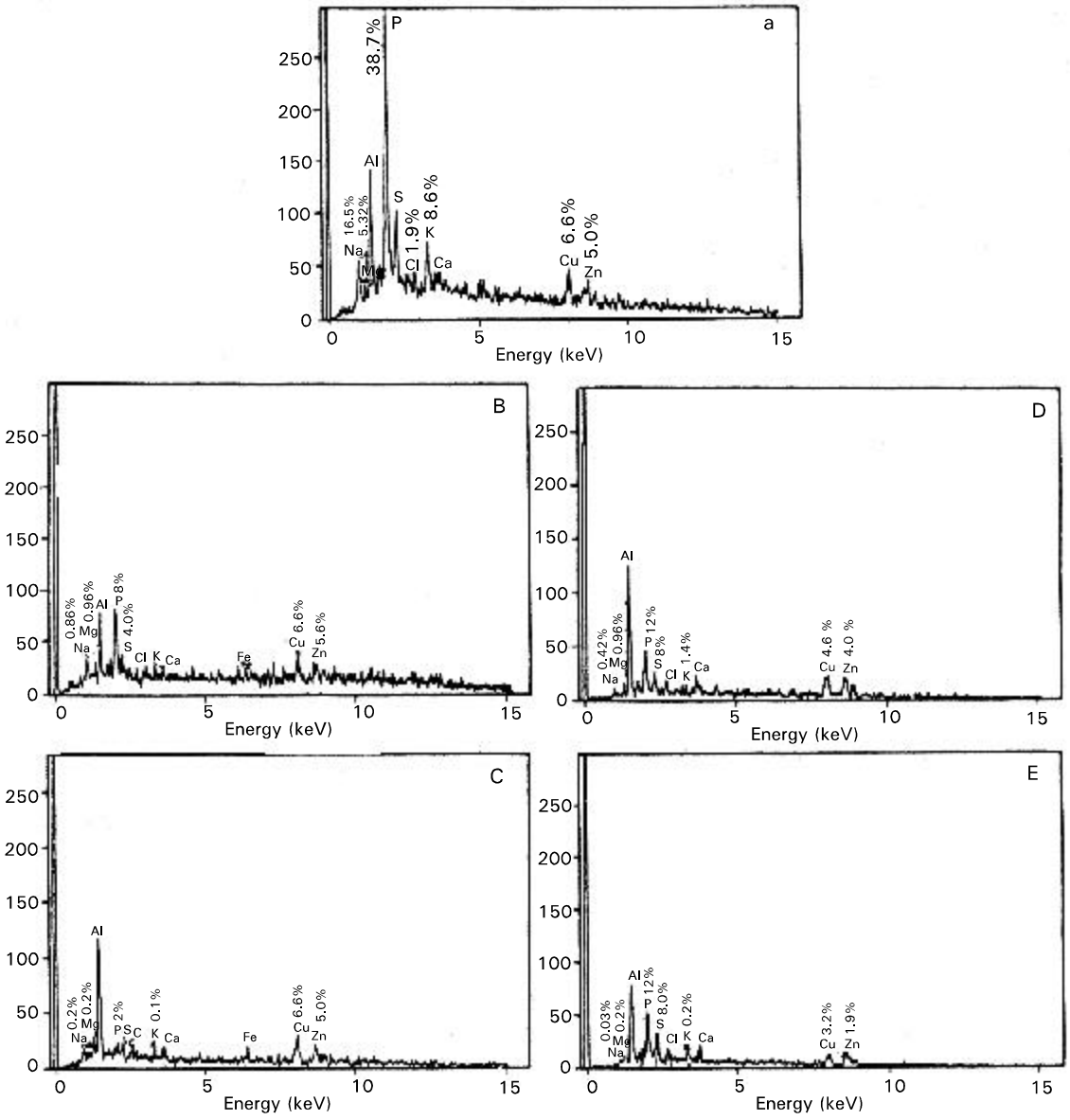

Fig. 1: X-ray emission spectra from the whole cells of *E. coli* of serotype 055: K39. (a) Control spectra for the untreated cells. (b) Cells treated with 0.007% TH4+ for 360min.(c) Cells treated with 0.015% TH4 for 360 min. (d) Cells treated with 0.007% Virkon-S for 360min and (e) Cells treated with 0.015% Virkon-S for 360 min.

of the bands in the region of 18.5-36 kDa (Lanes 1&2). Virkon-S treated cells showed no change when Virkon-S was added at 0.007%, whereas the removal of bands at 36-330 kDa occurred at 0.015% concentration (Lanes 3 and 4). TH4+ addition (Lanes 5 and 6) showed a typical protein banding matching that of the control (Lane 7). In addition, gel scans indicated that there was a consistent decrease in the amount of protein in treated as compared to the untreated cells (data not shown).

Discussion

The disruption of bacterial membranes function appears to be a common feature of the action of phenolic compounds and other antibacterial agents, which in turn is relatable to their antibacterial action (Hugo and Bloomfield, 1971; Chawner and Gilbert, 1989). This is mainly because the damage of cell wall alone does not kill the bacteria but an increase in the membrane permeability allows entry of bactericidal agents. In this investigation, Formalin did not affect the membrane potential, as there was no leakage at all detected. This confirms the electron microscope studies where no change in the morphology of *E. coli* O55: K39 serotype. The other two disinfectants altered the morphology of this serotype (El-Naggar *et al.*, 2001) differently at different concentrations, especially TH4+ which disrupted the cell surface membrane. TH4+ and Virkon-S are variably caused leakage of potassium(K), and 260nm absorbing materials.

This could be best explained on the ground that TH4+ consists of a hydrophilic biocidal glutaraldehyde which reacts strongly by cross linking with the amino groups of microbial proteins and a lipophilic biocidal quaternary ammonium compounds that disturbed the phospholipid bilayer to increase

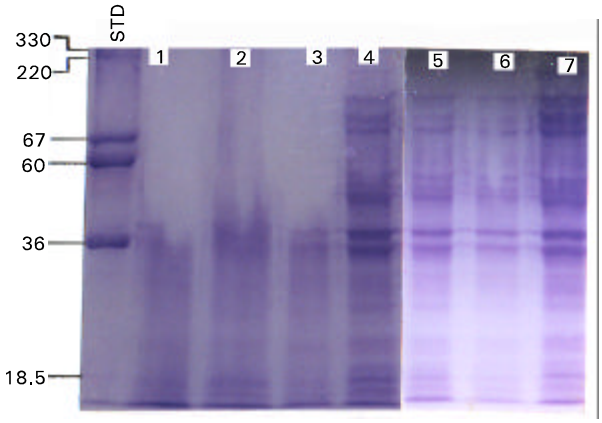

Fig. 2: SDS-PAE of *E. coli* 055: K39 serotype protein STD. Standard molecular weight marker. Lane 1: 0.0015% Formalin treated cells; Lane 2: 0.007% Formalin treated cells; Lane 3: 0.015% Virkon-S treated cells; Lane 4. 0.007% Virkon-S treated cells; Lane 5: 0.015% TH4+ treated cells, Lane 6: 0.007% TH4+ treated cells and Lane 7: Control. All *E. coli* 055: K39 cultures with or without disinfectant were incubated at 37°C for 360 min.

the leakage rate. On the other hand, Virkon-S is a powerful oxidizing agent that may cause an oxidation to any of the membrane components, which in turn helps, the leakage process to proceed. This is evidenced by the disappearance of certain protein bands from the electrogram and the appearance of spheroplasts as revealed by the transmission electron microscope (El-Naggar *et al*., 2001).

Research work on the membrane potential involved the influence of lactoferrin on the cell morphology of *E. coli* O157: H 7 was investigated by Shin *et al.* (1998) using Transmission electron microscopy. Their findings demonstrated that lactoferrin acts on the bacterial surface membrane and caused alterations in the cytoplasmic contents. Gudz and Pisko (1988) scrutinized the effect of thionium and ethonium (bis-quaternary ammonium compounds) which were found to increase the permeability of the cytoplasmic membranes of *E. coli* cells and to decline the activity of dehydrogenase complex enzyme. It has also been reported that some antibiotics promoted the extracellular release of verotoxins from enterohaemorrhagic *E. coli* (Bitzan *et al.,* 1992; Walterspiel *et al.,* 1992).

Among these antibiotics, some peptide antibiotics were found to induce membrane changes as staphylococci-like peptide Pep-5 produced by *Staphylococcus epidermidis* has been shown to cause the efflux of low-*Mr* cytoplasmic compounds (Sahl and Brandis, 1983). The peptide antibiotic nisin was also found to be a potential membrane active agent, where it caused a rapid efflux of amino acids from the cytoplasm of Gram-positive bacteria (Ruhr and Sahl, 1985). The data presented here collectively suggested that the primary target of TH4+ and Virkon-S is the cytoplasmic membrane. This is concluded from the observation that one of earliest events after disinfectant addition is rapid, non-specific efflux of different substrates like UV-absorbing materials and cations from *E. coli* cells and their membranes.

Leakage of sodium and potassium and some other ions was further confirmed by X-ray microanalysis. The later is a sensitive tool for intercellular element compartment, cell membrane damage, ion transport systems and ion shifts related to dynamic processes in cells (Zierold, 1988). This technique revealed the elemental composition of whole cell preparation and signified the low elemental level of the treated compared to untreated cells (control). The elemental composition of this serotype is of considerable interest in relation to a number of key aspects of its existence. For example, the low phosphorous content in both TH4+ and Virkon-S treated cells may reflect the low level of DNA synthesis and would justify the slowing growth rate. Changes in the concentration of specific cations, in particular, have been implicated in various functional processes-including bacterial changes during disease and chemical control measures (Sekizawa and Wakabayashi, 1990).

Although, reduction in the amount of the total protein after TH4+ treatment occurred, nevertheless, the protein pattern did not change which may suggest that this agent slows down the rate of protein synthesis rather than process inhibition, i.e., It affected the quantity rather than quality of the synthesized protein. On the other hand, Virkon-S and formalin stopped the expression of the protein between 36-330kDa. This could be supported by the finding of Paull and Fleming (1990) who reported that the exposure of W 3110 strain of *E. coli* K12 to low concentrations of glutaraldehyde or formaldehyde resulted in an unusual pattern of protein expression as determined by high resolution, two dimensional polyacrylamide gel electrophoresis.

References

Angelillo, I. F., A. Bianco, C. G. A. Nobile and M. Pavia, 1998. Evaluation of the efficacy of glutaraldehyde and per oxygen for disinfection of dental instruments. Lett. Appl. Microbiol., 27: 292 – 296.

Anon, 1991. Hospital stops glutaraldehyde use because of staff safety concern. The Pharmaceutical J., 247: 269.

Ares-Mazas, E., 1997. Effect of a commercial disinfectant ("Virkon") on mouse experimental infection by *Cryptosporidium parvum*. J. Hosp. Infect., 36: 141-145.

Bitzan, M., D. E. Muller-Wiefel, A. Schwazkopf and H. Karch, 1992. Zur rolle von antibiotika beim management des Verotxin-Assoziierten-Hamolytish- Uramichen Syndromes. Immunitat und Infektion, 20: 168-172.

Charleston, B., J. Gate, I. Aitken, B. Stephan and R. Froyman, 1998. Comparison of the efficacies of three fluoroquinolone antimicrobial agents, given as continuous or pulsed-water medication, against *Escherichia coli* infection in chickens. Anti. Agents Chemother., 42: 83-87.

Chawner, J. and P. Gilbert, 1989. A comparative study of the bactericidal and growth inhibitory activities of the bisbiguanides alexidine and chlorhexidine. J. Appl. Bacteriol., 66: 243- 252.

Coates, D., 1996. Sporicidal activity of sodium dichloro-isocyanurate, per oxygen and glutaraldehyde disinfectants against *Bacillus subtilis*. J. Hosp. Infect., 32: 283-294.

Coates, D. and M. Wilson, 1992. Powders, composed of chlorine-releasing agent acrylic resin mixtures or based on per oxygen compounds, for spills of body fluids. J. Hosp. Infect., 21: 241-252.

Dickson, A., 1991. Mammalian cells biotechnology, a practical approach. Ed. By Butler, M. Published by IRL Press, Oxford, UK.

El-Naggar, M. Y. M., M. A. Akeila, H. A. Turk, A. A. El-Ebady and M. Z. Sahaly, 2001. Evaluation of *in vitro* antibacterial activity of some disinfectants on E. coli serotypes. J. Gen. Appl. Microbiol., 47: 63-73.

Gasparini, R., T. Pozzi, R. Magnelli, D. Fatighenti, E. Giotti, G. Poliseno, M. Pratelli, R. Severini, P. Bonanni and L. De- Feo, 1995. Evaluation of *in vitro* efficacy of the disinfectant Virkon. Eur. J. Epidemiol., 11: 193 –197.

Gudz, O. V. and G. T. Pisko, 1988. Effect of quaternary ammonium salts on the functional state of cytoplasm membrane in *Escherichia coli.* Mikrobial. ZH, 50: 75- 78.

Hugo, W. B. and S. F. Bloomfield, 1971. Studies on the mode of action of the phenolic antibacterial agent fentichlor against *Staphylococcus aureus* and *Escherichia coli*. 1- the adsorption of fentichlor by the bacterial cell and its antibacterial activity. J. Appl. Bacteriol., 34: 557 – 567.

Laemmli, U. K., 1970. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature (London), 227: 680-685.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Paull, T. T. and J. E. Fleming, 1990. Upregulation of *E. coli* 38 kDa proteins induced by glutaraldehyde and formaldehyde. Curr. Microbiol., 21: 117-121.

Ruhr, E. and H. G. Sahl, 1985. Mode of action of the peptide antibiotic nisin and influence on the membrane potential of whole cells and on cytoplasmic and artificial membrane vesicles. Antimicrob. Agents Chemother., 27: 841-845.

Sagripanti, J. L., 1992. Metal- based formulations with high microbicidal activity. Appl. Environ. Microbiol., 58: 3157- 3162.

Sagripanti, J. L., C.A. Eklund, P. A. Trost, K.C. Jinneman, C. Jr. Abeyta, C. A. Kaysner and W. E. Hill, 1997. Comparative sensitivity of 13 species of pathogenic bacteria to seven chemical germicides. Am. J. Infect. Control, 25: 335-339.

Sahaly, M. E., 1995. Characterization of *E. coli* isolated from broilers. M. V. Sci. Thesis, Faculty of Veterinary Medicine, Alexandria University, Egypt.

Sahl, H.G. and H. Brandis, 1983. Efflux of low-M_r substances from the cytoplasm of sensitive cells caused by the staphylococci-like agent Pep-5. FEMS Microbiol. Lett., 16: 75-79.

Sekizawa, Y. and K. Wakabayashi, 1990. Bactericides. In Methods in phytobacteriology, ed. Z. Klement, K. Rudolph & D. C. Sands, pp.319-26. Budapest: Akademiai Kiado.

Shin, K., K. Yamauchi, S. Teraguchi, H. Hayasawa, M. Tomita, Y. Otsuka and S. Yamazaki, 1998. Antibacterial activity of bovine lactoferrin and its peptides against enterohaemorrhagic *Escherichia coli* O157: H7. Lett. Appl. Microbiol., 26: 407-411.

Sigee, D. C. and N. Hodson, 1993. Electron probe X-ray microanalysis of bacterial cells. In X-ray Microanalysis in Biology: Experimental Techniques and Applications (Sigee, D. C., A. J. Morgan, A. T. Sumner and A. Warley, editors). Cambridge University Press, Cambridge. UK.

Tanaka, H., K. Kawakita, H. Suzuki, P. S. Nakagawa and S. Omura, 1988. The mode of action of cervinomycin in *Staphylococcus aureus*. J. Antibiot., 42: 431-439.

Walterspiel, J. N., S. Ashkenazi, A. L. Morrow and T. G. Cleary, 1992. Effect of subinhibitory concentrations of antibiotics on extracellular Shiga-like toxin I. Infection, 20: 25-29.

Zierold, K., 1988. Electron probe microanalysis of cryosections from cell suspensions. In Methods in Microbiology, vol.20, Electron microscopy in microbiology, ed. F. Mayer, pp: 91-111. London; Academic Press.

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