RESISTANCE OF BACTERIA TO QUATERNARY AMMONIUM DISINFECTANTS

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SUMMARY

The resistance development to seven disinfectants containing quaternary ammonium compounds with addition of aldehydes and/or sequestrants was evaluated. Pseudomonas aeruginosa and Proteus vulgaris were used as tests organisms. In all cases after passaging an increase of resistance was observed: bacteria tolerated disinfectants in concentration lower (2 preparations) and higher (5 preparations) than those applied in practice.

Introduction

Disinfectants containing quaternary ammonium compounds (QACs) as active components are widely used in meat processing plants. Besides their numerous advantages like low toxicity, lack of specific color and smell, relatively weak corrosive effect, and good detergent characteristics - they have also some disadvantages. The most important are low bactericidal activity against Gram-negative bacteria and possibility of their resistance development to QACs. At the moment sequestrants and aldehydes are added to QACs disinfectants in order to increase their effectiveness, moreover sequestrants are added for additional protection against development of resistance [Mc. Gregor and Elliker, 1958].

In food industry the preparations with aldehydes content are used in limited scope because of their toxicity. They are exclusively recommended to disinfect surfaces having no contact with food (floors and walls in warehouses, production plants, etc.). The preparations are accepted to use in meat/food industry, among others in Germany [Reuter, 1993], Belgium, and lately in Poland.

Our investigations were aimed at estimation of resistance development to disinfectants of miscellaneous compositions from QACs group, and evaluation of the influence of additional substances (aldehydes, sequestrants) on resistance development.

Materials and methods

Test organisms: Proteus vulgaris NCTC 4635, Pseudomonas aeruginosa NCTC 6749 and ATCC 15442.

Disinfectants: Seven commercially available disinfectants were tested. The composition of preparations is presented in Table 1.

Method: The tests were performed according to the official Polish method [Krzywicka at al., 1987 a]. The bacteria were passaged at least 10 times on media with disinfectant added in increasing concentrations. After 3 days of incubation at 37°C the next passage was done. All tests were performed in triplicate.

Results and discussion

The addition of QACs disinfectants to the media caused turbidity, and in the highest examined concentrations a sediment in the bottom of the test tubes appeared. Bacteria in the medium with preparation created a mucous layer on the surface of the medium, encroaching on the tube's internal surface (P. aeruginosa), or formed clumps (P. vulgaris), however they did not grow regularly in all medium.

The results of the tests are presented in Table 2 and on the figures. It was found that the increase of bacteria resistance was higher in the case of P. vulgaris (7 to 375 times) than for P. aeruginosa (2 to 31 times). The resistant strains tolerated QACs in the concentration up to 0.6 - 0.7 %.

For preparations I and II (Fig. 1, Tab. 2) after passaging the level of bacteria resistance was small, practically not important. In the case of preparations III to VII the changes in resistance were significant: the disinfectants in the "use concentrations" were tolerated by P. vulgaris (III and VI - Tab. 2), P. aeruginosa (IV and V - Fig. 2, Tab. 2), and both bacterial strains (VII - Fig. 3). The "use concentrations" are concentrations recommended by manufacturer and evaluated through DGHM (Deutsche Gesellschaft fr Hygiene und Mikrobiologie) or DVG (Deutsche Veterinrmedizinische Gesellschaft) methods and estimated using modified AOAC carrier method obligatory in Poland [Krzywicka an al., 1987 b].

The phenomenon of resistance development to QACs is known for 25 years. The resistant bacterial strains were obtained in laboratories [Jaszczuk and Krzywicka, 1973] and isolated in hospitals [Kêdzia and Lipiñski, 1974]. In extreme cases disinfectant depended strains were isolated, which could grow only in the presence of disinfectants [Krzywicka an al., 1987 c].

According to the Codex Alimentarius [1988] - QACs should be used in food industry at a concentration 200 to 1200 milligrams per liter (0.02 to 0.12 %). Recently it was shown, that bacteria isolated from meat plants could grow in the presence of 0.15 % QAC [Lane, 1991]. Therefore the maximal QAC concentration recommended by the Codex Alimentarius (0.12 %) is slightly lower, than concentrations tolerated by resistant bacteria (0.15 %) isolated from meat plants, and much lower, than concentrations tolerated by resistant bacteria obtained by us in the laboratory (0.6 - 0.7 %).

During the last few years some aspects of the bacterial resistance to QAC were elucidated. It was shown, that Gram- negative bacteria producing mucous capsules, for example enterobacteria belonging to KES group (Klebsiella, Enterobacter, Serratia) can develop resistance. Capsules and cell walls act as partially permeable barriers for sublethal doses of QAC, to which the organisms can react. It is known, that mucous capsules are stabilized by calcium and magnesium ions. The addition of sequestrants "disintegrates" these structures leading to increased permeability of bacterial surfaces and higher activity (effectiveness) of the QAC [Lane, 1991].

Some authors suggest that development of resistance can be totally prevented by the addition of sequestrants binding bivalent metal ions [Lane, 1991]. Our investigations only partially have confirmed this thesis. We have observed increase of resistance to the QAC with and without addition of sequestrants (EDTA, Sequion, phosphates), but changes in the resistance in the presence of sequestrants were lower, sometimes practically insignificant (preparations I and II).

The addition of aldehydes did not prevent the development of resistance (preparations IV and V - Fig. 2, Tab. 2), but the increase of resistance was relatively low. In both cases P. aeruginosa strains were obtained, which tolerated preparations in "use concentrations".

Conclusions:

1. Gram-negative bacteria may become resistant to disinfectant of QACs group containing the addition of aldehydes and sequestrants.
2. Resistant bacteria can tolerate disinfectants in concentration higher then those applied in practice.
3. It is possible to compose the preparations in such a way that the resistance changes are minor, practically insignificant.
4. Resistance tests should be included in the methods of disinfectants' testing.

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List of tables and figures