ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. XXIX (4) SECTIO EE 2011

Department of Animal and Environment Hygiene, University of Life Sciences in Lublin,
Akademicka 13, 20-950 Lublin
e-mail: magdalena.pyrz@up.lublin.pl

MAGDALENA POPIOŁEK-PYRZ,
ANNA CHMIELOWIEC-KORZENIOWSKA, MONIKA SERAFIN-KOZAK, KAROL ABRAMCZYK

Estimate of the effectiveness assessment of selected disinfectants against *Staphylococcus aureus* strains isolated *in vitro* from bovine mammary gland

Ocena skuteczności działania wybranych preparatów dezynfekcyjnych wobec szczepów *Staphylococcus aureus* wyizolowanych z gruczołu mlekowego krów w badaniach *in vitro*

Summary. The effectiveness of five disinfecting preparations widely used in clinical practice against *Staphylococcus aureus* strains isolated from inflammatory secretion of the cow mammary gland was estimated. The paper presents a comparative analysis of the disinfecting agents in terms of their cidal activity, used at the concentration recommended (100%, 50%, 10%) by the manufacturer. The determinations were performed by the cylinder method. After the 18h-incubation time, it was found that all the disinfectants tested without dilution showed the strongest bactericidal potential against *S. aureus* strains. The disinfecting agents composed of active ingredients comprising phenols and hydrogen peroxide with chlorhexidine, irrespective of its concentration, exhibited the strongest bactericidal effect on *S. aureus*.

Key words: Staphylococcus aureus, mammary gland, disinfection, biocidal product

INTRODUCTION

Proper microbiological and cytological standards for raw milk ensure the safety of the consumers health and determine economic benefits of dairy cattle breeders. A problem of bovine udder disorders, especially mastitis proves to be the most common illness in dairy herds and to cause most important financial losses [Borkowska *et al.* 2006]. The disease occurs wherever and whenever the dairy cows are managed under the intensive

production system [Czerw *et al*. 2004, Sawa 2004]. In Poland, it is estimated that 30–80% of cows are affected by mastitis.

The countries that export milk and its products to the European Union member states are obliged to observe all the rules set out in the EU directives stating that drinking milk must come from healthy animals, managed in farms according to basic veterinary hygiene practices.

The most common and challenging infectious agent involved in bovine mastitis is *Staphylococcus aureus*. Staphylococci are ubiquitious in the environment, occurring on the skin and mucous membranes of human and animals. Beside live organisms, the microbes exist in air, on objects, in soil, sewage, milk and milk products. These bacteria survive well in the dry environment, are present in dust that constitutes another infection source for farm animals. In the case of compromised immunity system or penetration of host tissues by staphylococci, the partnership turns into parasitism. [Markiewicz and Kwiatkowska 2006]. Mammary gland infection caused by this bacteria is usually long lasting and may be transmitted to the successive lactations causing a milk yield decrease. Infections of the cow udder induced by coagulase – positive staphylococci are critical from an economic and sanitary aspects. A clinical form of mastitis is very difficult to successful treat and cure. Importantly, subclinical infections are for the most part asymptomatic and that poses a serious threat for healthy animals exposed to infectious agent in the herd [Fox *et al*. 1991].

One of most important mastitis-prevention practices is teat disinfection. Choice of a suitable bactericidal agent decreases the risk of new infection incidence. Combating the infection is extremely difficult due to low efficacy of the antimicrobial therapy associated with resistance of many *S. aureus* strains to antibiotics applied in the veterinary medicine [Borkowska *et al*. 2006].

The objective of the studies was to estimate the effectiveness of selected disinfectants against the *Staphylococcus aureus* strain isolated from bovine mammary gland with mastitis.

MATERIAL AND METHODS

The study was conducted on *Staphylococcus aureus* strain isolated from the inflammatory secretion of the mastitic cow udder. The microbial identification was performed by the biochemical tests API STAPH (Biomerieux, Poland) according to the manufacturer`s instructions.

The studies on the susceptibility to disinfectant agents included the following preparations:

Preparation 1 – active substance: ethanol, hydrogen peroxide and chlorhexidine;

Preparation 2 – active substance: ethanol, propan-2-ol, benzyl alcohol;

Preparation 3 – active substance: glucoprotamine – amine derivative;

Preparation 4 – active substance: hydrogen peroxide and chlorhexidine;

Preparation 5 – active substance: triclosane-phenol.

The chosen disinfectants were evaluated at three different concentrations: 100%, 50% and 10%. The studies were performed using the cylinder method on Petri dishes of 100 mm diameter. There was added 0.02 ml of 18h-broth culture medium of the isolated

Staphylococcus aureus strain to the 14 ml of 2% enriched agar (BIOMED, Poland) heated until it melted into liquid (48ºC). After the careful mixing of agar and the bacterial suspension, the mixture was poured into Petri dishes. Then, once the medium has solidified, cylinders made of stainless and acid-proof (1H18N9T) of 10 × 6 mm were placed on it and filled with the analyzed disinfectants, 0.3 ml each. Having filled the cylinders, the plates were incubated at 37ºC temperature for 18 hours in an incubator. Afterwards, the presence or absence of microbial growth inhibition zones surrounding the cylinders was checked and the diameters of the obtained zones of inhibition were measured. There were 10 replications performed for each preparation and dilution. A total of 150 determinations were made. The obtained research results were analyzed statistically by t-Student test.

RESULTS AND DISCUSSION

A problem of disinfecting agents applicability in the zootechnical sector and assessment of their effectiveness in bacteria growth inhibition, including major mastitis pathogens, like *Staphylococcus aureus*, is of primary importance. Importantly, increasing resistance developed by bacteria against disinfectants resulting from massive use of chemotherapeutical drugs makes the problem crucial.

The prophylactic measures against mastitis include washing the skin with a liquid disinfectant prior to milking and dipping the teats with a suitable liquid agent immediately after it. Besides, cleanliness and strict hygienic measures applied at the cow housing facility play a key role as well [Skrzypek *et al*. 2004]. The present paper analyzes the in-vitro tested efficacy of five disinfectants commonly applied in the medical practice to disinfect hospitals, outpatient clinics and health centers. All these preparations belong to the multi-component disinfecting agents comprising several active biocidal ingredients, in that oxidizing substances, phenols, alcohols, amine derivatives or biguanides.

The present experiment indicated that the activity of the analyzed preparations was concentration-dependent and thus, increasing concentration caused gradual inhibition of bacteria growth (Tab. 1). Among the tested undiluted substances, the highest inhibitory activity was shown by Preparation 5 and slightly lower – Preparation 4. A similar trend was observed with the preparation at 50% concentration. Evaluating the growth intensity at 10% concentration the complete lack of an inhibitory effect of Preparation 3 was found. Alike, Preparation 2 exhibited much weaker activity against the tested strain. Figure 1 presents the tested disinfectants in the rank order of their efficacy. The strongest bactericidal activity towards *Staphylococcus aureus*, irrespective of concentration, was shown by phenols (Preparation 5) and hydrogen peroxide with chlorhexidine (Preparation 4). The alcohol addition to Preparation 1 reduced its overall bactericidal activity. However, Matuska and Moulis [2000] reported that the combination of oxidizer, chlorhexidine and alcohol applied for hygienic hand disinfection had high bactericidal activity. Only slight effect on inhibition of *Staphylococcus aureus* growth tested in-vitro was stated by Głuszek [2010]. Ethanol applied at concentrations ranging from 10 to 60% did not inhibit the growth of staphylococci. These findings agree with the results reported by Bocian and Tyski [2004] who indicated that the optimal cidal concentration of alcohols

ranges between 60 and 90%. However, at the concentration below 50%, their activity is considerably reduces.

In the present study, some controversy arose over the results concering Preparation 3 composed mainly of glucoprotamine – an amine derivative. This preparation applied at 10% concentration showed a complete inhibition of bactericidal activity against the analyzed strain, whereas Tyski *et al*. [2009] and Grzybowska *et al*. [2010] reported its high effectiveness even at much lower concentrations. Grzybowska *et al*. [2010] determining bactericidal capacity with the quantitative suspension and carrier tests indicated a high bactericidal potential of glucoprotamine towards multidrug resistant clinical isolates of bacteria as well. The tested strains were recovered from the isolated human clinical material.

Table 1. Growth inhibition zones of *Staphylococcus aureus* of tested disinfection preparations (mm)
Tabela 1. Strefy hamowania wzrostu *Staphylococcus aureus* badanych preparatów dezynfekcyjnych (mm)

Preparation Preparaty	Concentration of the preparations Koncentracja preparatów								
	100%			50%			10%		
	M	SD	V%	M	SD	V%	M	SD	V%
1	37.31	1.06	2.84	18.04	0.99	5.53	7.19	4.97	65.19
2	30.21	1.00	3.31	14.91	1.06	7.14	4.04	5.21	129.11
3	26.28	0.66	2.54	18.06	1.25	6.92	0	0	0
4	45.51	1.23	2.7	34.94	1.97	5.65	12.4	0.86	6.94
5	59.89	1.92	3.21	39.97	2.96	7.40	10.27	3.74	36.47

M – mean zone of growth inhibition/średnia strefa hamowania wzrostu
SD – standard deviation/odchylenie standardowe
V% – variability coefficient/współczynnik zmienności

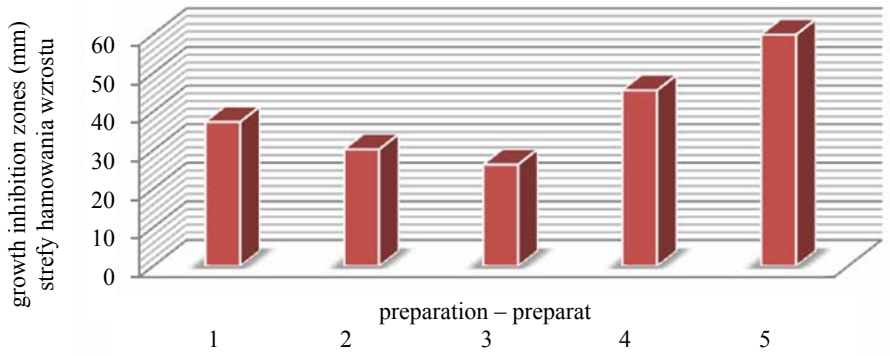

Fig. 1. Effectiveness of chosen preparations against *Staphylococcus aureus*
Ryc. 1. Skuteczność działania wybranych preparatów wobec *Staphylococcus aureus*

The monitoring studies to check the effectiveness of the commercial disinfectants seem to be indispensable due to the fact that disorders of bovine mammary gland have

remained major disease affecting cow udder. The use of inappropriate active substances against the specific strains characteristic of a given environment (here mastitis pathogens), especially preparations applied at improper concentration may cause the development of antimicrobial resistance – AMR in bacterial pathogens [Truszczński and Pejsak 2010]. Consequently, the effectiveness of mastitis prophylaxis is reduced and the incidence rate increases.

CONCLUSIONS

1. *Staphylococcus aureus* strains exhibit lowered susceptibility to disinfecting agents. Therefore, it is necessary to develop novel disinfecting formulas as well as to continuously change the disinfection preparation used.

2. Only well chosen and applied at the appropriate concentration disinfecting agents guarantee achieving the desired results. Disinfection practices contribute to breaking the routes of bacterial transmission, substantial decline of the infection rate and mastitis incidence.

3. Statistical analysis revealed no significant differences between the effectiveness of the disinfecting agents.

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Streszczenie. Oceniono skuteczność działania pięciu preparatów dezynfekcyjnych, stosowanych powszechnie w praktyce klinicznej, wobec szczepów *Staphylococcus aureus* wyizolowanych z wydzieliny zapalnej gruczołu mlekowego krów. W pracy dokonano analizy porównawczej wymienionych środków pod względem ich aktywności bójczej, stosując je w stężeniach zalecanych przez producenta (100%, 50%, 10%). Oznaczenia wykonano metodą cylinderkową. Po 18-godzinnej inkubacji stwierdzono, że najsilniejsze działanie bakteriobójcze wobec szczepów gronkowca złocistego wykazały wszystkie preparaty zastosowane bez rozcieńczenia. Najsilniejsze działanie bakteriobójcze wobec *Staphylococcus aureus,* niezależnie od stężenia wykazywały środki dezynfekcyjne, gdzie substancję aktywną stanowiły fenole oraz nadtlenek wodoru z chlorheksydyną.

Słowa kluczowe: *Staphylococcus aureus*, gruczoł mlekowy, dezynfekcja, produkty biobójcze