UNIVERSITA' DEGLI STUDI DI MILANO
ISTITUTO DI MICROBIOLOGIA MEDICA
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Perstorp Chemitec S.p.A.

EVALUATION OF THE ANTI-MICROBIAL ACTIVITY OF UREA RESIN SAMPLES WITH AND WITHOUT ADDITION OF A 2nd GENERATION ANTISEPTIC

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INTRODUCTION

Perstorp Chemitec S.p.A., a firm based in Castellanza (VA) Via Sempione 13, entrusted us to carry out an evaluation study on the anti-microbial activity of traditional urea resin with and without the addition of a 2nd generation antiseptic.

The customer supplied us with a proper number of rectangular pebbles (sides 5.2 x 6.2 cm) marked with the following letters:

- A= urea resin
- C = urea resin with addition of a 2nd generation antiseptic

MATERIAL AND METHODS

The following microbial strains, recently isolated from biological materials obtained from hospitalised patients, were selected:

- *Streptococcus pyogenes* group A (isolated from pharyngeal swab)
- *Enterococcus faecalis* (isolated from urine)
- *Staphylococcus aureus* (isolated from surgical wound pus)
- *Escherichia coli* (isolated from urine)
- *Klebsiella pneumoniae* (isolated from sputum)
- *Proteus vulgaris* (isolated from urine)
- *Salmonella typhi* (isolated from faeces)
- *Candida albicans* (isolated from vaginal swab)
- *Pseudomonas aeruginosa* (isolated from sputum)

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These strains, excluding *Candida albicans*, were cultured in Brain Heart Infusion broth (DIFCO) and incubated at 37°C for 18-24 h in order to obtain an abundant bacterial growth giving rise to a bacterial concentration of approximaly 10^9 C.F.U./ml.

C. albicans was grown on Sabouraud dextrose broth (DIFCO) and incubated at 37°C for 24 h.

Broth-cultures were the appropriately diluted with sterile physiological solution in order to obtain a final microbial concentration ranging from 4.6×10^4 to 1.4×10^5 CFU/ml.

3 ml of each broth-culture were added on the surface of different plates containing the urea resin samples with and without the addition of the 2nd generation antiseptic, as well as on a sterile Petri dish. The latter was used as a control to quantify the microbial inoculum in contact with the different urea resin samples and to monitor for any bacterial growth in the broth-culture in contact with an inert matter such as plastic.

The tests were performed in strict conditions of asepsis. The microbial concentration of each strain kept in contact with the different urea resin samples was verified at time 0, 4, 8 and 24 hours.

During the tests, the plates containing the urea resin samples were placed inside sterile Petri dishes and kept at room temperature.

At different times 100 µl of inoculum was collected from the surface of the different plates and Petri dishes, diluted 1:10, 1:100 and 1:1000 and transferred to Mueller

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Hinton agar plates (DIFCO) or dextrose Sabouraud agar plates in case of *Candida albicans*.

The different dilutions of the microbial strains were uniformly streaked on the surface of each culture plate in order to obtain a suitable microbial growth to allow an adequate colony count. Culture plates were then incubated at 37°C for 24 - 48 h.

Culture plates were read only when containing from 30 to 300 colonies. The number of colonies counted in each culture plate was multiplied by the inverse factor of dilution in order to determine the number of microbial cells on the urea resin samples at different times. The bactericidal effect was defined as occurring when there was a reduction of at least 99.9% of the original bacterial inoculum.

RESULTS

The results are shown in table 1.

After 4 h of contact with the two urea resins, Group A *Streptococcus pyogenes* showed a slight decrease of bacterial concentration; this effect was much more evident after 8 h with both resin samples. After 24 h the antiseptic supplemented resin demonstrated a complete bactericidal effect. Antibacterial activity of resin containing the antiseptic resulted always higher than that of the resin without antiseptic.

With regards to *Enterococcus faecalis*, notoriously resistant to most antibiotics, a slight reduction of the bacterial inoculum was observed after 4 and 8 h, in urea resin

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both with and without antiseptic. A complete bactericidal effect was achieved only for the antiseptic added resin after 24 h.

Urea resin without antiseptic showed a marginal reduction in the number of *Staphylococcus aureus* bacterial cells after 4 h and a more marked reduction after a period of 8 h, reaching a decrease of one logarithm (90% reduction) as compared to the control examined after 24 h. On the other hand, the resin with antiseptic determined a more marked decrease in the bacterial inoculum after 24 h, achieving the complete bacterial killing.

In the case of *Escherichia coli*, a significant increase in the viable cells (from 100.000/ml to 8.000.000/ml) was noted in the control during the first 24 hrs. Starting from the 4th h of contact, both resin samples showed a decrease of bacterial inoculum as compared to the control, reaching a bactericidal effect after 24 h with the resin without antiseptic and complete bacterial killing in the case of the resin with antiseptic.

With *Klebsiella pneumoniae* an increase of bacterial cells was noted after 24 h. Both resin samples proved active already after a contact of 4 h. The resin with antiseptic showed a clearly higher effect in comparison with the one without antiseptic, reaching also in this case complete bacterial killing after 24 h.

For *Proteus vulgaris* , an increase in the number of colonies present in the control after 4, 8 and 24 h was found, while both resins determined a significant decrease of bacterial inoculum after 24 h; in particular, the resin with antiseptic determined a complete bactericidal effect.

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Versus *Salmonella typhi*, urea resin with antiseptic proved clearly more active than the resin without antiseptic; a good activity was already detectable after 8 h and a complete bactericidal effect was achieved after 24 h.

The 24 h control showed a marked increase in the bacterial count for *Pseudomonas aeruginosa.* Both resins showed bactericidal activity versus this strain, with a more marked effect for the antiseptic containing resin with 100% killing of bacterial inoculum after 24 h.

Versus *Candida albicans* both resin samples showed a moderate activity during the first 8 h. After 24 h the resin with antiseptic showed a better activity, causing a fall in the bacterial inoculum concentration of more than a logarithm, even if not determining a bactericidal effect. At 24 h the resin without antiseptic resulted almost inactive.

CONCLUSIONS

The results obtained in this study allow us to make some conclusive considerations.

The urea resin added with the 2nd generation antiseptic showed a very good antimicrobial activity during the 24 h test, determining complete bacterial killing (as to reach a complete drop of microbial concentration) of all considered strains with the exception of *Candida albicans*.

The resin without antiseptic showed a lower ability to cause a fall in the bacterial inoculum so that the bactericidal effect was achieved after 24 h only for *E. coli* and *P. aeruginosa*.

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For *C. albicans* strains, which are known to be resistant to many antimicrobial agents, a bactericidal effect was not observed during the first 24 h of contact with either the considered urea resins.

In conclusion, the urea resin containing the 2nd generation antiseptic determined a better antimicrobial effect than the resin alone on all microbial species examined, isolated from hospitalised patients and therefore more resistant to antibacterial agents than micro-organisms isolated in the community.

Milan, 14th November 2001

Prof. Roberto Mattina

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Table1 – Antibacterial activity of urea resin samples without antiseptic (A) and with antiseptic (C).

Micro-organisms	h	Control CFU/ml	Urea resin A CFU/ml	Urea resin C CFU/ml
Streptococcus pyogenes				
	0	140.000	140.000	140.000
	4	69.000	65.000	49.000
	8	45.000	2.600	2.400
	24	7.400	20	0
Enterococcus faecalis				
	0	110.000	110.000	110.000
	4	81.000	76.000	69.000
	8	76.000	64.000	55.000
	24	66.000	31.000	0
Staphylococcus aureus				
	0	100.000	100.000	100.000
	4	67.000	65.000	32.000
	8	61.000	35.000	8.800
	24	39.000	3.900	0
Escherichia coli				
	0	100.000	100.000	100.000
	4	130.000	120.000	79.000
	8	250.000	85.000	20.000
	24	8.000.000	6.600	0
Klebsiella pneumoniae				
	0	100.000	100.000	100.000
	4	130.000	120.000	79.000
	8	250.000	85.000	20.000
	24	8.000.000	6.600	0
Proteus vulgaris				
	0	94.000	94.000	94.000
	4	110.000	120.000	64.000
	8	160.000	100.000	15.000
	24	1.400.000	16.000	0
Salmonella typhi				
	0	100.000	100.000	100.000
	4	88.000	83.000	61.000
	8	110.000	77.000	35.000
	24	970.000	26.000	0
Pseudomonas aeruginosa				
	0	84.000	84.000	84.000
	4	83.000	47.000	51.000
	8	270.000	46.000	4.500
	24	6.100.000	2400	0
Candida albicans				
	0	46.000	46.000	46.000
	4	52.000	12.000	18.000
	8	41.000	13.000	18.000
	24	49.000	41.000	4.000