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Comparison of Dey and Engley (D/E) Neutralizing Medium to Letheen Medium and Standard Methods Medium for recovery of *Staphylococcus aureus* from sanitized surfaces

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SUMMARY

The ability of Dey and Engley (D/E) Neutralizing Medium to recover *Staphylococcus aureus* ATCC 6538 from tile surfaces exposed to a commercial phenol (Mikro-Bac) and a quaternary ammonium compound (Mikro-Quat) was compared to recovery with Letheen Medium. Standard Methods Medium was used as a control recovery medium. Organisms were exposed to both antimicrobials for varying time periods, then were recovered by swab and Rodac plate on both test media. The recovery by either procedure was significantly higher with Dey and Engley (D/E) Neutralizing Medium than with Letheen and Standard Methods Medium. The D/E Medium shows promise for evaluating antimicrobial chemicals used in environmental sanitation.

INTRODUCTION

The antimicrobial activity of a sanitizing chemical is assessed by allowing the agent to come in contact with organisms for a given period of time [1]. The remaining viable organisms are then recovered on a medium containing a specific neutralizer with capacity to inactivate the carried-over test agent [1,7]. In the absence of a neutralizer, the chemicals inhibit the growth of viable organisms [2]. The ability of a recovery medium to inactivate an agent is therefore essential to assess the antimicrobial activity of the agent used for controlling microbial contaminants on environmental surfaces.

The recovery of chemically-exposed organisms in a medium depends upon the neutralizing capacity of a medium which, in turn, is controlled by the specificity and the concentration of a neutralizer in the medium [7]. Lack in either requirement allows the inhibitory action of a carried-over chemical to continue in the recovery medium and inhibit viable organisms. As a result, a bacteriostatic chemical may appear bactericidal. Therefore, a recovery medium must have a specific neutralizer present at the appropriate concentration for the antimicrobial chemical under evaluation [7].

An earlier study showed that recovery of chemically-treated *S. aureus* by Dey and Engley (D/E) Neutralizing Medium was significantly higher than Standard Methods Agar with or without Tween 80 and lecithin [4]. The organism recovery rate after various times of exposure indicated that the increases were due to the concentration and specificity of neutralizers present in Dey and Engley (D/E) Neutralizing Medium [5]. The present research compares the capacity of Dey and Engley (D/E) Neutralizing Medium [3] with Letheen Medium [1] for recovering surface organisms exposed to two sanitizing chemicals, using a modified swab technique and Rodac plate procedure to transfer the exposed organisms to the recovery medium.

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MATERIALS AND METHODS

Organism

Staphylococcus aureus ATCC 6538 was used. An inoculum of approximately 150 organisms was denoted as low inoculum (LI) whereas an inoculum of approximately 300 organisms was denoted as high inoculum (HI).

Media

Nutrient Broth (Difco, Detroit, MI, USA) was used for growing the organism and for preparing the culture inoculum. With Standard Methods Medium [8] as the control recovery medium, the recovery of chemically-treated organisms at various time intervals by Dey and Engley (D/E) Neutralizing Medium [3] was compared with recovery on Letheen Medium [1].

Antimicrobial agents

The aqueous solutions of a 1:128 dilution of a commercial phenol 'Mikro-Bac' and a commercial quaternary ammonium compound 'Mikro-Quat' (Economics Laboratories, St Paul, MN, USA) were used in the study. The dilution used was the dilution suggested by the manufacturer.

Procedure

The procedure for recovering chemically-exposed *S. aureus* with swab and Rodac plate is presented diagrammatically (Fig. 1). Floor tiles 2″ by 2″ wide were sterilized with ethylene oxide gas. One-tenth milliliter of nutrient broth previously adjusted to contain approximately 150 *S. aureus* ATCC 6538 cells (LI) was pipetted to two sets of 15 floor tiles, then evenly spread with separate glass angle rods. Each of three tiles in a set serving as a control was swabbed with a sterile cotton swab, then the swab was transferred to 5 ml of broth made with one of the three kinds of media being tested, shaken thoroughly and then plated with 20 ml of the corresponding agar.

One-tenth milliliter of Mikro-Bac was applied to each of 12 tiles in one set and 0.1 ml of Mikro-Quat to each of 12 in the other set. After 0, 5, 15, or 30 min of exposure, all three tiles in one set were swabbed with a sterile cotton swab. These swabs were handled in the same manner as the control swabs. The plates were incubated at 37 °C; after 24 h colonies on each plate were counted.

The above procedure was repeated with another two sets of tiles with approximately 300 *S. aureus* ATCC 6538 cells (HI). The procedure with Rodac plate was the same except each tile in each set was sampled with a plate containing a test agar.

RESULTS

The organism recovery by swab and Rodac plates with Dey and Engley (D/E) Neutralizing Medium (D/E medium), Letheen Medium (LT medium) and Standard Methods Medium (SM medium) at various time intervals were plotted as curves, an example of which is shown in Fig. 2.

The raw data indicated that by either procedure the number of organisms recovered with SM medium [8] were few and no recovery was made after 5 min exposure. Thus, the average and the mean response of recovery data were analyzed statistically at the four exposure intervals for D/E medium [3] and LT medium (1). There was a significantly higher mean recovery with D/E medium in every case (P-value <0.001).

To show the difference in the recovery rate over time between D/E and LT medium more explicitly, estimates of recoveries and change in the average relative recovery with time of exposure were made using linear regression analysis for all treatments (number of organisms exposed, antimicrobials and method of recovery). As the variances for higher mean recoveries were larger than for smaller recoveries (variability should be proportional to the square root of the mean response), a weighted linear regression of the natural logarithm of mean recovery versus the time of exposure was computed, where the weight was proportional to the mean recovery raised to the 1.5 power.

The regression equation used to derive the intercept and slope was:

$$\ln(x) = a + b \text{ (time of exposure)}$$

where $\ln(x)$ is the natural logarithm of the mean recovery at the specific time of expose, a is the intercept and b is the slope. The regression analyses were performed on the SAS-PC, release edition 6.4 (SAS, Carey, NC, USA).

The regression analysis result is presented in Table 1. The anti-natural logarithm of the intercepts estimates the recovery of organisms at time zero. The slope multiplied by 100 estimates the change in the recovery of organisms over time of exposure in units of percent change per minute of exposure. The regression was calculated for those exposure times where a positive recovery was made with a medium and procedure when a certain level of inoculum was treated with an antimicrobial.

The slope values for the D/E medium were higher than those for the LT medium for all seven cases where a comparison was possible (no comparison could be made for Mikro-quat, with Rodac plate). The averages for the D/E and the LT slopes, −3.06% per minute and −6.31% per minute respectively, indicate that decrease in the recovery with time of exposure was approximately 50% less for the D/E medium than that for the LT medium.

Using a paired sample t-test, with six degrees of freedom, the statistical significance of the difference between the two averages is P-value ~0.001. Thus, the average difference between the relative change in recovery for the two media is highly significant.

The estimated recoveries at time zero were higher for the swab than those for Rodac plate in all seven cases. The ratios of the recovery using the swab to the recovery with Rodac plates were not large except for Mikro-Quat with the LT medium, where the ratio is approximately 5. The geometric mean of the remaining ratios for recovery using the swab to recovery with the Rodac plates is approximately 1.5. This difference in paired sample t-test with five degrees of freedom on the intercepts derived from the regressions, is statistically significant at $P = 0.02$ level.

DISCUSSION

The recovery of organisms was significantly higher with D/E and LT medium compared to SM medium. This indicates that the carried-over Mikro-Bac and Mikro-Quat were respectively inactivated by Tween 80 and lecithin present in LT and D/E media. However, the organism recovery with D/E medium over time was significantly higher than LT medium. This may be due to the increased concentration of lecithin in D/E medium which is known to have phenol-neutralizing capacity [5,6]. The higher concentration of lecithin could also be the reason for the higher recovery rate for the Mikro-Quat-treated organisms on D/E medium [5].

The recovery for a medium increased with swab sampling compared to the Rodac plate procedure for two reasons. First, the mechanical action of the swabbing procedure removes more organisms from surfaces than the Rodac plate, which relies on simple contact with the surface. Secondly, rinsing of the swab in broth before plating, dilutes the effect of a carried-over antimicrobial agent. Some viable organisms exposed to Mikro-Bac for only a short time were recovered by swab on SM medium which contains no specific neutralizers for phenol (Fig. 2). However, the increase in organism recovery with D/E and LT by swab was due to stepwise reduction in the strength of carried-over antimicrobial agents. This reduction was

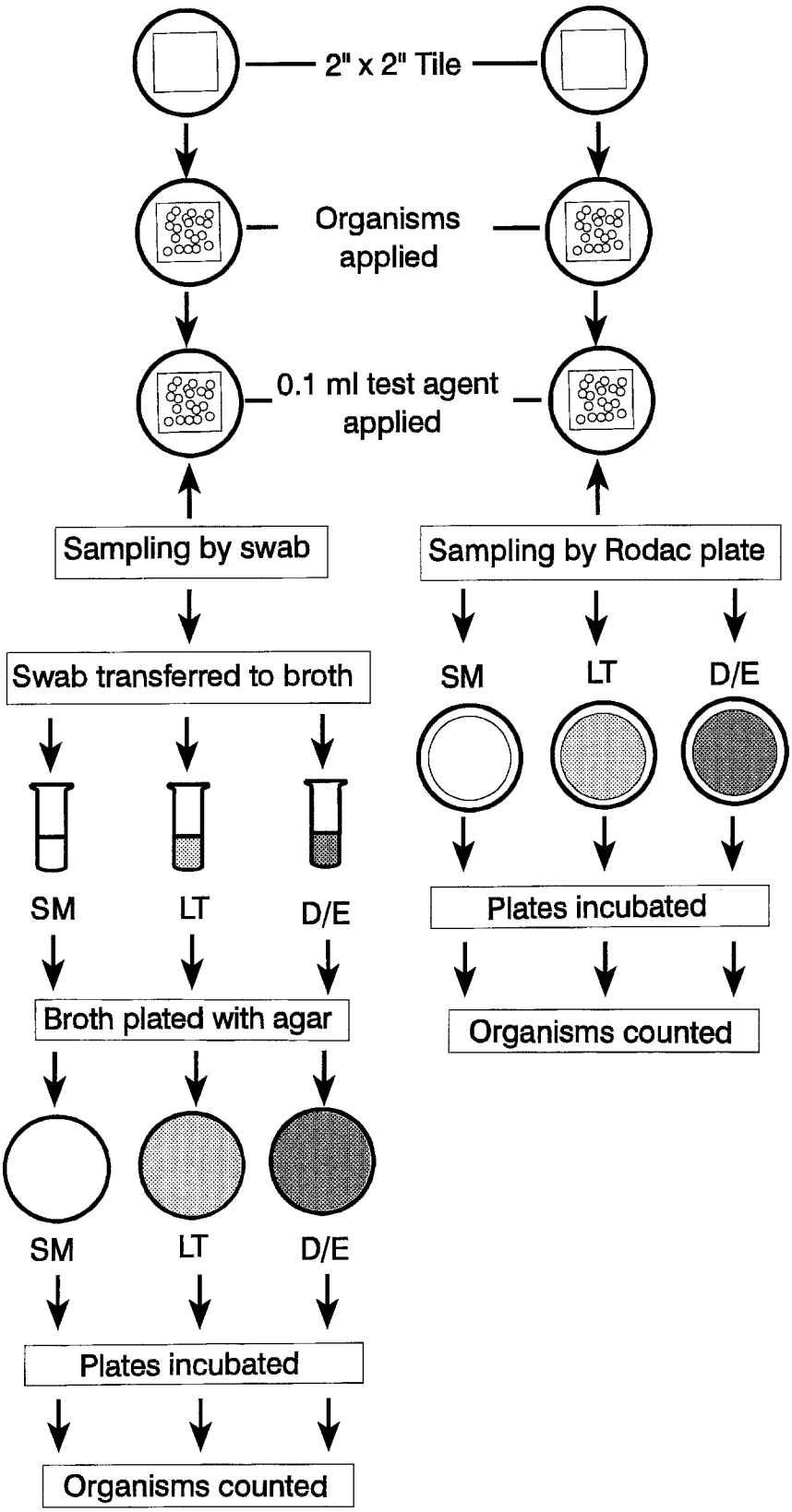

Fig. 1. Scheme for the recovery of chemically-exposed *S. aureus* ATCC 6538.

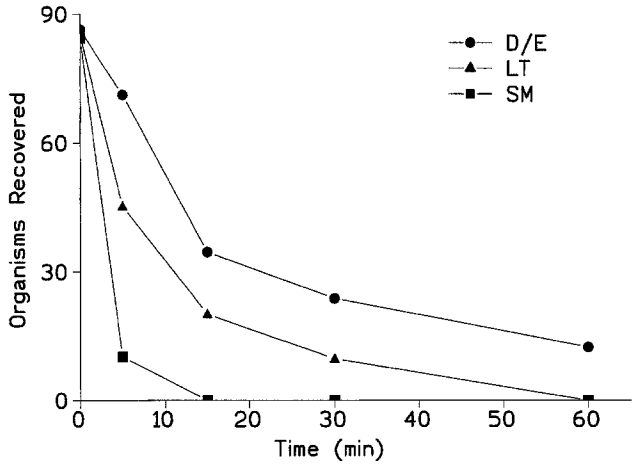

Fig. 2. Recovery of Mikro-Bac-exposed *S. aureus* ATCC 6538 by swab at various time intervals with D/E, SM and LT medium. (Inoculum level 300 organisms).

to preparations of a phenol and a quaternary ammonium compound depends largely on the antimicrobial neutralizing capacity of the recovery medium. Significantly higher organism recovery with D/E medium by either sampling method shows that D/E medium is more efficient in neutralizing carried-over commercial phenol and quaternary ammonium compound than the LT medium. An earlier study also showed that the recovery of Mikro-Bac- and Mikro-Quat-treated *S. aureus* with D/E medium was significantly higher than the Standard Methods Medium, with or without Tween 80 and lecithin [4].

Easy detection of recovered organisms on D/E medium is an added advantage. Due to bromcresol purple indicator in the D/E medium, an *S. aureus* colony appears yellow against a purple background [3,5]. The medium shows promise for evaluating antimicrobial agents used in surface disinfection.

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TABLE 1

Regression analysis of *S. Aureus* ATCC 6538 recovery

Antimicrobial	No. of organisms exposed	Recovery		Number of time intervals organisms recovered	Antilog of intercept[a]	100 × slope[b]
		Method	Medium			
Mikro-Bac	127.5	Swab	LT	4	72.2	−4.21
	123.6	″	D/E	4	103.0	−2.63
	85.2	″	LT	3	62.0	−6.69
	86.4	″	D/E	4	78.5	−3.64
Mikro-Quat	127.5	″	LT	3	54.1	−5.63
	123.6	″	D/E	4	99.3	−2.87
	85.2	″	LT	2	14.8	−5.80
	86.4	″	D/E	4	63.8	−3.02
Mikro-Bac	152.4	Rodac	LT	3	63.7	−6.77
	158.1	″	D/E	4	81.0	−2.87
	89.4	″	LT	2	29.1	−9.60
	87.1	″	D/E	4	42.8	−3.60
Mikro-Quat	152.4	″	LT	3	11.0	−5.47
	158.1	″	D/E	4	62.0	−2.80
	89.4	″	LT	0	–	–
	87.1	″	D/E	4	54.5	−5.28

[a] Estimated number of organisms recovered at time = 0.
[b] Relative % change in recovery per minute of exposure.

achieved first by dilution and then by inactivation of carried-over chemicals by the neutralizers present in the broth and in the recovery agar. On the other hand, a five times higher ratio between the swab to Rodac recoveries for Mikro-Quat with the LT medium show that many of the organisms sampled on Rodac plates made with a medium that lacks adequate neutralizer were suppressed by the Mikro-Quat carried over.

The results indicate that the recovery of organisms exposed

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