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TWO METHODS FOR THE EVALUATION OF SIX DISINFECTANTS USING *PSEUDOMONAS AERUGIONOSA AND SERRATIA MARCESCENS* AS THE TEST ORGANISMS

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ملخـــــص

استعملت في هذه الدراسة ٥٠ عزله من *Pseudomonas aeruginosa* و ٦ عزلات من *Serratia marcescens* عزلت من عينات طبية مختلفة . اختبر تأثير انواع مختلفة من المطهرات التجارية على هذه العزلات باستعمال طريقتي تخفيف مختلفة . وقد اظهرت نتائج هذه الدراسة ان جميع عزلات *S, marcescens* قد توقفت عن النمو تماما باستعمال ٠ر١٥ بالمئة اسيل أو ٠ر٤٤ بالمئة سفلون . بينما توقف نمو عزلات *P, aerugionosa* باستعمال ٠ر٣ بالمئة اسيل أو ٠ر٨٨ بالمئة سفلون . وقد لوحظ أن نمو كل من الميكروبيتين المذكورتين قد توقف تماما باستعمال ٠ر٦٥ بالمئة وستاسبت أو ٠ر١٢٥ بالمئة فينول او ٠ر٥ بالمئة اكريفلفين أو ٠ر٢٥ بالمئة ميكروبك .

ABSTRACT

A total of 50 isolates of *Pseudomonas aeruginosa* and 6 isolates of *Serratia marcescens* from various clinical specimens were used in this study. The action of various commerical disinfectants on these isolates was studied using the agar and broth dilution methods. All *S. marcescens* strains were inhibited by 0.15% O-syl or 0.44% Savlon. While *P. aeruginosa* strains were inhibited by 0.3% O-syl or 0.88% Savlon. Both organisms were inhibited by 0.65% Westaspet, 0.125% Phenol, 0.5% Acriflavin or 0.25% Micro - Bac.

INTRODUCTION

Pseudomonas aeruginosa as a cause of a wide variety of nosocomial infections is attracting much attention in the clinical field. It is a frequent source of trouble in hospitals because of its exceptional ability to survive and multiply in the most unpropitious and unlikely enviromments such as 1% cetavlon, (sterile water), cork of bottles, sinks, as well as in wounds, the renal tract and bronchial tree (Faydi, 1981, unpublished data). It's spread from contaminated resusscitation equipment to premature infants have been reported by various investigators[1,9]. Primary infections in dults are less common but serious. The organism may cause minor localized infections, such as otitis externa, or highly generalized infections that occur as a consequence to trauma or some debilitating states. The organism was reported to cause fatal generalized infections following severe burns[11], and leukemia[4]. The mucoid variants of the organism cause chronic cheast disease[12].

Serratia marcescens is one of the several species of microorganisms, formerly considered non-pathogenic[6]. It is increasingly recognized as causing clinical disease and is asuming an increasing medical importance. It is a pathogen frequently involved in nosocomial bacteremia[7]. An increasing incidence of isolation of *S. Marcescens* in the clinical bacteriology laboratory was noted in the past few years. It has also been observed that non-pigmented strains were more common than pigmented ones[2,3]. There is a great need for the correct identification of *Serratia* species particularly the non pigmented ones.

EXPERIMENTAL

Source of cultures: A total of 50 strains of *P. aeruginosa* and 6 strains of *S. marcescens* were isolated from clinical specimens such as urine, sputum, blood, eye, ear feces and others. Some of the *P. aeruginosa*

strains were isolated from the nursery and delivery suite environments of the American University Hospital of Beirut. They were maintained on trypticase soy agar slants and stored at room temperature (24 ±1° C) until needed. *Pseudomonas aeruginosa* strains were identified according to the scheme of Nabbut and Faydi[10] (Fig. 1). *Serratia marcescens* strains were identified according to a series of biochemical reactions[8] (Table 1).

Fig. 1 Simplified scheme for the identification of P. aeruginosa.

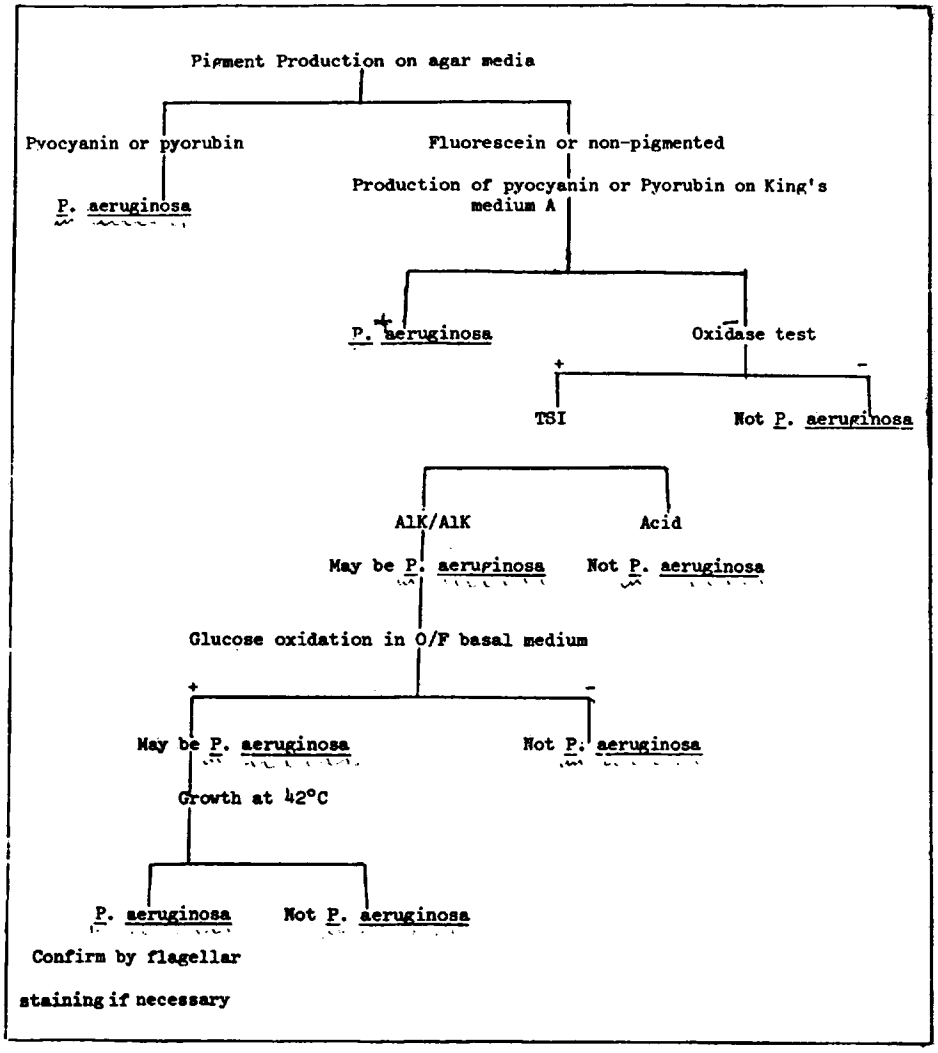

Table 1. Identification of Serratia marcescens. O, doubtful.

Test	Sign	Percent Positive
Glucose		
Acid	+	100
Gas	+ or −	61.3
Inositol		
Acid	d	73.8 (12.6)
Gas	−	0
Glycerol		
Acid	+	94.6 (4.4)
Gas	−	0
Cellobiose		
Acid	d	
Gas	−	
Esculin		
Acid	d	71.3 (0.2)
Gas	−	0
Raffinose		
Acid	−	0.9 (2.6)
Gas	−	0
Arabinose		
Acid	−	0.2 (1.9)
Gas	−	0
Xylose		
Acid	d	7.9 (18.6)
Gas	−	0
Erythritol (acid)	d	1.7 (22.8)
a-Methyl Glucoside	−	0.9 (0.6)
Methyl red		
37° C	+ or −	14
22° C	+ or −	8.8
Voges Proskauer		
37° C	+	100
22° C	+	100

Culture media: Trypticase soy agar (TSA) and trypticase soy broth (TSB), both Difco products were used in this study. MacConkey agar (Difco) was used for the isolation of *S. marscescens.* Other media used for identification purposes are shown in Figure 1 and Table 1.

Disinfectants: O-syl, a phenolic disinfectant (Lehn and Fink Industrial Products); Savlon contains chlorhixidin and cetrimide (ICI); Westaspet, a hexachlorophene disinfectant (West Chemical Products); Micro-Bac, a phenolic disinfectant (Economics Laboratory, INC.); Acriflavin (Winthrop) and Phenol (British Drug Houses Ltd.) were used.

Test procedures: The actions of the various disinfectants on *P. aeruginosa* and *S. marcescens* were tested by the two following methods:

1. *Agar dilution method:* Trypticase soy agar plates with different disinfectants concentrations were prepared. Phenol and Acriflavin plates contained the following concentrations of each disinfectant 0.06%, 0.125%, 0.25%, 0.5% and 1%. TSA plates with O-syl, Savlon, Westasept or Micro-Bac contained concentrations of 1%, 2%, 3%, 4%, and 5%. Plates were dried for about 10 minutes in the incubator at 37° C before they were used. A loopful of 4-hour TSB culture of each test organism was streaked with one line of streaking on the plates with disinfectant, using a 4-mm calibrated loop. A control plate without disinfectants was also streaked in the same manner. All plates were incubated overnight at 37° C and were then read for growth inhibition.

2. *Broth dilution method:* Two fold dilution series of each disinfectant were prepared in TSB using screw capped culture tubes (10 X 10). The final volume in each tube was 0.5 ml. One-half ml of 10^{-3} dilution of overnight TSB culture containing $5.9 \cdot X\ 10^{4}$ organism/ml was added to every tube. Tubes were then incubated overnight at 37° C. They were then usually examined for evidence of growth. The minimal bactericidal concentration (MBC) of every disinfectant was determined by streaking a loopful from each tube showing no visible turbidity on TSB plates. Plates were incubated for 24 hours at 37° C and were examined for growth.

RESULTS AND DISCUSSION

There is a great need for an effective reliable disinfectant(s) to eradicate microbiological contaminations in hospital environments, specially those caused by *P. aeruginosa*. A good testing system for the evaluation of these

disinfectants is required. The phenol coefficient does not indicate the relative effictiveness of the product for use in disinfection of floors or walls[13]. Thus in this paper two methods were used for the evaluation of 6 disinfectants using both *P. aeruginosa* and *S. marcescens* as the test organisms.

In the agar dilution method different concentrations of each disinfectant were incorporated in TSA plates. They were added in the form of milliliters and the total active ingrediant for each disinfectant present in the TSA plates was calculated as percentage. It is evident from Table 2 that all*S. marcescens* strains were inhibited by 0.15% O-syl or 0.44% Savlon. However *P. aeruginosa* strains were more resistant and required 0.3% O-syl or 0.88 % Savlon to inhibit their growth. For the remaining disinfectants both organisms were inhibited by 0.065% Westaspet, 0.125% Phenol, 0.5% Acriflavin or 0.25% Micro-Bac.

It was not possible to visually determine the minimum inhibitory concentration by the use of the broth dilution method due to the turbidity

Table 2. Disinfectants concentrations required to inhibit the growth of *P. aeruginosa* and *S. marcescens.*

Organisms	O-syl.	Savlon	Westasept	Acriflavin	Micro-Bac	Phenol
P. aeruginosa	0.3 /	0.88 /	0.065 /	0.5 /	0.25 /	0.125 /
S. serratia	0.15 /	0.44 /	0.065 /	0.5 /	0.25 /	0.125 /

of the broth caused by the addition of the disinfectants. For this reason I had to rely on the determination of the MBC. O-syl, Savlon, Westasept and Acriflavin showed varied MBCs for different strains of the test organisms. As shown in Table 3 the MBC for phenol was 0.125% for 9 P. aeruginosa *strains*

and 3 S. marcescens strains. In case of Micro-Bac, 2 *P. aeruginosa* strains and 5 *S. marcescens* strains showed an MBC of 0.125%. However, 8 *P. aeruginosa* strains and 1 *S. marcescens* strain showed an MBC of 0.25%. So the agar dilution method is easier to perform. It does not require much equipment and several strains can be tested at the same time on one single plate.

Table 3. Minimum bactericidal concentration (MBC) of Phenol and Micro-Bac for *P. aeruginosa* and *S. marcescens*. * Refers to number of organisms

Disinfectant	MBC	*P. aeruginosa*	*S. marcescens*
Penlol	0.125%	9*	3
	0.25%	1	3
Micro-Bac	0.125%	2	5
	0.25%	8	1

My results agree with the work of the Association of Official Analytical Chemists (AOAC) who used dilution method which is superior to the phenol coefficient procedure [13].

I suggest to use both O-syl and Micro-Bac for disinfecting hospital environment and equipment. The proper concentrations and the time of exposure of the disinfectant should be taken into consideration. Also the inhibitory effect of the disinfectants used in hospitals should be tested from time to time, as some resistant organisms may arise. The hospital environment and equipment should be sampled before and after the application of the disinfectant.

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