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SURVIVAL OF DIFFERENT BACTERIAL SPECIES ON CEMENT AND PORCELAIN SURFACES. AT LOW TEMPERATURE.

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SUMMARY

Fourteen different bacterial species were used in a study of their abilities to survive on cement and porcelain surfaces. A dose of 5×10^4 cfu/drop (50 ul) for each species was used to contaminate each surface. The bacteria used included: Bacillus subtilis, Brucella abortus, Coraynebacterium pyogenes, E.coli, Klebsiella pnemmoniae, Listeria monocytogenes, Pasteurella multocida, Proteus vulgaris, Pseudomonas aergenosa, Salmonella typhi, Staphylococus aureus, Staph. Epidermidis, Streptococcus equi & Strept. Zooepidemicus. After 7, 14 and 21 days of storage at 4°C, subsamples were cultured onto different types of media, and after 24-48 hr. of incubation at 37°C, growth or no growth were scored for each type of bacteria used.

Results showed that B.subtilis, Staph.epidermidis & Strept. zooepdemicus were recovered from all surfaces up till 21-day-storage. E. coli, Pr.vulgaris, Staph.aureus & Strept.equi were recovered only from Porcelaim surfaces throughout the whole experiment, whereas Past. multocida was recovered at all times from cement surfaces only. Br. abortus was recovered only from cement surfaces after 7 & 14 days of storage, but was never isolated from contaminated Porcelain surfaces. Kl. pneumoniae was isolated from

Porcelain surfaces at all times, but only after 7 -day- storage from cement surfaces. On the other hand, L. monocytogenes was never recovered from any surface at any time of storage.

INTRODUCTION

Bacterial adhesion to a surface is known to play an important role in a wide variety of situations, e.g. infection of various tissues (Woods et al 1980). However, the fundamental mechanisms governing bacterial adhesion are poorly understood and have not been well defined. Most work done on microbial adhesion has dealt with growth of the adhering microbes and other subsequent behavior (Fletcher 1997). On the other hand, adhesion of a bacterium to a solid surface is dependent upon attractive forces between the two surfaces (Rutter & Vincent 1980). At the same time, repulsive forces can occur which may offset the attractive interaction or even inhibit adhesion. These physicochemical forces of attraction and repulsion include long-range forces, e.g. electrostatic interactions, like vanderwaals forces etc.

Most bacteria have a net negative charge (Harden & Harris 1953), as do most surfaces. Therefore, close attraction between similar charges will result in repulsion (McEldowney & Fletcher 1986).

Accordingly, this work has been done to determine the abilities of different pathogens bacterial species to liven 2 different surfaces for variable periods of storage at room temperature, in a way to simulate conditions of food storage, and the effect of temperature on microbial numbers.

MATERIALS & METHODS

A) *Bacteria used*: 14 bacterial species obtained from different pathological condition were used. The species included: B.subtilis (obtained from the laboratory); Br.abortus (obtained from human with Malta fever); C.pyogenes (from pus in the skin of a cow); E.coli (from the stool of a cow); E.coli (from the stool of a calf with diarrhea); Kl.pneumoniae (from a child pneumonia) ; L.monocytogenes (from the cerbrospinal fluid of child with meningitis); Past. multocida (from a calf with pneumonia); Pr. vulgaris (from urine of a dog with cystitis); Ps. aeruginosa (from the ear of a man with otitis media); Sal.typhi (from a case of human salmonllosis); Staph.aureus (from mastitic milk); Staph. epidermidis (from normal human skin); Strept. equi (from horse nostril) & Strept.zooepidemicus (from a mare genital tract with vaginitis). All bacterial species were preserved on Brain-heart infusion (BHI) agar slants prior to use.

B) *Surfaces used*: 2×2 cm^2 pieces of each of porcelain and cement surfaces were cleaned by water and soap and then soaked in 70% ethanol in screw-capped containers until use.

C) *Preparation of inocula*: For checking of purity and identity of the isolates used in the experiment, deferent biochemical tests were done according were cultured onto 5% sheep blood agar, checked by Gram's stain for purity and then transferred to tryptic soy births (10 ml. Amounts) to be diluted of the contamination inoculate were done according to the method described by Cruickshank (1975). 5×10^4 colony forming units (CFU) per 50 ul drops was used as a dose for contamination of each of 3 surfaces, consisting of 3 Porcelain & 3 Cement surfaces. Contaminated surfaces were stored in refrigerator (4-5oC) in plastic covered containers.

D) *Collection of sample*: The contaminated areas on each surface were swabbed after 7, 14 & 21 days of storage. For isolation of Staphylococci, swabs were streaked onto Mannitol salt agar; for

Streptococci, culturing was done on Azide blood agar; for Gram negative bacilli, MacConkey agar was used for isolation; for Brucella abortus tryptic soy agar was used, and for the isolation of C.pyogenes, L.monocytogenes, P.multocida and B.suotilis 5% SBA was used. Cetrimide agar (Difco) was used for selective isolation of Ps.aeruginosa. Salmonella-Shigella agar was also used for selective isolation of Salmonella and Shigalla species.

Growth was recorded +ve when clear and characteristic growth for each species was observed; otherwise, on growth or suspected type of growth for the particular organism was recorded as -ve result.

RESULTS

Results (Table. 1) showed that B.subtilis, Ps. aeruginosa, S.typhi, Staph epidermidis & Strept.zooepidemicus were all recovered successfully from all surfaces throughout the whole sampling periods.

On the other hand, C.pyogenes & L.monocytogenes were never recovered from any surface at any time.

P.multocida & Br.abortus were isolated from cement surfaces only, and that was at all sampling periods, except that Br.abortus was not isolated from either surfaces after 21 days of storage.

The reverse was obtained with E.coli, Pr.vulgaris, Staph.aureus & Strept.equi which were continuously isolated from porcelain surfaces at all times.

Kl.pneumoniae was isolated from both surfaces after 7 days of storage, but, after that, it was isolated only from porcelain surfaces.

The uninoculated (control) dishes did not show any growth, except fungal growth which was seen after 21 days of storage.

DISCUSSION

It is very interesting to demonstrate such differences in the abilities of survival of a heterogeneous group of bacterial species, on 2 different types of surfaces; one is very smooth, glistaening and

lacks the property of having fissures, the Porcelain surface. The other is a very rough, corrugated and opaque, merely the Cement surface.

Some bacterial species are well adapted to survive any adverse conditions, on any surface and for prolonged periods of time, being protected by many mechanisms, like: Spore-formation (Rose 1976) as in our case with B.subtilis (Table. 1); capsule formation (Rose 1976), as in case of Kl.pneumoniae, and so on.

In their studies on adhesion of many types of bacteria to different polymeric surfaces, Absolom and his colleagues (1983) found that the extent of adhesion depends on several factors, including: surface tensions of the adhering particles of the substrate, and of the suspending liquid medium. They studied the adhesion of Staph.aureus, Staph.epidermidis, E.coli & L.monocytogenes onto different surfaces, and they found many differences in the adhesion abilities of the different bacterial species used. Our results coincided very well with their results (Table. 1).

More studies are needed to explore the duration of survival of other potentially pathogenic bacteria on different surfaces, particularly, those related to public health.

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Table. 1: Survival of different bacterial species in Cement and Porcelain surfaces for different periods of time.

Bacterial species *	7-days storage**		14-days storage		21-days storage	
	Porcelain	cement	Porcelain	cement	Porcelain	cement
1. B subtilis	+	+	+	+	+	+
2. Br abortus	-	+	-	+	-	-
3. Coryn pyogenes	-	-	-	-	-	-
4. E.coli	+	-	+	-	+	-
5. Kl pneumoniae	+	+	+	-	+	-
6. L monocytogenes	-	-	-	-	-	-
7. Past multocida	-	+	-	+	-	+
8. Pr vulgaris	+	-	+	-	+	-
9. Ps aeruginosa	+	+	+	+	+	+
10. Sal typhi	+	+	+	+	+	+
11. Staph aureus	+	-	+	-	+	-
12. = epidermidis	+	+	+	+	+	+
13. Strept equi	+	-	+	-	+	-
14. = zooepidemicus						
15. Control	-	-	-	-	-	-

* Contamination dose for each type of bacteria use in the experiment was 5×10^4 cfu/50 ul drops.

** Swabs from each surface were pre-enriched in : Typtic soy broth (TSB) for Gram +ve species and MacConkey's (MC) broth for gram -ve ones, prior to culture onto each of : Chocolate agar (for Past multocida) , 5% SBA (For all species), Mannitol salt agar (MSA for Staphylococci) and MacConkey agr (for enteric bacteria)

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تعايش أنواع مختلفة من البكتريا على سطحي السمنت والبورسلين في درجة حرارة منخفظة

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الخلاصة

تم استخدام (14) نوعا مختلفا من البكتريا لغرض دراسة مدى قابلية تلك الانواع على البقاء حية على سطحي السمنت والبورسلين وقد استخدمت جرعة مقدارها 1×10^4 بكتريا لكل قطرة (مقدارها مايكروليتر) لكل نوع وذلك بتلويث كل من السطحين وقد استخدمت الانواع البكتيرية التالية:

B.subtilis, Br.abortus, C.pyogenes, E.coli, Kl.pneumonuae, L.monocytogenes, past.multocida, pr.vulgaris, Ps.aeruginosa, Sal.typhi,Sta.aureus, Sta.epidermidis, Str.equi, Str.zooepidemicus.

وقد خزنت جميع السطوح الملوثة بدرجة حرارة (4) مئوية ثم أخذت منها بعد (7) و (14) و (21) يوما من الخزن مسحات حيث زرعت على أوساط زرعية مختلفة وحضنت بدرجة حرارة (37) مئوية ولمدة (48–24) ساعة ثم سجلت النتائج بوجود نمو للبكتريا أو بعدمه.

أظهرت النتائج بأن كل من البكتريا B.subtilis و Steph. Epidermidis و Str.zooepidemicus قد تم عزلها من كلا السطحين الملوثين حتى اليوم الحادي والعشرون من الخزن أما الـ E.coli و Pr.vuigaris و Staph.aureus و Strep.equi فقد عزلت من سطوح البورسلين فقط أثناء فترة التجربة بينما الـ Past.multocida عزلت من سطوح السمنت فقط خلال أوقات الخزن الثلاثة.

أما بكتريا الـ Br.abortus فقد عزلت من سطوح السمنت الملوثة فقط وكان ذلك بعد (7) و (14) يوما فقط من الخزن ولكنها لم تعزل أبدا من سطوح البورسلينز وعزلت الـ Kl.pneumoniae سطوح البورسلين خلال كل فترات الخزن ومن سطوح السمنت بعد سبعة أيام فقط من الخزن. من ناحية أخرى لم تعزل بكتريا الـ L.monocytogenes قط من أي من السطوح الملوثة.